CRC PRESS
PHARMACY EDUCATION SERIES

BASIC STATISTICS AND PHARMACEUTICAL STATISTICAL APPLICATIONS

THIRD EDITION

James E. De Muth

CRC Press
Taylor & Francis Group

BASIC STATISTICS AND PHARMACEUTICAL STATISTICAL APPLICATIONS

THIRD EDITION

CRC PRESS
PHARMACY
EDUCATION
SERIES

RECENTLY PUBLISHED BOOKS

Basic Statistics and Pharmaceutical Statistical Applications, Third Edition
James E. De Muth

Basic Pharmacokinetics, Second Edition
Mohsen A. Hedaya

Pharmaceutical Dosage Forms and Drug Delivery, Second Edition
Ram I. Mahato and Ajit S. Narang

Pharmacy: What It Is and How It Works, Third Edition
William N. Kelly

Essentials of Law and Ethics for Pharmacy Technicians, Third Edition
Kenneth M. Strandberg

Essentials of Human Physiology for Pharmacy, Second Edition
Laurie Kelly McCorry

Basic Pharmacology: Understanding Drug Actions and Reactions
Maria A. Hernandez and Appu Rathinavelu

Managing Pharmacy Practice: Principles, Strategies, and Systems
Andrew M. Peterson

Essential Math and Calculations for Pharmacy Technicians
Indra K. Reddy and Mansoor A. Khan

Pharmacoethics: A Problem-Based Approach
David A. Gettman and Dean Arneson

Pharmaceutical Care: Insights from Community Pharmacists
William N. Tindall and Marsha K. Millonig

Essentials of Pathophysiology for Pharmacy
Martin M. Zdanowicz

Quick Reference to Cardiovascular Pharmacotherapy
Judy W. M. Cheng

Essentials of Pharmacy Law
Douglas J. Pisano

Pharmacokinetic Principles of Dosing Adjustments: Understanding the Basics
Ronald D. Schoenwald

Please visit our website ***www.crcpress.com*** *for a full list of titles*

BASIC STATISTICS AND PHARMACEUTICAL STATISTICAL APPLICATIONS

THIRD EDITION

James E. De Muth
Professor, School of Pharmacy, University of Wisconsin-Madison

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Version Date: 20140117

International Standard Book Number-13: 978-1-4665-9674-0 (eBook - PDF)

Dedicated to
Elin Ann Burns

Contents

Preface

The first two editions of this book were published thirteen and eight years ago. The first edition was a fairly successful attempt to provide a practical, easy-to-read, basic statistics book for two primary audiences, those in the pharmaceutical industry and those in pharmacy practice. Reviewing the contents and current uses of the first edition, several shortcomings were identified, corrected and greatly expanded in the second edition. This third edition represents not only an update of the previous two editions, but a continuing expansion on topics relevant to both intended audiences. As described later, most of the expanded information in this third edition related to allowing statistical software to accomplish the same results as identified through hand calculations.

The author has been fortunate to have taught over 100 statistics short courses since the 1999 release of the first edition. Valuable input through the learners attending these classes and new examples from these individuals have been helpful in identifying missing materials in the previous editions. In addition, the author had the opportunity to work closely with a variety of excellent statisticians. Both of these activities have helped contribute to the updating and expansions since the first book.

The continuing title of the book, *Basic Statistics and Pharmaceutical Statistical Applications*, is probably a misnomer. The goal of the first edition was to create an elementary traditional statistical textbook to explain tests commonly seen in the literature or required to evaluate simple data sets. By expanding the contents, primarily in the second edition, the material in this edition well exceeded what would be expected in a basic statistics book.

A Book for Non-Statisticians

As stated in the preface of the first edition, statistics provide useful methods to analyze the world around us, evaluate the findings and hopefully make beneficial decisions. These various tests provide a methodology for answering questions faced by pharmacists and members of the pharmaceutical industry. Statistics provide a means for summarizing data and making constructive decisions about the observed outcomes and their potential impact. This organized approach to evaluating observed data help us avoid jumping to conclusions and making choices that may be unwise or even dangerous to individuals served by our profession.

In 2005, at one of the author's Land O'Lakes Conferences, Wendell C. Smith, formerly a statistician with Eli Lilly, made two interesting statements during his presentation. The first was that statistics "provides methods and tools for decision

making in the face of uncertainty". As will be seen throughout this book, these statistical "tools" help identify differences or relationships in data where variability or uncertainty exists. An analogy from the preface in the first edition was used to describe the materials in the first eight chapters of this book. The analogy related to a heavy object suspended in midair, held in place by a rope. By definition a rope is a flexible line composed of fibers twisted together to give tensile strength to the line. The strength of a rope is based on the interwoven nature of this series of fibers. The individual fibers by themselves can support very little weight, but combined and wrapped with other fibers can form a product capable of supporting a great deal of weight. Statistics can be thought of in similar terms. A very useful and powerful device, a statistical test is based on a number of unique interwoven areas, such as types of variables, random sampling, probability, measures of central tendency and hypothesis testing. In order to understand how statistical tests work, it is necessary to have a general understanding of how these individual areas (fibers) work together to make the test (rope) a strong and effective procedure. At the same time a poorly knotted rope will eventually weaken and untie. Similarly, poorly designed experiments and/or inappropriate statistical tests will eventually fail, producing erroneous results. Treating Smith's reference to statistics as a tool in the face of uncertainty, the information in the first section of this book will briefly explore some of the basic fibers involved in strengthening this rope or tool we call statistics. The later chapters deal with specific tests. The incorrect use of statistics (through their inappropriate application) or misinterpretation of the results of the statistical test can be as dangerous as using faulty or biased data to reach the decision. Our statistical rope could quickly fray and the object come crashing to the ground.

Wendell Smith's second statement was that research involves "collaboration among participating scientists". His definition for scientists covered both traditional scientists (e.g., medicinal chemistry, pharmacology, pharmacy practitioner) and the statistical scientists (e.g., professional statistician). In research, the assistance and guidance of a professional statistician can help in avoiding certain problems and pitfalls. Conversely, to be effective, the statistician needs the input and expertise of scientists involved with the product, service or data being evaluated. It is a collaborative effort with shared expertise.

Unfortunately, many individuals fear, even hate, statistics. Why? There appear to be two major reasons for this dislike. The first is the naive belief that statistics is associated with higher mathematics and therefore difficult to learn. On the contrary, as seen in the following pages, most basic statistical tests involve four-function math (+, -, x, ÷), with a few square roots thrown in for good measure. Hopefully, even the mathematically challenged learner will benefit and increase his or her confidence using these procedures. The second major reason for disliking this area of mathematics is the association with unpleasant past experiences with statistics. In many cases, undergraduate and graduate courses are taught by individuals who are deeply concerned and interested in how statistical formulae work and the rationale behind the manipulation of the data. Unfortunately they may spend too much time on the derivation of the statistical tests, rather than focusing on practical day-to-day uses for these tools and successful interpretation of their results. One of the primary goals of this book is to dispel some of the fear and anxiety associated with the basic statistical tests used in the pharmacy profession and to assist individuals using

statistical computer software to help them interpret their results correctly.

By using worked out examples, individuals can understand how the mathematics work and the logic behind many of the equations used in this book. By seeing and doing the formulae the learner can better understand the outcome of the test rather than just letting the computer report the end reportable values.

Purpose of This Book

The purpose of this book has not changed since the first edition. It is to serve as an introduction to statistics for undergraduate and graduate students in pharmacy, as well as a reference guide for individuals in various pharmacy settings, including the pharmaceutical industry. It is designed for individuals desiring a brief introduction to the field of statistics, as well as those in need of a quick reference for statistical problem solving. It is a handbook, a guide and a reference for researchers in need of methods to statistically analyze data. It does not deal with the theoretical basis or derivation of most of the formulae presented; rather, it serves as a quick and practical tool for the application of the most commonly employed statistical tests. Now with the third edition, it also provides information on software applications to assist with the evaluation of data.

A greater knowledge of statistics can assist pharmacy students, pharmacists and individuals working in the pharmaceutical industry in at least four ways:

1. When reading articles in a refereed journal we assume that the material has been thoroughly checked and the information presented is accurate. Unfortunately, reviews of the medical literature have found numerous errors and these will be discussed in Chapter 24. It is important to be cognizant of possible statistical mistakes when reading the literature.
2. Pharmacists and pharmacy decision makers are constantly gathering data to improve or justify their professional services, or are involved in clinical trials to help identify more effective therapies for their patient clientele. Use of the appropriate statistical test and correct interpretation of the results can assist in supporting new programs or expanded services.
3. Scientists working in the pharmaceutical industry are constantly presented with data and knowledge of the use of both descriptive and inferential statistics can be helpful for preparing reports, submitting regulatory documentation, or other problem-solving activities.
4. For pharmacists, the Board of Pharmaceutical Specialties has developed board certification for pharmacotherapy with the designation “Board Certified Pharmacotherapy Specialist.” Certification requires the candidate to pass a rigorous examination that includes therapeutics, research design, basic data analysis and biostatistics, clinical pharmacokinetics and knowledge of physical examination findings. An increased comfort level with statistics and greater understanding of the appropriate tests can assist with this endeavor.

How is This Book Similar to the First Two Editions?

The approach to presenting the topic of statistics has not changed since the first two editions. The book is still divided into three major sections: 1) the underpinnings required to understand inferential statistical tests; 2) inferential statistics to help in problem solving; and 3) supportive materials in the form of flow charts and tables.

The second section presents the various statistical tests commonly found in pharmacy and the pharmaceutical literature. A cursory view of today's literature indicates that these same tests are still commonly used. Each chapter includes example problems. The problems are derived from the areas of pharmacy, analytical chemistry, and clinical drug trials. The focus in these chapters is on: 1) the most commonly used tests; 2) when these tests should be used; 3) conditions that are required for their correct use; and 4) how to properly interpret the results. Some sections of the second edition and this edition present a variety of tests to accomplish the same purpose and which rarely appear together in other available statistics books. All are intended to provide a useful guide or reference for evaluating research data or understanding the published literature.

The last section of the book consists of a flow chart to aid in the selection of the most appropriate test given the types of variables involved, tables for the interpretation of the significance of the statistical results, and a quick reference list of steps to follow to perform tests on two types of computer software.

How Does This Book Represent an Improvement over Previous Editions?

There are still the same number of chapters as the last edition, but each chapter in the second edition has been reviewed and edited to clarify or expand on information previously discussed. Virtually every chapter has some new information either in the form of additional paragraphs or entirely new sections.

Some of the smaller enhancements in this edition include: 1) a discussion of nonprobability sampling procedures (Chapter 3); 2) determining if data is normally distributed (Chapter 5); 3) evaluation of covariances (Chapter 13); 4) expanding the discussion of regression analysis to include confidence intervals around the intercept point, lack-of-fit test; discussion of curvilinear and nonlinear models and expansion of the discussion on multiple linear regression (Chapter 14); 5) expansion of the chi square tests associated with goodness-of-fit and test for trends with ordinal data (Chapter 16); 6) expansion of the use of the Wilcoxon and other tests related to survival statistics (Chapter 20); 7) major additions nonparametric procedures including the one-sided sign test, Wilcoxon signed-ranks test and Mood's median test (Chapter 21); and 8) a discussion of testing for precision equivalence (Chapter 22).

The majority of the new information relates to the use of Excel® and Minitab® for performing statistical analysis. There are many books available on how to use Excel, but very few mention or go into detail on the use of the "Data Analysis" add-in available on with this Microsoft Windows® program. These limited statistical analysis programs are available on most individuals' laptop or desktop computers without the requirement of purchasing additional software. The other software package discussed is Minitab 16. It has many more statistical programs than available on Excel and has been available since the late 1960s. It also was chosen because of the author's

experience with Minitab due to periodical teaching short courses for a major pharmaceutical manufacturer where medical liaisons have Minitab on their laptops. These courses consisted of teaching both basic statistics and how to use this software. So rather than dealing with multiple packages, Minitab became the preferred software. Is there any best software available? That question is hard to answer. Minitab is easy to use, provides simple and concise output and covers a wide variety of tests. The important thing for users to determine is which software is acceptable for the type of data they commonly encounter and if it has the required applications.

All chapters except the last one have example problems related to the tests discussed in each chapter. Another major change in this edition of the book has been the removal of the answers from each chapter and placement in Appendix D. This was intended to streamline the chapters and concentrate on essential information in each chapters.

Acknowledgments

As noted in previous editions of this book, many people have contributed directly or indirectly to the completion of all three editions of this book. Thanks to all the participants in the numerous short courses since the first edition of this book. They have represented audiences of both pharmacists and pharmaceutical scientists, and I have been blessed to not only educate/stimulate these audiences in the United States, but also in Brazil, Canada, China, India, Jordan, Saudi Arabia and throughout Western Europe. Through their excellent questions and my sometimes response of "I'm not sure", they have stimulated problem-solving activities that have resulted in many of the new sections in later editions. Without their insightful and challenging questions, there would not have been a need for a second or third edition.

Serving as Chair of the USP Expert Committee on Biostatistics from 2000 to 2005 was a fantastic learning experience. The members of this Committee were extremely professional and volunteered their expertise not only for the improvement of public standards for pharmaceuticals, but helping educate the Committee Chair. I miss working with these individuals and publicly thank them for help during the five-year cycle. Also, thanks to the USP staff and liaisons who supported the activities of the Committee and provided guidance and input as I continued to Chair committees on General Chapters (2005-2010) and Pharmaceutical Dosage Form (2010-2015). Involvement with USP also provided the opportunity for me to meet and work with Walter Hauck, the organization's statistical specialist. Walter continually served as a source for clarifying statistical issues and has an amazing ability to express clearly explained statistical through the written word. The knowledge and insight brought to the table by Walter and the former USP Committee members are examples of why I classify myself as a "statistical hobbyist" and not a professional statistician!

Thanks to Russell Dekker, former Chief Publishing Officer for Marcel Dekker, Inc. (publishers of the first edition of this book). Russell suggested and encouraged (even hassled) me into preparing both editions of this book.

One of the frustrations with the first two editions of this book was its listing as part of a biostatistics series and primarily promoted to professional statisticians instead of the intended audience. Thanks to Taylor & Francis for moving this book to

the CRC Press Pharmacy Education Series and promoting the book to the intended audience.

As with the previous two editions of this book, the accomplishment of completing the following materials is directly attributable to the love and support of my family. A very special thank you to my wife Judy, to our daughters, Jenny and Betsy and my son-in-law Erik, for their continued encouragement and patience as this third edition has evolved over the past two years.

James E. De Muth

Symbols

α (alpha)	type I error; probability used in statistical tables, *p*
α'	Bonferroni adjusted type I error, Sidák test statistic
α_{ew}	experimentwise error rate = $1 - (1 - \alpha)^C$
β (beta)	type II error; population slope
$1 - \beta$	power
β_1, β_2, β_3	regression coefficients (beta weights)
Γ (gamma)	Goodman-Kruskal's gamma statistic
δ (delta)	difference
η (eta)	correlation ratio
θ (theta)	equivalence interval
κ (kappa)	Cohen's kappa statistic
μ (mu)	population mean
μ_0	target mean in control charts
μ_d	population mean difference (matched-pair t-test)
$\mu_{\overline{X}}$	mean of the sampling distribution of $\overline{X}$
ν (nu)	degrees of freedom in analysis of variance
ρ (rho)	Spearman rank correlation coefficient; population correlation coefficient
ρ_α	Cronbach's alpha statistic
ρ_{KR20}, ρ_{KR21}	Kuder-Richardson test statistics
σ (sigma)	population standard deviation
σ^2	population variance
$\sigma_{\overline{X}}$	standard deviation of the sampling distribution of $\overline{X}$
τ_b (tau)	Kendall's tau-b statistic
τ_c	Kendall's tau-c statistic
ϕ (phi)	phi coefficient, phi statistic
χ^2 (chi)	chi square coefficient
χ^2_{CMH}	Cochran-Mantel-Haenszel chi square test statistic
$\chi^2_{corrected}$	Yates' correction for continuity statistic
$\chi^2_{McNemar}$	McNemar test statistic
χ^2_{MH}	Mantel-Haenszel chi square test statistic
χ^2_r	Friedman two-way analysis of variance test statistic

ψ_I (psi)	estimator for Scheffé's procedure
ϖ^2 (omega)	coefficient of determination for nonlinearity
a	y-intercept, intercept of a sample regression line
A_n^2	Anderson-Darling test statistic
AD_i	absolute deviation
ARR	absolute risk reduction
b	sample slope, slope of a sample regression line
c or C	number of columns in a contingency table; number of possible comparison with two levels; Cochran's C test statistic; Pearson's C statistic, contingency coefficient
C^*	Sakoda's adjusted Pearson's C statistic
cf	cumulative frequency
CI	confidence interval
CEO	control event odds
CER	control event rate
C_p, C_{pk}, C_{pm}	process capability indexes
CV	coefficient of variation
D	difference between pairs of values or ranks; Durbin-Watson coefficient
D	Komogorov-Smirnov goodness-of-fit test statistic
$\overline{d}$	sample mean difference (matched-pair t-test)
df	degrees of freedom
d_{xy}, d_{yx}	Somers' D statistic
e	2.7183, the base of natural logarithms
E	event or expected frequency with chi square
E^2	coefficient of nonlinear correlation
$E(T)$	expected total value for Wilcoxon matched-pairs test
$E(x)$	expected value
EEO	experimental event odds
EER	experimental event rate
f	frequency, frequency count
F	analysis of variance coefficient, test statistic
F_{max}	Hartley's F-max test statistic
FN	false-negative results
FP	false-positive results
H	Kruskal-Wallis test statistic
H'	Kruskal-Wallis test statistic corrected for ties
H_0	null hypothesis, hypothesis under test
H_1	alternate hypothesis, research hypothesis
$\hat{h}(t_i)$	hazard rate
$K_{intervals}$	number of class intervals in a histogram
L	Lord's range test statistic
LCL	lower control line in a control chart
LSL	lower specification limit for capability indices
LTL	lower tolerance limit
LR^+, LR^-	likelihood ratio

Log	logarithm to the base 10
M	median, huge rule outlier test statistic
MS_B	mean square between
MS_E	mean squared error
MS_R	mean squared residual
MS_{Rx}	mean squared treatment effect
MS_W	mean square within
n	number of values or data points in a sample
N	number of values in a population, total number of observations
n!	factorial
$\binom{n}{x}$	combination statement
NNT	number needed to treat
O	observed frequency with chi square
OR	odds ratio
p	probability, level of significance, type I error; Fisher's exact test statistic; median test statistic
p(E)	probability of event *E*
p(x)	probability of outcome *x*
$p(E_1 \text{ and } E_2)$	probability that both events E_1 and E_2 will occur
$p(E_1 \cap E_2)$	probability that both events E_1 and E_2 will occur
$p(E_1 \text{ or } E_2)$	probability that either event E_1 or E_2 will occur
$p(E_1 \cup E_2)$	probability that either event E_1 or E_2 will occur
$p(E_1 \mid E_2)$	probability that event E_1 will occur given E_2 has occurred
${}_nP_x$	permutation notation
PVN	predicted value negative
PVP	predicted value positive
q	studentized range statistic
Q	Cochran's Q test statistic; Yule's Q statistic
Q_1	25th percentile
Q_3	75th percentile
r	correlation coefficient, Pearson's correlation
r or *R*	number of rows in a contingency table
R	range
r^2	coefficient of determination
R^2	coefficient of multiple determination
r_{xy}	reliability coefficient, correlation statistic
R_1, R_2	sum of ranks for samples of n_1, n_2 in Mann-Whitney U test
rf	relative frequency
RR	relative risk
RR_{MH}	Mantel-Haenszel relative risk ratio
RRR	relative risk reduction
RSD	relative standard deviation
S or *SD*	sample standard deviation
S^2	sample variance or Scheffé's value
S_p	pooled standard deviation

S_p^2	pooled variance
$S_{y/x}$	standard error of the estimate for linear regression
S_r	residual standard deviation
$\hat{S}_i$	survival function estimate
SE	standard error term
SEM	standard error of the mean, standard error
$SE(\hat{h}_i)$	standard error of the hazard rate
$SE(\hat{S}_i)$	standard error of the survival function estimate
SIQR	semi-interquartile range
t	t-test statistic
T	Wilcoxon signed rank test statistic; Tshuprow's T statistic; extreme studentized deviate test statistic, Grubbs' test statistic
TN	true-negative results
TP	true-positive results
U	Mann-Whitney U test statistic
UC	Theil's uncertainty coefficient
UCL	upper control line in a control chart
USL	upper specification limit for capability indices
UTL	upper tolerance limit
V	Cramer's V statistic
x	variable used to predict *y* in regression model
x_i	any data point or value
x'_i	transformed data point
$\overline{X}$	sample mean
$\overline{X}_G$	geometric mean; grand mean
w	width of a class interval
W	Shapiro-Wilk's W test statistic; Kendall's coefficient of concordance
y	variable used to predict *x* in regression model
Y	Yule's Y statistic
z	z-test statistic
Z_0	reliability coefficient for repeatability and reproducibility
z_x	standardized score for an abscissa
z_y	standardized score for an ordinate

1

Introduction

Statistics can be simply defined as the acquisition of knowledge through the process of observation. We observe information or data about a particular phenomenon and from these observations we attempt to increase our understanding of the event that data represents. According to Conover (1999), it provides a means to measure the amount of subjectivity that goes into researcher's conclusions, separating "science" from "opinion." Physical reality provides the data for this knowledge and statistical tests provide the tools by which decisions can be made.

Types of Statistics

As noted by Daniel (1978) "...statistics is a field of study concerned with (1) the organization and summarization of data, and (2) the drawing of inferences about a body of data when only a part of the data are observed." All statistical procedures can be divided into two general categories: descriptive or inferential. **Descriptive statistics**, as the name implies, describe data that we collect or observe (**empirical data**). They represent all of the procedures that can be used to organize, summarize, display, and categorize data collected for a certain experiment or event. Examples include: the frequencies and associated percentages; the average or range of outcomes; and pie charts, bar graphs or other visual representations for data. These types of statistics communicate information, they provide organization and summary for data, or afford a visual display. Such statistics must: 1) provide an accurate representation of the observed outcomes; 2) be presented as clear and understandable as possible; and 3) be as efficient and effective as possible. In order to perform inferential statistics described below, one must first calculate the descriptive statistics because these values or numbers will be used in calculations required for the inferential statistic.

Inferential statistics (sometimes referred to as analytical statistics or inductive statistics) represent a wide range of procedures that are traditionally thought of as statistical tests (e.g., student t-tests, analysis of variances, correlation, regression or various chi square and related tests). These statistics infer or make predictions about a larger body of information based on a sample (a small subunit) from that body. It is important to realize that the performance of an inferential statistical test involves more than simple mathematical manipulation. The reason for using these statistical

tests is to solve a problem, answer a question or at a minimum provide direction to an answer. Therefore, inferential statistics actually involves a series of steps: 1) establishing a research question; 2) formulating a hypothesis that will be tested; 3) selecting the most appropriate test based on the type of data collected; 4) selecting the data correctly; 5) collecting the required data or observations; 6) performing the statistical test; and 7) making a decision based on the result of the test. This last step, the decision making, will result in either the rejection of or failure to reject the statement (hypothesis) being tested and will ultimately answer the research question posed in the first step of the process. These seven steps will be discussed in more detail at the end of this chapter.

The first sections of this book will deal mainly with descriptive statistics, including presentation modes (Chapter 4) and with data distribution and measures of central tendency (Chapters 5 and 6). These measured characteristics of the observed data have implications for the inferential tests that follow. Chapter 8 on hypothesis testing provides guidance for the development of statements that will be evaluated through the inferential statistics. The information beginning with Chapter 9 covers specific inferential statistical tests that can be used to make decisions about an entire set of data based on the small subset of information selected.

In fact, statistics deal with both the known and unknown. As researchers, we collect data from experiments and then we present these initial findings in concise and accurate compilations (known – the descriptive statistics). However, in most cases the data that we collect represent only a small portion (a **sample**) of a larger set of information (a **population**) for which we desire information. Through a series of mathematical manipulations the researcher will make certain guess or statement (unknown – the inferential statement) about this larger population.

Parameters and Statistics

As mentioned, statistical data usually involve a relatively small portion of an entire population, and through numerical manipulation, decisions and interpretations (inferences) are made about that population. To illustrate the use of statistics and some of the terms presented later in this chapter, consider the following example:

> A pharmaceutical manufacturing company produces a specific dosage form of a drug in batches (lots) of 50,000 tablets. In other words, one complete production cycle is represented by 50,000 tablets.

Parameters are characteristics of populations. In this particular case the population would be composed of one lot of 50,000 units. To define one of the population's parameters, we could weigh each of the 50,000 tablets and then be able to: 1) calculate the average weight for the entire lot; and 2) determine the range of weights within this one particular batch by looking at the difference between the two extreme weights (both lightest and heaviest tablet). This would give us the exact weight parameters for the total batch; however, it would be a very time-consuming process. An even more extreme situation would be to use a Stokes or Strong-Cobb hardness tester to measure the hardness of each tablet. We could then determine the

average hardness of the total batch, but in the process we would destroy all 50,000 tablets. This is obviously not a good manufacturing procedure.

In most cases, calculating an exact population parameter may be either impractical or impossible (e.g., required destructive testing as shown in the previous example). Therefore, we sample from a given population, perform a statistical analysis on this information, and make a statement (inference) regarding the entire population. **Statistics** are characteristics of a sample we create by selecting a subset of the population, They represent summary measures computed on observed sample values. For the above example, it would be more practical to periodically withdraw 20 tablets during the manufacturing process, then perform weight and hardness tests on the tablets, and assume these sample statistics for this subset are representative of the entire population of 50,000 units.

Continuing with our manufacturing example, assume that we are interested in the average weight for each tablet (the research question). We assume there is some variability, however small, in the weights of the tablets. Using a process described in Chapter 3, we will sample 20 tablets that are representative of the 50,000 tablets in the lot and these will become our best "guess" of the true average weight. These 20 tablets are weighed and their weights are averaged to produce an average sample weight. With some statistical manipulation (discussed in Chapter 7) we can make an educated guess about the actual average weight for the entire population of 50,000 tablets. As explained in Chapter 7, we would create a confidence interval and make a statement such as "with 95% certainty, the true average weight for the tablets in this lot is somewhere between 156.3 and 158.4 milligrams." Statistical inference involves the degree of confidence we can place on the accuracy of the sample measurements to represent the population parameter.

It is important to note (and will be further discussed in Chapter 8) that if we are good scientists and are careful and accurate about our sample collection and summary, then our descriptive statistic should be 100% accurate for the sample information. However, when we make inferences or statements about a larger population from which we have sampled, because they are educated guesses, we must accept a certain percentage of chance (risk) that an inference may be wrong. Therefore, descriptive statistics can be considered accurate, but inferential statistics are always associated with a certain (hopefully small) chance of error or being wrong in our decision (Figure 1.1).

For consistency in this book, parameters or population values are represented by Greek symbols (e.g., μ, σ, ψ) and sample descriptive statistics are denoted by letters (e.g., $\overline{X}$, S^2, r).

Samples, which we have noted are only a small subset of a much larger population, are used for nearly all inferential statistical tests. Through the use of formulas these descriptive sample results are utilized to make predictions (inferences) about the population from which they were sampled. Examples will be presented with all inferential statistics starting with Chapter 9.

Sampling and Independent Observations

One of the underlying assumptions for any inferential test is that the data obtained from a population are collected through some random **sampling** process. As

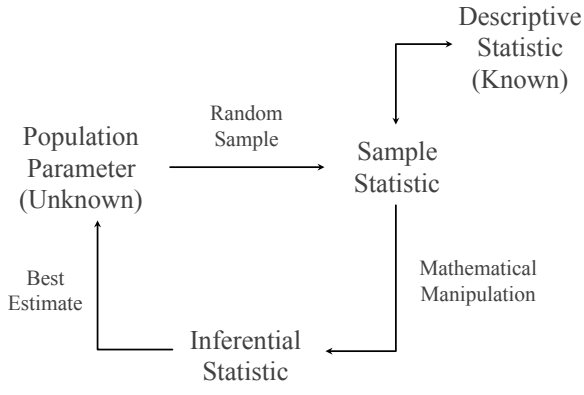


Figure 1.1 Descriptive and inferential statistics.

discussed in Chapter 3, in a completely random sample, each individual member or observation in the population has an equal chance of being selected for the sample. In the above example, sampling was conducted in such a matter that theoretically each of the 50,000 tablets has an equal chance (probability) of being selected.

The second required assumption for any inferential statistical test is that the observations be measured independent of each other. Therefore, no member of the sample should affect the outcome of any other member of the sample. The simplest example of this type of **independence** would be the proctoring of an examination to ensure that students do not cheat, thereby assuring independent performance by each person being tested. In the case of laboratory analysis, equipment should be properly cleaned and calibrated, so that the seventh sample assayed is not influenced by the sixth sample and the seventh sample does not affect any remaining assays. In other words, an independent observation or result must represent an outcome not dependent on the result of any other observation, either past or future.

Formulas used in this book assume that the sample is obtain by random sampling or equivalent procedure and that there is independence among observations in the sample.

Types of Variables

A **variable** is any attribute, characteristic, or measurable property that can vary from one observation to another. Any observation could have an infinite number of variables, such as height, weight, color, or density. For example, consider pharmacy students in a specific graduating class (at the moment the degree is awarded). Just a few of the numerous variables that could be associated with each student include:

gender
height
weight
marital status

class rank
previous undergraduate degree (yes/no)
systolic blood pressure
blood type (A, B, AB, O)
blood glucose level
accepted into graduate school (yes/no)
final examination score in physical pharmacy

The number of possible variables is limited only by our imagination. Also, the fact that we can measure a certain characteristic implies that students will differ with respect to that characteristic, and thus the characteristic becomes a variable (sometime referred to as a variate). Variables may be simply dichotomized as either discrete or continuous. The determination of whether a variable is discrete or continuous is critical in selecting the appropriate test required for statistical analysis.

A **discrete variable** is characterized by gaps or interruptions. These types of variables are also referred to as "qualitative," "category," or "nominal" variables. These variables involve placing observations into a specific, finite number of categories or classifications. Examples include distinct colors, dosage form (tablets versus capsules), and passage or failure of a specific assay criteria. Discrete variables can represent predetermined blocks of data, such as above and below a midpoint in a distribution. With relationship to the population, discrete variables for a sample must be both exhaustive and mutually exclusive. Levels of a discrete variable are **exhaustive** when the categories of that variable account for all possible outcomes. For example, males and females are exhaustive for the population of human beings based on gender; whereas age groups 0-20, 21-40, 41-60, and 61-80 are not exhaustive because there are humans over 80 years old. Similarly, levels of a discrete variable must be created that are **mutually exclusive** where categories do not have members in common with each other. Age groupings 0-20, 20-40, 40-60, and 60-80 are not mutually exclusive because ages 20, 40, and 60 are each included in two of the discrete groups. To represent a mutually exclusive and exhaustive set of categories, the age groupings should be as follows: 20 years or less, 21-40 years, 41-60 years, or 61 and older. A second example for a discrete variable might be a predetermined dissolution criterion for tablets. In this case the outcomes are represented by two mutually exclusive and exhaustive results; either the tablet passes or fails the specified criteria. From the above list of possible variables for pharmacy graduates, discrete variables include:

gender
marital status
previous undergraduate degree (yes/no)
blood type (A, B, AB, O)
accepted into graduate school (yes/no)

In contrast, a **continuous variable** has no gaps or interruptions. Also referred to as "quantitative" variables, they are probably the most commonly encountered variables in pharmacy research. Where discrete variables usually imply some form of counting, continuous variables involve measurements. Examples include age, percent,

viscosity, or blood glucose levels. In the case of our pharmacy graduates, continuous variables would include:

height
weight
class rank
systolic blood pressure
blood glucose level
final examination score in physical pharmacy

With a discrete variable, outcomes or measures are clearly separated from one another (e.g., males and females). With continuous variables it is possible to imagine more possible values between them. Theoretically, no matter how close two measures are together, a difference could be found if a more precise instrument were used. Consider age, which is a continuous variable; it can be measured by years, months, days, hours, minutes, seconds, or even fractions of a second. Time in years may be appropriate for a person's age, but for measures of disintegration or time to complete a task, minutes and seconds would be more beneficial. Any measurement result for a continuous variable actually represents a range of possible outcomes and in theory, this range for a continuous variable is considered the distance or interval from half a unit below to half a unit above the value. These numbers ("real limits") are useful in providing an accurate interpretation of statistical tests using interval or ratio scales, which are discussed below. To illustrate this, assume the most precise analytical balance in a laboratory measures the weight of a sample to be 247 mg. If we could locate a more exact balance we might find that the sample actually weighs 247.2 mg. An even more precise instrument could identify the weight in micrograms or nanograms. Therefore, our original weight of 247 mg actually represents an infinite range of weights from the real limits 246.5 to 247.5 mg. The major limitation in measuring a continuous variable is the sensitivity or precision of the instrumentation used to create a measured value.

Occasionally, a continuous variable is presented on a **rating scale** or modified into a discrete variable. For example, study results may be: 1) dichotomized either above or below the midpoint, 2) arbitrarily classified as high, medium, or low results, or 3) measured on a continuum that either "passes" or "fails" a predefined level. Even though each of these examples represents the results of a continuous measurement, by placing them *a priori* (before the test) on a rating scale they can be handled as discrete variables.

Parallel nomenclature for measurements of a variable could be in terms of types of scales, with a **scale of measurement** implying a set of numbers. As mentioned, discrete variables would involve the simplest type. Also called a **nominal scale** (from the Latin word *nominalis* meaning "of a name")**,** observations are qualitatively classified based on a characteristic being measured. They differ only in kind and cannot be arranged in any meaningful order (e.g., largest to smallest). Examples of nominal scale measurements would be male versus female, a tablet versus a capsule versus a solution, or survival versus death.

The second type of measure scale is the **ordinal scale**, in which quantitative observations are related to each other or some predetermined criteria. There is a

hierarchy to the levels of the scale with some type of rank order. We are not concerned here with the amount of difference between two observations, but their relative positions (for example, if the second observation is less than, equal to, or greater than the first observation). Ordinal scales may be used when it is not possible to make more precise measurements. For example, seen below is a scale for measuring the state of cognitive impairment in Alzheimer's patients using a seven-point scale (Morris, 1994).

	Cognitive Performance Scale Description
0	Intact
1	Border-Line Intact
2	Mild Impairment
3	Moderate Impairment
4	Moderate to Severe Impairment
5	Severe Impairment
6	Very Severe Impairment

The numbers are attached simply to show the arranged order, not the degree of difference between the various measures. With ordinal scales, even though order exists among categories, the magnitude of the difference between two adjacent levels is not the same throughout the scale. For example, is the magnitude of difference between mild and moderate impairment (previous scale), the same as the magnitude between severe and very severe impairment? Ordinal scales are extremely important in nonparametric statistical procedures (Chapter 21). Both nominal and ordinal scales are sometimes referred to as **nonmetric scales**. Also, for both of these nonmetric scales it is possible to have only two possible levels. These are termed **dichotomous** or **binary** variables. If there are no relative positions (i.e., males versus females) it is a dichotomous nominal variable. If there is a relative position (e.g., passing or failing a criterion) the variable is a dichotomous ordinal scale.

The third type of measurement scale is the **interval scale**, where the difference between each level of the scale is equal. The scales represent a quantitative variable with equal differences between scale values; however, ratios between the scale values have no meaning because of an arbitrary zero. For example the ratio between 40°F and 20°F does not imply that the former measure is twice as hot as the second.

If a genuine zero is within an interval scale it becomes a **ratio scale**, for example, measures of weight or height. If an object weighs 500 mg and a second object weighs 250 mg, the first object is twice the weight of the second. Other examples of ratio scales would include percentage scales and frequency counts. With interval and ratio scales most arithmetic operations (e.g., addition and subtraction) are permissible with these numbers. Ratio and interval scales are sometimes referred to as **metric scales**.

Independent and Dependent Variables

In addition to a variable being defined as continuous or discrete, it may also be considered independent or dependent. Most statistical tests require one or more **independent variables** that are established in advance and controlled by the

researcher. Also called a **predictor variable**, the independent variable allows us to control some of the research environment. At least one **dependent variable** is then measured against its independent counterpart(s). These **response** or **criterion variables** are beyond our control and dependent on the levels of the independent variable used in the study. Independent variables are usually qualitative (nominal) variables but also may be continuous or ordinal. For example, subjects in a clinical trial are assigned to a new drug therapy or control group, their selection is made before the study and this becomes the independent variable (treatment versus control). The therapeutic outcomes (e.g., decreased blood pressure, pharmacokinetic data, length of hospitalization) are variables dependent on the group to which they were assigned. A second example is a measure of the amount of active ingredient in the core tablet portion of an enteric-coated tablet for the same medication, using the same process, at three different manufacturing facilities (New Jersey, Puerto Rico and India). The independent variable is the facility location (a discrete variable with three levels) and the dependent variable would be the average content (amount of active ingredient) of the drug at each facility. Note in the second example that only three facilities are used in the study and each sample must come from one of these sites and cannot come from two different locations at the same time; thus representing mutually exclusive and exhaustive observations that fulfill the requirements for a discrete variable. It is assumed that samples were selected appropriately (through some random process, discussed in Chapter 3), content is measured using the same apparatus and using the same procedures, and conducted in such a manner that each result is independent of any other sample.

In designing any research study, the investigator must control or remove as many variables as possible, measure the outcome of only the dependent variable, and compare these results based on the different levels or categories of the independent variable(s). The extraneous factors that might influence the dependent variable's results are known as **confounding** or **nuisance variables**. In the previous example, using different instruments to measure the contents at different sites may produce different results even though the tablets are the same at all three sites.

Selection of the Appropriate Statistical Test

In order to select the correct inferential test procedure, it is essential that as researchers, we understand the variables involved with our data. Which variables are involved for a specific statistical test? Which variable or variables are under the researcher's control (independent) and which are not (dependent)? Is the independent variable discrete or continuous? Is the dependent variable continuous or discrete? As seen in Appendix A, answering these questions automatically gives direction toward the correct inferential statistical procedure to use in a given situation. All the statistical procedures listed in the flow chart in Appendix A will be discussed in Chapters 9 through 23. To illustrate the use of this Appendix, consider the previous example on clinical trials (measure of therapeutic outcomes based on assignment to the treatment or control group). Starting in the box in the upper left corner of Panel A in Appendix A, the first question would be: Is there an independent, researcher-controlled variable? The answer is yes, we assign volunteers to either the experimental or control groups. Therefore, we would proceed down the panel to the

next box: is the independent variable continuous or discrete? It is discrete, because we have two nominal levels that are mutually exclusive and exhaustive. Continuing down Panel A, are the results reported as a percentage or proportion of a certain outcome? Assuming that our results represent length of hospital stay in days, the answer would be no and we again continue down the page to the next decision box. Is the dependent variable continuous or discrete? Obviously number of days is a continuous measure; therefore we proceed to Panel B. The first question in Panel B asks the number of discrete independent variables. In this example there is only one, whether the volunteer received the study drug or control. Moving down Panel B, what is the number of levels (categories) within the independent variable? There are only two, therefore we continue down this panel. The next decision will be explained in Chapter 9, but for the moment we will accept the fact that the data are not paired and move down once again to the last box on the left side of Panel B. Similarly, for the point of our current discussion we will assume that the population variance is unknown and that our sample is from a population in which the dependent variable is normally distributed and that both levels produce a similar distribution of values (these will be explained in Chapter 6). Thus, we continue to the right and then down to the last point on the right side of the panel and find that the most appropriate inferential statistical test for our clinical trial would be a two-sample t-test.

Procedures for Inferential Statistical Tests

Most individuals envision statistics as a labyrinth of numerical machinations. Thus, they are fearful of exploring the subject. As mentioned in the Preface, the statistics in this book rely primarily on the four basic arithmetic functions and an occasional square root. The effective use of statistics requires more than knowledge of the mathematical required formulas. This is especially true today, when personal computers can quickly analyze sample data. There are several important parts to completing an appropriate statistical test.

1. **Establish a research question**. It is impossible to acquire new knowledge and to conduct research without a clear idea of what you wish to explore. For example, we would like to know if three batches of a specific drug are the same regarding their content uniformity. Simply stated: are these three batches equal?

2. **Formulate a hypothesis**. Although covered in a later chapter, we should formulate a hypothesis that will be either rejected or not rejected based on the results of the statistical test. In this case, the hypothesis that is being tested is that Batch A equals Batch B equals Batch C. The only alternative to this hypothesis is that the batches are not all equal to each other.

3. **Select an appropriate test**. Using information about the data (identifying the dependent and independent variables) the correct test is selected based on whether these variables are discrete or continuous. For example, batches A, B, and C represent an independent variable with

three discrete levels and the assay result for the drug's contents is a continuous variable (%) dependent upon the batch from which it was selected. Therefore, the most appropriate statistical test would be one that can handle a continuous dependent variable and a discrete independent variable with three categories. If we once again proceeded through Appendix A we would conclude that the "analysis of variance" test would be most appropriate (assuming normality and homogeneity of variance, terms discussed later in this book). A common mistake is to collect the data first, without consideration of these first three requirements for statistical tests, only to realize that a statistical judgment cannot be made because of the arbitrary format of the data.

4. **Sample correctly**. The sample should be randomly selected from each batch (Chapter 3). An appropriate sample size should be selected to provide the most accurate results (Chapter 8).

5. **Collect data**. The collection should ensure that each observed result is independent of any other assay.

6. **Perform test**. Only this portion of the statistical process actually involves the number crunching associated with statistical analysis. Many commercially available computer packages are available to save us the tedium of detailed mathematical manipulations.

7. **Make a decision**. Based on the data collected and statistical manipulation of the sample data, a statement (inference) is made regarding the entire population from which the sample was drawn. In our example, based on the results of the test statistics, the hypothesis that all three batches are equal (based on content uniformity), is either rejected or the sample does not provide enough information to reject the hypothesis. As discussed in Chapter 8, the initial hypothesis can be rejected, but never proven true.

To comprehend the principles underlying many of the inferential statistical tests it is necessary that we have a general understanding of probability theory and the role that probability plays in statistical decision making. The next chapter focuses on this particular area.

Applications of Computer Software

Many commercial software packages are available for presenting descriptive statistics or doing inferential statistical analysis. They are easier, quicker, and more accurate compared to hand calculations (as will be illustrated in later chapters). With easy access to computer software, step 6 in the previous section may be the least important component of a statistical test. However, commercial software can give the user a false sense of security and it is important to understand the software and how to enter and query the data. The availability of software makes the task easier but does not eliminate the need for a good understanding of basic statistics and which test is

appropriate for the given situation. Even using sophisticated packages the researcher still needs to interpret the output and determine what the results mean with respect to the model used for the analysis.

Two commercially available packages will be demonstrated in this book - Excel® by Microsoft and Minitab® 16 by Minitab, Inc. These were chosen because of the easy access to Excel and the fact that the author uses Minitab for teaching statistics to selected groups of pharmacists. Many other software packages are available. Examples include BMDP, JMP, SAS, SPSS, Statgraphics and Systat (current websites for each are listed in the references at the end of this chapter). No software package is "perfect" and before purchasing any package the potential users should determine the types of applications they commonly require, access a demonstration version of the software, and determine if it meets their needs.

The format used for discussing the software will start with a line indicating the terms appearing in the title bar of the software and any subsequent dropdown menu selections from the title bar.

Title Bar ➤ First Dropdown ➤ Second Dropdown ➤ etc.

Note with Minitab that the underscored letters or numbers when combined with the ALT key will produce the same results as left clicking on the dropdown option. Selections on various intermediate menus will be in *italic* and areas requiring information or final command options will be in "quotation marks". For example, using Minitab to perform a two-sample t-test in Chapter 7 the access to the application would be:

Stat ➤ Basic Statistics ➤ 2-sample t…

Followed by moving of the selected independent variable form the left column to the "Subscripts:" box and the dependent variable to the "Samples:" box. The *Options* menu can be selected for one-tailed test where the "Alternative:" selection can be made for "greater than" or "less than". For Excel, all function commands will be presented as **BOLDED.CAPS**.

Excel is available on must computers using Microsoft software and should represent a negligible cost for the interested user. Applications discussed are available with both Excel 97-2003 and Excel 2010. It is assumed that the reader knows how to initiate and enter data into Excel. Many of the actions will require the functions box in the upper center of the screen (Figure 1.2). This is initiated by entering an equals sign in a cell and selecting from the functions listed on the left hand side of the screen. Excel 2010 also includes statistical add-ins which appear as "Data Analysis" in the upper right corner when "Data" is initiated on the top application title bar (Figure 1.3). If the "Data Analysis" does not appear, it can be added by selecting "File" on the application title bar and choosing "Option", followed by Add-Ins, selecting "Analysis ToolPak" and OK (Figure 1.4). Notation used in the following chapters will be:

File ➤ Options ➤ Add-Ins ➤ Analysis ToolPak ➤ OK

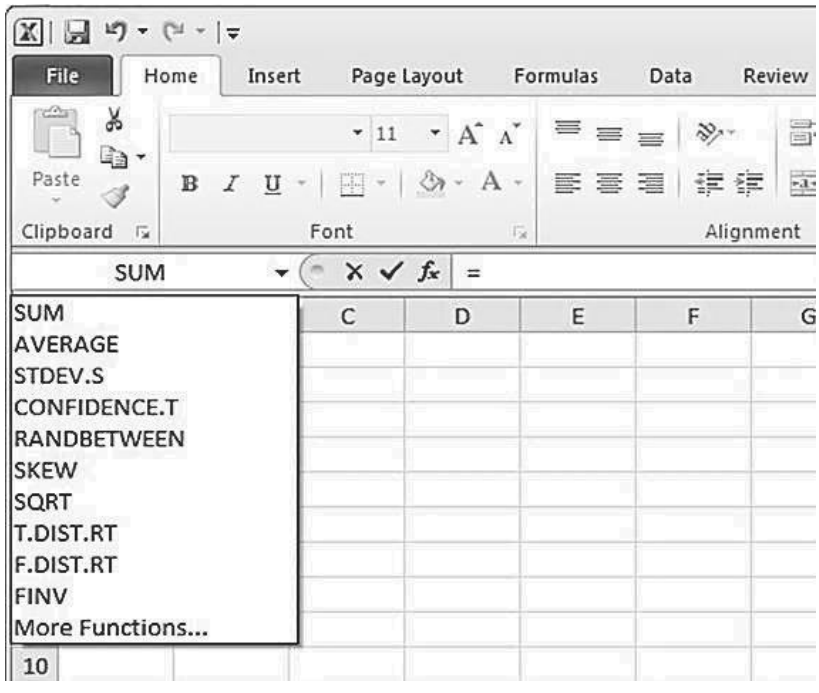


Figure 1.2 Example of Excel function window and dropdown menu.

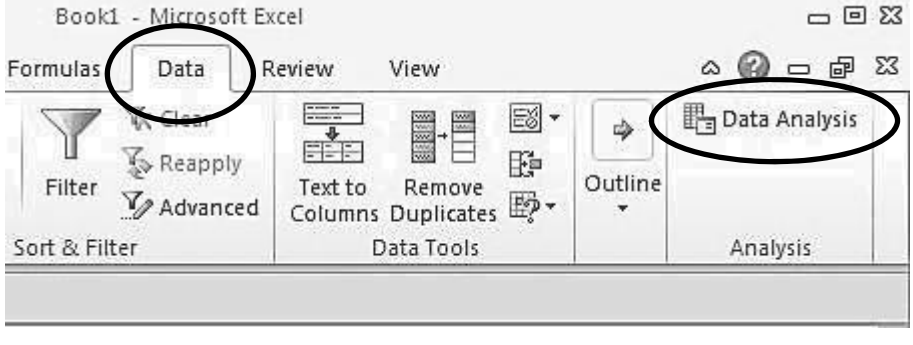


Figure 1.3 Data analysis location with Excel 2010.

This will place the "Data Analysis' on the second bar to the right. The same application is available with Excel 97-2003:

Tools ➤ Add-Ins ➤ Analysis ToolPak ➤ OK

To initiate the "Data Analysis" with Excel 97-2003 select "Data Analysis" under the "Tools" menu. Both versions provide the menu in Figure 1.5.

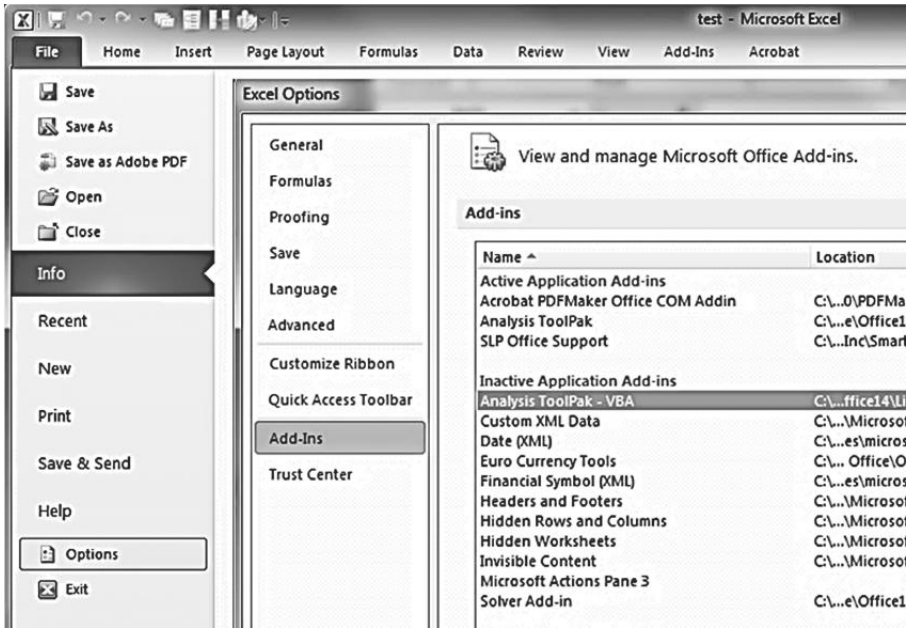


Figure 1.4 Add-in Options with Excel 2010.

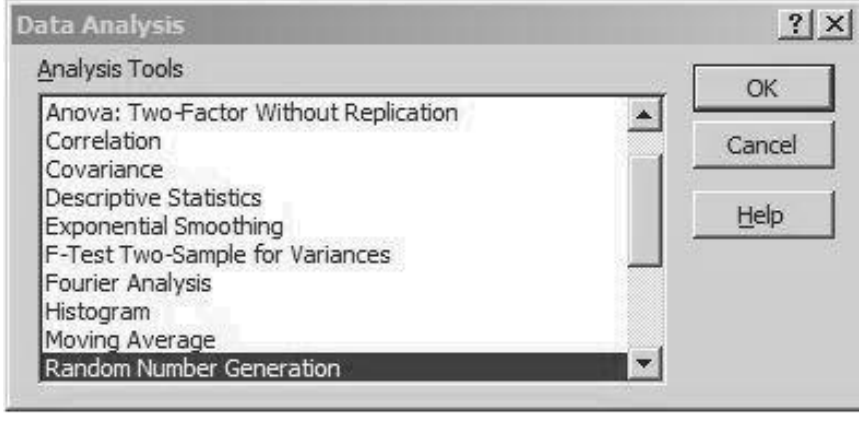


Figure 1.5 Data analysis menu with Excel.

With Minitab most of the statistical operations will involve pointing and clicking on the "Stats" and "Graph" commands on the application title bar (Figure 1.6). Once again, it is assumed that reader is familiar with data entry and manipulation using Minitab. If not, you are encouraged to explore the downloadable user's guide "Meet Minitab 16" listed in the suggested readings, especially the sections on "Opening a Worksheet".

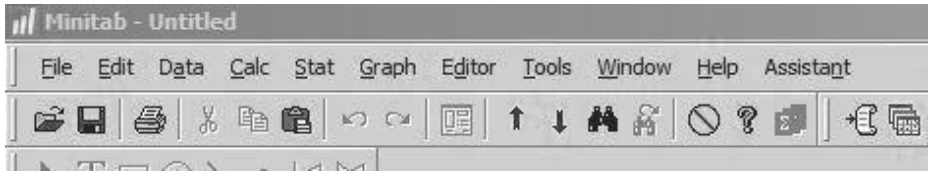


Figure 1.6 Application title bar in Minitab.

References

Conover, W.J. (1999). *Practical Nonparametric Statistics*, John Wiley and Sons, New York, p. 2.

Daniel, W.W. (2008). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Ninth edition, John Wiley and Sons, New York, p. 1.

Morris, J.N. , Fries B.E., Mehr, D.R., et al. (1994) "MDS Cognitive Performance Scale" *Journal of Gerontology*: *Medical Sciences,* 49:M174-182.

BMDP software information, www.statistical-solutions-software.com

JMP software information, www.jmp.com

SAS software information, www.sas.com

SPSS software information, www-01.ibm.com/software/analytic/spss

Statgraphics software information, www.statlets.com

Systat software information, www.systat.com

Suggested Supplemental Readings

Billo, J. (2001), *Excel for Chemists: A Comprehensive Guide*, Second edition, Wiley-VCH, New York, pp. 3-58.

Bolton, S. (1997). *Pharmaceutical Statistics: Practical and Clinical Applications*, Third edition, Marcel Dekker, Inc., New York, pp. 538-541.

Meet Minitab 16, *www.minitab.com/uploadedFiles/Shared_Resources/Documents/MeetMinitab/EN16_MeetMinitab.pdf,* pp. 1-1 - 2-13.

Zar, J.H. (2010). *Biostatistical Analysis*, Fifth edition, Prentice Hall, Upper Saddle River, NJ, pp. 1-5, 16-17.

Example Problems (Answers are provided in Appendix D)

1. Which of the following selected variables, associated with clinical trials of a drug, are discrete variables and which are continuous?

   Experimental versus control (placebo)
   Dosage form – table/capsule/other
   Bioavailability measurements (C_{max}, T_{max}, AUC)
   Test drug versus reference standard
   Fed versus fasted state (before/after meals)
   Prolactin levels (ng/l)
   Manufacturer (generic versus brand)
   Male versus female subjects
   Age (in years)
   Smoking history (cigarettes per day)
   "Normal" versus geriatric population

2. Which of the following selected variables associated with a random sample of 50,000 tablets, mentioned earlier in this chapter, are discrete variables and which are continuous?

   Amount of active ingredient (content uniformity)
   Dissolution test – pass or fail criteria
   Disintegration rate
   Change in manufacturing process – old process versus new
   Friability – pass or fail criteria
   Hardness
   Impurities – present or absent
   Size – thickness/diameter
   Tablet weight
   Immediate release or sustained release
   Formulation A, B, or C

3. The ability to identify independent and dependent variables, and determine if these variables are discrete or continuous is critical to statistical testing. In the examples listed below, identify the following:

   Is there an independent variable? Is this independent variable continuous or discrete? What is the dependent variable? Is this dependent variable continuous or discrete?

   a. During a clinical trial, volunteers were randomly divided into two groups and administered either: 1) the Innovator antipsychotic medication or 2) Acme Chemical generic equivalent of the same drug. Listed below are the results of the trial (C_{max}). Is there any difference between the two manufacturers' drugs based on this one pharmacokinetic property?

Result of Clinical Trial for C_{max} (ng/ml)

	Innovator	Acme Chemical
Mean	289.7	281.6
S.D.	18.1	20.8
n	24	23

b. During a cholera outbreak in a war-devastated country, records for one hospital were examined for the survival of children contracting the disease. These records also reported the children's nutritional status. Was there a significant relationship between their nutrition and survival rate?

	Nutritional Status	
	Poor (N_1)	Good (N_2)
Survived (S_1)	72	79
Died (S_2)	87	32

c. Samples were taken from a specific batch of drug and randomly divided into two groups of tablets. One group was assayed by the manufacturer's own quality control laboratories. The second group of tablets was sent to a contract laboratory for identical analysis.

Percentage of Labeled Amount of Drug

Manufacturer		Contract Lab	
101.1	98.8	97.5	99.1
100.6	99.0	101.1	98.7
100.8	98.7	97.8	99.5

d. An instrument manufacturer ran a series of tests to compare the pass or fail rate of a new piece of disintegration equipment. Samples were taken from a single batch of uncoated tablets. Two different temperatures were used and tested for compendia recommended times. Success was defined as all six tablets disintegrating in the disintegration equipment.

	Success	Failure	
39°C	96	4	100
35°C	88	12	100
	184	16	200

e. Three physicians were selected for a study to evaluate the length of stay for patients undergoing a major surgical procedure. All these procedures occurred in the same hospital and were without complications. Eight records were randomly selected from patients treated over the past twelve months. Was there a significant difference, by physician, in the length of stay for these surgical patients?

Days in the Hospital

Physician A	Physician B	Physician C
9	10	8
12	6	9
10	7	12
7	10	10
11	11	14
13	9	10
8	9	8
13	11	15

f. Acme Chemical and Dye received from the same raw material supplier three batches of oil from three different production sites. Samples were drawn from drums at each location and compared to determine if the viscosity was the same for each batch.

Batch A	Batch B	Batch C
10.23	10.24	10.25
10.33	10.28	10.20
10.28	10.20	10.21
10.27	10.21	10.18
10.30	10.26	10.22

g. Two different scales were used to measure patient anxiety levels upon admission to a hospital. Method A was an established test instrument, while Method B (which had been developed by the researchers) was quicker and an easier instrument to administer. Was there a correlation between the two measures?

Method A	Method B	Method A	Method B
55	90	52	97
66	117	36	78
46	94	44	84
77	124	55	112
57	105	53	102
59	115	67	112
70	125	72	130
57	97		

2

Probability

As mentioned in the previous chapter, statistics involve more than simply the gathering and tabulating of data. Inferential statistics are concerned with the interpretation and evaluation of data and making statements about larger populations. The development of the theories of probability resulted in an increased scope of statistical applications. Probability can be considered the "essential thread" that runs throughout all statistical inference (Kachigan, 1991).

Classic Probability

Statistical concepts covered in this book are essentially derived from probability theory. Thus, it would be only logical to begin our discussion of statistics by reviewing some of the fundamentals of probability. The **probability** of an event [*p(E)*] is the likelihood of that occurrence. It is associated with discrete variables. The probability of any event is the number of times or ways an event can occur (*m*) divided by the total number of possible associated events (*N*):

$$p(E) = \frac{m}{N} \qquad \text{Eq. 2.1}$$

In other words, probability is the fraction of time in which the event will occur, given many opportunities for its occurrence. For example, if we toss a fair coin, there are only two possible outcomes (a head or a tail). The likelihood that one event, for example a tail, is 1/2 or $p(T_{ail}) = 0.5$.

$$p(T_{ail}) = \frac{1}{2} = 0.50$$

A synonym for probability is **proportion**. If the decimal point is moved two numbers to the right, the probability can be expressed as a percentage. In the previous example, the proportion of tails is 0.5 or there is a 50% chance of tossing a tail or 50% of the time we would expect a tail to result from a toss of a fair coin.

The **universe** (*N*), which represents all possible outcomes, is also referred to as the **outcome space** or **sample space**. Note that the outcomes forming this sample space are mutually exclusive and exhaustive. The outcomes that fulfill these two

requirements are called **simple outcomes**. Other common examples of probabilities can be associated with a normal deck of playing cards. What is the probability of drawing a red card from a deck of playing cards? There are 52 cards in a deck, of which 26 are red; therefore, the probability of drawing a red card is

$$p(R_{ed}) = \frac{26}{52} = \frac{1}{2} = 0.50$$

Note that cards must be red or black, and cannot be both; thus, representing mutually exclusive and exhaustive simple outcomes. What is the probability of drawing a queen from the deck? With four queens per deck the probability is

$$p(Q_{ueen}) = \frac{4}{52} = \frac{1}{13} = 0.077$$

Lastly, what is the probability of drawing a diamond from the deck? There are 13 diamonds per deck with an associated probability of

$$p(D_{iamond}) = \frac{13}{52} = \frac{1}{4} = 0.25$$

Does this guarantee that if we draw four cards one will be a diamond? No. Probability is the likelihood of the occurrence of an outcome over the "long run." However, if we draw a card, note its suit, replace the card, and continue to do this 100, 1000, or 10,000 times we will see the results become closer to if not equal to 25% diamonds.

There are three general rules regarding all probabilities. First, a probability cannot be negative. Even an impossible outcome would have $p(E) = 0$. Second, the sum of probabilities of all mutually exclusive outcomes for a discrete variable is equal to one. For example, with the tossing of a coin, the probability of a head equals 0.50, the probability of a tail also equals 0.50 and the sum of both outcomes equals 1.0. Thus the probability of an outcome cannot be less than 0 or more than 1.

$$0 \leq p(E) \leq 1$$

A probability equal to zero indicates that it is impossible for that event to occur. For example, what is the probability of drawing a "blue" card from a standard deck of playing cards? Such an outcome would be impossible and have a probability of zero. This is sometime referred to as an **empty set**. In contrast, a probability of 1.0 means that particular event will occur with utter certainty or a **sure event**.

At times our primary interest may not be in a single outcome, but with a group of simple outcomes. Such a collection is referred to as a **composite outcome**. The third general rule, because of the **addition theorem**, is that the likelihood of two or more mutually exclusive outcomes equals the sum of their individual probabilities.

$$p(E_i \text{ or } E_j) = p(E_i) + p(E_j) \qquad \text{Eq. 2.2}$$

For example, the probability of a composite outcome of drawing a face card (jack, queen, or king) would equal the sum of their probabilities.

$$p(F_{ace\,card}) = p(K_{ing}) + p(Q_{ueen}) + p(J_{ack}) = \frac{1}{13} + \frac{1}{13} + \frac{1}{13} = \frac{3}{13} = 0.231$$

For any outcome *E*, there is a complementary event ($\overline{E}$), which can be considered "not *E*" or "*E* not." Since either *E* or $\overline{E}$ must occur, but both cannot occur at the same time, then $P(E) + P(\overline{E}) = 1$ or written for the complement

$$p(\overline{E}) = 1 - p(E) \qquad \text{Eq. 2.3}$$

The complement is equal to all possible outcomes minus the event under consideration. In one of the previous examples, it was determined that the probability of drawing a queen from a deck of cards is 0.077. The complimentary probability, or the probability of "not a queen" is

$$p(\overline{Q}_{ueen}) = 1 - p(Q_{ueen}) = 1 - 0.077 = 0.923$$

Our deck of cards could be considered a universe or a population of well-defined objects. Probabilities can then be visualized using simple schematics as illustrated in Figure 2.1. Figure 2.1-A illustrates the previous example of the likelihood of selecting a queen or a card that is not a queen. Note that the two outcomes are visually mutually exclusive and exhaustive. This type of figure can be helpful when more than one variable is involved.

Probabilities can be either theoretical or empirical. The previous examples with a deck of cards can be considered **theoretical probabilities** because we can base our decision on formal or logical grounds. In contrast, **empirical probabilities** are based on prior experience or observation of prior behavior. For example, the likelihood of a 55 year-old female dying of lung cancer cannot be based on any formal or logical considerations. Instead, probabilities associated with risk factors and previous mortalities would contribute to such an empirical probability.

A visual method for identifying all of the possible outcomes in a probability exercise is the **tree diagram**. Branches from the tree correspond to the possible results. Figure 2.2 displays the possible outcome from tossing three fair coins.

Probability Involving Two Variables

In the case of two different variables (e.g., playing card suit and card value), it is necessary to consider the likelihood of both variables occurring, *p(A)* and *p(B)*, which are not mutually exclusive. A **conjoint** or **union** $(A \cup B)$ is used when calculating the probability of either *A* or *B* occurring. An **intersect** $(A \cap B)$ or **joint probability** is employed when calculating the probability of both *A* and *B* occurring at the same time. The probability of an intersect is either given, or in the case of theoretical

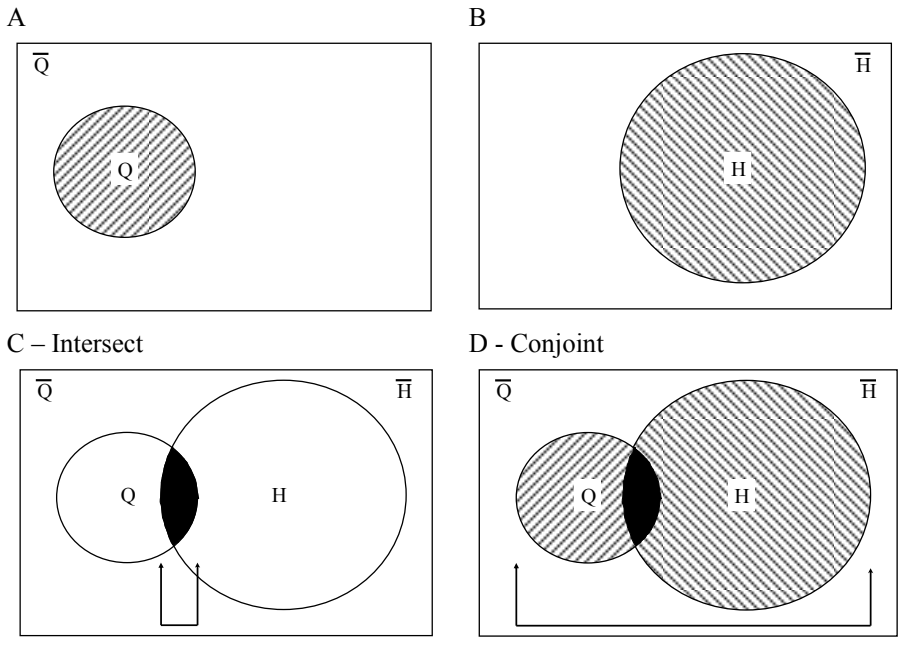


Figure 2.1 Schematics of various probability distributions.

probabilities, easily determined using the **multiplication theorem**, in which *p(A and B) = p(A) × p(B)* if *A* and *B* are independent of each other.

$$p(A \cap B) = p(A) \times p(B) \qquad \text{Eq. 2.4}$$

For example what is the probability of drawing a card that is both a queen and a heart (Figure 2.1-C)?

$$p(\text{queen and heart}) = p(Q \cap H) = 1/52$$

$$p(\text{queen and heart}) = p(\text{queen}) \times p(\text{heart}) = 1/13 \times 1/4 = 1/52$$

In this case there is obviously only one queen of hearts in a deck of cards. What is the probability of drawing either a queen or a red card from the deck? Looking at Figure 2.1-D it is possible to see that using the addition theorem the probability of queen and the probability of a heart could be added together. However, the intersect represents an overlapping of the two probabilities or the p(*A* or *B*) equals the sum of the two probabilities minus the probability associated with the intersect.

$$p(A \cup B) = p(A) + p(B) - p(A \cap B) \qquad \text{Eq. 2.5}$$

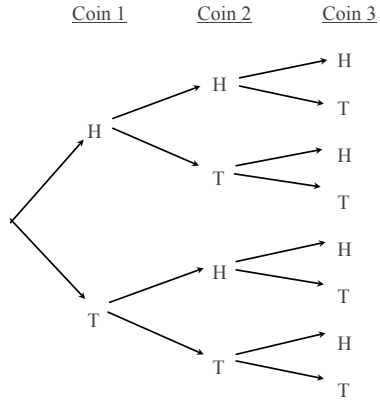


Figure 2.2 Tree diagram of the result of tossing three fair coins.

Therefore, if we subtract one of the two intercept areas seen in Figure 2.1-C we can compute the conjoint:

$$p(\textit{queen or heart}) = p(Q \cup H) = p(Q) + p(H) - p(Q \cap H)$$

$$p(\textit{queen or heart}) = 4/52 + 13/52 - 1/52 = 16/52$$

Here there are 13 heart cards and four queens for a total of 17, but one of the queens is also a heart, thus the 16 possible outcomes. The conjoint is sometimes referred to as the additive rule for two events that are not mutually exclusive.

To illustrate these points further, consider the following example using empirical probability data. In a national survey, conducted in the early 1990s, on the availability of various types of hardware required to utilize different methods of programming for continuing pharmaceutical education, it was found that out of the 807 respondents: 419 had access to a personal computer capable of downloading external software; 572 had cable television in their homes; and 292 had both personal computers and cable television. Assuming that this sample is representative of all pharmacists nationally, what was the probability (at that point in time) of selecting a pharmacist at random and finding that this individual had access to a personal computer?

$$p(PC) = \frac{m(PC)}{N} = \frac{419}{807} = 0.519$$

What is the probability of selecting a pharmacist at random and finding that this individual had cable television?

$$p(TV) = \frac{m(TV)}{N} = \frac{572}{807} = 0.709$$

What is the probability of selecting a pharmacist at random and finding that this individual did not have cable television?

$$p(noTV) = \frac{m(noTV)}{N} = \frac{(807 - 572)}{807} = 0.291$$

or considering *p(noTV)* as a complement

$$p(noTV) = 1 - p(TV) = 1 - 0.709 = 0.291$$

Note that the sum of all possible outcomes for cable television equals 1.

$$Total\ p(cable\ TV) = p(TV) + p(noTV) = 0.709 + 0.291 = 1.000$$

What is the probability of selecting a pharmacist at random who had both access to a personal computer and cable television?

$$p(PC \cap TV) = \frac{m(PC \cap TV)}{N} = \frac{292}{807} = 0.362$$

Conditional Probability

Many times it is necessary to calculate the probability of an outcome, given that a certain value is already known for a second variable. For example, what is the probability of event *A* occurring given the fact that only a certain level (or outcome) of a second variable (*B*) is considered.

$$p(A)\ given\ B = p(A \mid B) = \frac{p(A \cap B)}{p(B)} \qquad \text{Eq. 2.6}$$

For example, what is the probability of drawing a queen of hearts from a stack of cards containing only the heart cards from a single deck?

$$p(queen \mid heart) = \frac{p(Q \cap H)}{p(H)} = \frac{1/52}{13/52} = 1/13$$

In this example, if all the hearts are removed from a deck of cards, 1/13 is the probability of selecting a queen from the extracted hearts.

Another way to consider the **multiplication theorem** in probability for two events that are not mutually exclusive is based on conditional probabilities. The probability of the joint occurrence *(A∩B)* is equal to the product of the conditional probability of *A* given *B* times the probability of *B* (if $p(B) > 0$):

$$p(A \cap B) = p(A \mid B)\ p(B) \qquad \text{Eq. 2.7}$$

From the previous example, if a selected pharmacist had a personal computer, what is the probability that this same individual also had cable television?

$$p(TV \mid PC) = \frac{p(PC \cap TV)}{p(PC)} = \frac{(0.362)}{(0.519)} = 0.697$$

If the selected pharmacist had cable television, what is the probability that this same individual also had access to a personal computer?

$$p(PC \mid TV) = \frac{p(PC \cap TV)}{p(TV)} = \frac{(0.362)}{(0.709)} = 0.511$$

Conditional probability can be extremely useful in determining if two variables are independent of each other or if some type of interaction occurs. For example, consider the above example of pharmacists with cable television and/or personal computers. The data could be arranged as follows, with those pharmacists having both cable television and personal computers counted in the upper left box.

	Cable TV	No Cable TV
Computer		
No Computer		

Assume for the moment that only 300 pharmacists were involved in the sample and by chance 50% of these pharmacists had personal computers:

	Cable TV	No Cable TV	
Computer			150
No Computer			150
	200	100	300

If there is no relationship between cable TV and personal computer ownership (independence) then we would expect the same proportion of computer owners and those not owning computers to have cable TV service (100 and 100 in each of the left boxes) and the same proportion of individuals not receiving cable:

	Cable TV (A)	No Cable TV ($\overline{A}$)	
Computer (B)	100	50	150
No Computer ($\overline{B}$)	100	50	150
	200	100	300

In this example:

$$p(Cable\,TV \mid Computer) = p(Cable\,TV \mid No\,Computer) = p(Cable\,TV)$$

Thus, $p(A \cap B)$ will equal $p(A)$ if the outcomes for A and B are independent of each

Table 2.1 Outcomes Expected from Rolling Two Dice

Outcome	Die 1	Die 2	Freq.	Outcome	Die 1	Die 2	Freq.
2	1	1	1	8	2	6	5
					3	5	
3	1	2	2		4	4	
	2	1			5	3	
					6	2	
4	1	3	3				
	2	2		9	3	6	4
	3	1			4	5	
					5	4	
5	1	4	4		6	3	
	2	3					
	3	2		10	4	6	3
	4	1			5	5	
					6	4	
6	1	5	5				
	2	4		11	5	6	2
	3	3			6	5	
	4	2					
	5	1		12	6	6	1
7	1	6	6				
	2	5					
	3	4		Total possible ways = 36			
	4	3					
	5	2					
	6	1					

other. This aspect of conditional probability is extremely important when discussing the chi square test of independence in Chapter 16.

Probability Distribution

A **discrete random variable** is any discrete variable with levels that have associated probabilities and these associated probabilities can be displayed as a distribution. Many times a graph or table can be used to illustrate the outcomes for these discrete random variables. For example, consider the rolling of two fair dice. There is only one possible way to roll a two: a one (on die 1) and a one (on die 2). Two outcomes could produce a three: a one (on die 1) and a two (on die 2); or a two (on die 1) and a one (on die 2). Table 2.1 represents all the possible outcomes from rolling two dice.

Knowing the frequency of each possible outcome and the total number of possible events (N), it is possible to calculate the probability of any given outcome

Table 2.2 Probability of Outcomes Expected from Rolling Two Dice

Outcome	Frequency	Probability	Cumulative Probability
2	1	0.0278	0.0278
3	2	0.0556	0.0834
4	3	0.0833	0.1667
5	4	0.1111	0.2778
6	5	0.1389	0.4167
7	6	0.1666	0.5833
8	5	0.1389	0.7222
9	4	0.1111	0.8333
10	3	0.0833	0.9166
11	2	0.0556	0.9722
12	1	0.0278	1.0000
Σ =	36	1.0000	

(Eq. 2.1). If fair dice are used the probability of rolling a two is:

$$p(2) = \frac{1}{36} = 0.0278$$

Whereas the probability of a three is:

$$p(3) = \frac{2}{36} = 0.0556$$

Therefore it is possible to construct a table of probabilities for all outcomes for this given event (rolling two dice). As seen in Table 2.2, the first column represents the outcome, and the second and third columns indicate the associated frequency and probability for each outcome, respectively. The fourth column is the accumulation of probabilities from smallest to largest outcome. For example, the cumulative probability for four or less is the sum of the probabilities of one, two, three, and four (Eq. 2.2). Obviously the probabilities for any discrete probability distribution when added together should add up to 1.0 (except for rounding errors) since it represents all possible outcomes and serves as a quick check to determine that all possible outcomes have been considered. In order to prepare a probability table, two criteria are necessary: 1) each outcome probability must be equal to or greater than zero and less than or equal to one; and 2) the sum of all the individual probabilities must equal 1.00. Note once again that these are mutually exclusive and exhaustive outcomes. If two dice are rolled on a hard flat surface there are only 11 possible outcomes (3.5, 6.7, or 11.1 are impossible outcomes). Also, two different results cannot occur at the same time.

Table 2.3 Probabilities of Various Poker Hands

Possible Hands	Ways to Make	p
Royal flush (ace through ten, same suit)	4	.000002
Straight flush (five cards in sequence, same suit)	40	.000015
Four of a kind	624	.00024
Full house (three of a kind and a pair)	3,744	.0014
Flush (five cards, same suit)	5,108	.0020
Straight (five cards in sequence)	10,200	.0039
Three of a kind	54,912	.0211
Two pairs	123,552	.0475
One pair	1,098,240	.4226
Nothing	1,302,540	.5012
Totals	2,598,964	.99996

Modified from: Kimble, G.A. (1978). *How to Use (and Misuse) Statistics*. Prentice-Hall, Englewood Cliffs, NJ, p. 91.

Many of the founders of probability were extremely interested in games of chance and in some cases were compulsive gamblers (Bernstein, 1996). Therefore, for those readers interested in vacationing or attending conventions in Las Vegas or Atlantic City, Table 2.3 presents a summary of the possible hands one could be dealt during a poker game. Notice these also represent mutually exclusive and exhaustive events. Half the time you will get a hand with nothing, only 7.6% of the time will you receive two pairs or better (1 - 0.9238). Note also that we are dealt only one hand at a time. Each hand that is dealt should be independent of the previous hand, assuming we have an honest dealer and that numerous individual decks are combined to produce the dealer's deck. Therefore, the cards received on the tenth deal should not be influenced by the ninth hand. This fact dispels the **gambler's fallacy** that eventually the cards will improve if one plays long enough. As a parallel, assume that a fair coin is tossed ten times and the results are all heads. The likelihood of this occurring is 0.1%, which will be proven later. Would it not be wise to call tails on the eleventh toss? Not really; if the coin is fair you still have a 50/50 chance of seeing a head on the eleventh toss, even though there have been ten previous heads.

Counting Techniques

With the previous example, it is relatively easy to calculate the number of possible outcomes of rolling two dice. However, larger sets of information become more difficult and time consuming. The use of various counting techniques can assist with these calculations.

Factorials are used in counting techniques. Written as $n!$, a factorial is the product of all whole numbers from *1* to n.

$$n! = n(n-1)(n-2)(n-3)\ldots(1) \qquad \text{Eq. 2.8}$$

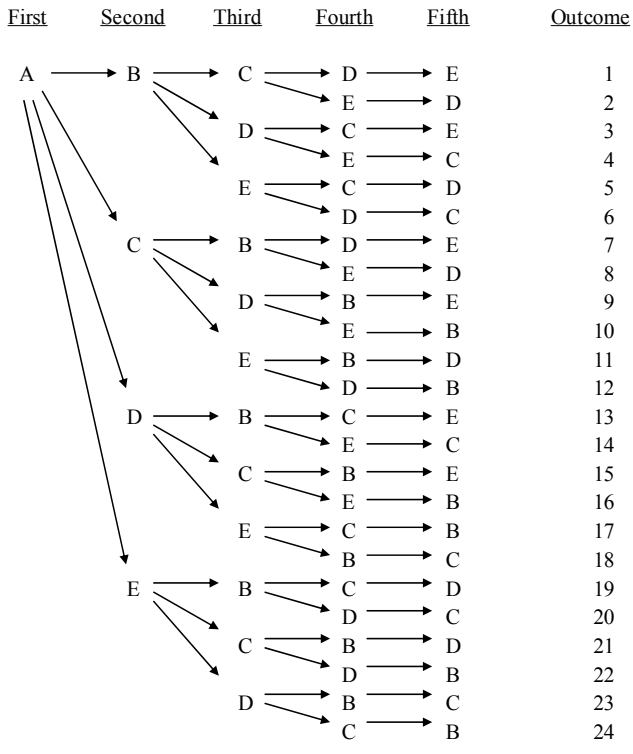


Figure 2.3 Possible ways to arrange five tablets with tablet "A" first.

For example:

$$8! = 8 \cdot 7 \cdot 6 \cdot 5 \cdot 4 \cdot 3 \cdot 2 \cdot 1 = 40{,}320$$

Because an explanation is beyond the scope of this book, we will accept by definition that:

$$0! = 1.0 \qquad \text{Eq. 2.9}$$

Permutations represent the number of possible ways objects can be arranged where *order is important*. For example, how many different orders (arrangements) can be assigned to five sample bottles in a row (bottles labeled A, B, C, D and E)? First let us consider the possible arrangements if bottle A is selected first (Figure 2.3). Thus, if A is first, there are 24 possible ways to arrange the remaining bottles. Similar results would occur if bottles B, C, D, or E are taken first. The resultant number of permutations being:

$$24 - 5 = 120 \text{ possible arrangements}$$

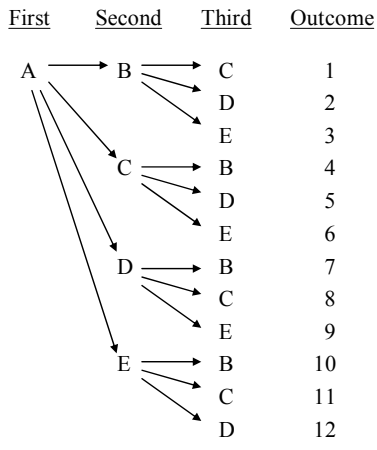


Figure 2.4 Possible ways to arrange three out of five tablets with tablet "A" first.

This is identical to a five-factorial arrangement:

$$5! = 5 \cdot 4 \cdot 3 \cdot 2 \cdot 1 = 120$$

Thus, when order is important, a permutation for n objects is $n!$. In other words, there are $n!$ possible ways to arrange n distinguishable objects.

If the permutation involves less than the total n, a factorial adjustment is easily calculated. In the above example how many possible ways could three of the five bottles can be arranged? Once again, let us look at the possibilities if bottle A is selected first (Figure 2.4). In this case, there are 12 possible ways to arrange the bottles when A is assayed first. Thus, the total possible ways to assay three out of five bottles is:

$$12 \cdot 5 = 60 \; ways$$

An easier way to calculate these permutations is to use the formula:

$$_n P_x = \frac{n!}{(n - x)!} \qquad \text{Eq. 2.10}$$

where n is the total number of possible objects and x is the number in the arrangement. In the example cited above, the possible number of arrangements for selecting five bottles, three at a time, is:

$$_5 P_3 = \frac{n!}{(n - x)!} = \frac{5!}{2!} = \frac{5 \times 4 \times 3 \times 2 \times 1}{2 \times 1} = 60$$

Combinations are used when the order of the observations is not important. For example, assume we want to assay the contents of three of the five bottles described above instead of arranging them in a row. The important feature is which three are selected, not the order in which they are chosen. As discussed in the previous chapter, independence is critical to any statistical analysis. Therefore, the order in which they are selected is irrelevant.

In the above example of five sample bottles, the results of the assay the contents for three out of five bottles is the important aspect, not the order in which the bottles were assayed. Orders A-B-C (*1* in Figure 2.4), B-C-A, C-A-B, B-A-C, A-C-B (*4* in Figure 2.4), and C-B-A would yield the same results. Thus the total possible combinations, regardless of order, can be reduced from 60 to only 10 possibilities. Using factorials for calculating larger combinations, the formula would be as follows:

$$\binom{n}{x} = \frac{n!}{x!\,(n-x)!} \qquad \text{Eq. 2.11}$$

Once again, n is the total number of possible objects and x is the number of objects selected for the combination. In the example previously cited:

$$\binom{n}{x} = \frac{n!}{x!\,(n-x)!} = \frac{5!}{3!2!} = \frac{5\times4\times3\times2\times1}{(3\times2\times1)(2\times1)} = 10$$

Consider the following example. During the production of a parenteral agent, the manufacturer samples 25 vials per hour for use in various quality control tests. Five of these vials sampled each hour are used for tests of contamination. How many possible ways could these vials be selected for contamination testing for one specific hour?

$$\binom{25}{5} = \frac{25!}{20!\,5!} = \frac{25\times24\times23\times22\times21\times20!}{5\times4\times3\times2\times1\times20!} = 53{,}130$$

In this particular case, the order with which the samples are evaluated is unimportant and therefore produces 53,130 possible sample combinations.

In a second example involving a dose proportionality study, 60 volunteers are randomly assigned to ten groups of six subjects each for the various segments (or legs) of a study. The first group receives the lowest dose, the second group receives the second lowest dose, up to the last group which receives the largest dose. At the last minute the sponsor of the study decides to reduce the maximum dose and will require only the first six segments of the study. How many ways can the assigned groups be selected for this abbreviated study?

$${}_6P_{10} = \frac{10!}{10-6!} = \frac{10\times9\times8\times7\times6\times5\times4!}{4!} = 151{,}200$$

With the groupings of subjects, order is important since each group will receive progressively larger dosages of the drug. With the order being important, there are

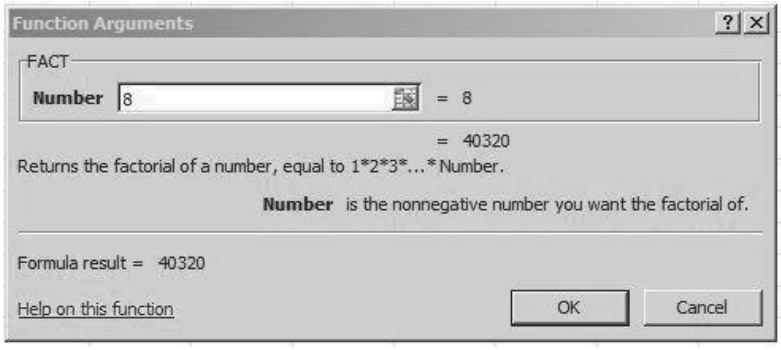


Figure 2.5 FACT function using Excel.

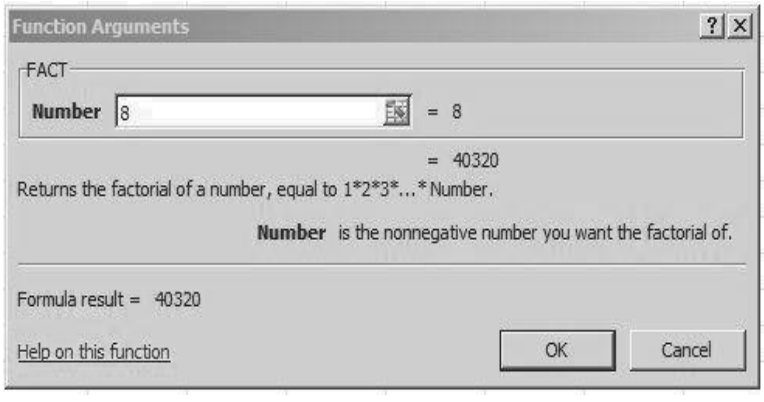


Figure 2.6 PERMUT function using Excel.

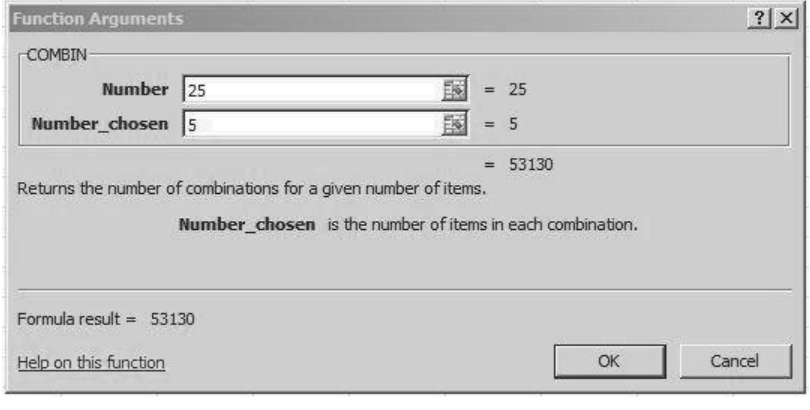


Figure 2.7 COMBIN function using Excel.

151,200 different way of selecting six of the ten groups of volunteers.

Excel can be used for all the previous counting techniques by selecting different "function" options. For factorials use function argument **FACT** and enter the number for which a factorial value is required (Figure 2.5). For permutations select function argument **PERMUT**, enter the total number of possible objects (*n* – "number") and the possible number in the arrangement (*x* – "Number_chosen") as seen in Figure 2.6. For a combination use function argument **COMBIN** (Figure 2.7), enter the total number of possible objects (*n* – "number") and the possible number in the arrangement (*x* – "Number_chosen").

Binomial Distribution

The binomial distribution is one of the most commonly encountered probability distributions. It consists of two mutually exclusive outcomes, sometimes referred to as **Bernoulli trials**. The distribution was developed by the Swiss mathematician Jakob Bernoulli in the 1600s (Dawson and Trapp, 2001). The simplest example would be a coin toss, where the probability of tossing a head is .50 and a tail is .50. If we toss two fair coins the possible results are displayed in the upper half of Figure 2.8. Note that these probabilities are excellent examples of the multiplication theorem. The first example is an example of two mutually exclusive outcomes (heads on the first coin and heads on the second coin).

$$p(H_1 \cap H_2) = p(H_1)p(H_2) = (0.50)(0.50) = 0.25$$

Two Coins

Coin 1	Coin 2	Outcome	Probability
H	H	1/4	0.25 of 2 heads
H	T	1/2	0.50 of 1 head
T	H		
T	T	1/4	0.25 of 0 heads

Three Coins

Coin 1	Coin 2	Coin 3	Outcome	Probability
H	H	H	1/8	0.125 of 3 heads
H	H	T	3/8	0.375 of 2 heads
H	T	H		
T	H	H		
H	T	T	3/8	0.375 of 1 head
T	H	T		
T	T	H		
T	T	T	1/8	0.125 of 0 heads

Figure 2.8 Probability of outcomes from tossing two or three coins.

Frequency Matrix

n														f
1						1		1						2
2					**1**		**2**		1					4
3				1		**3**		3		1				8
4			1		4		6		4		1			16
5		1		5		10		10		5		1		32
6	1		6		15		20		**15**		**6**		1	64
7		1		7		21		35		35		**21**		7		1	128

Probability Matrix

n														p
1						.5000		.5000						1.00
2					.2500		.5000		.2500					1.00
3				.1250		.3750		.3750		.1250				1.00
4			.0625		.2500		.3750		.2500		.0625			1.00
5		.0313		.1562		.3125		.3125		.1562		.0313		1.00
6	.0156		.0938		.2344		.3125		.2344		.0938		.0156	1.00
7		.0078		.0547		.1641		.2734		.2734		.1641		.0547		.0078	1.00

Figure 2.9 Pascal's triangle.

This is identical to the third possible outcome of zero heads, as seen in Figure 2.8. In the case of one head, we see a conditional probability.

$$p(H_2 \mid H_1) = \frac{p(H_2 \cap H_1)}{p(H_1)} = \frac{0.25}{0.50} = 0.50$$

The total outcomes for two coins are three combinations and four permutations. If we increase the number of fair coins to three we see the results in the bottom of Figure 2.8, where there are four combinations and eight permutations.

Obviously, the possible combinations and permutations become more difficult to define as the number of coins or observations increase. In 1303 Chu Shih-chieh, a Chinese mathematician, created what he called the "precious mirror of the four elements" (Bernstein, 1996). This later became known as **Pascal's triangle** and provides a method for calculating outcomes associated with events where the likelihood of success is 50% and failure is 50%. Figure 2.9 illustrates this triangle, the numbers in the upper portion represent frequency counts, and the lower half show proportions or probability. With respect to the frequencies, the two numbers in the top

line of the bolded triangles are summed to create the third lower point of the triangle. The total of all the frequencies for each row is summed in the far right column. To create the lower triangle in Figure 2.9, each frequency is divided by the sum of frequencies for that row. The result is a matrix that gives the probability of various outcomes (given a 50% chance of success). Notice the second and third rows in the probability matrix are identical to the results reported in Figure 2.8 for two and three coin tosses.

For example, assuming we toss a coin six times, what is the probability that we will get two heads? Referring to Figure 2.9, we would go down the sixth row of the probability matrix. The first probability (0.0156) is associated with no heads, the second (0.0938) only one head, the third (0.2344) for two heads, and so on to the last probability (0.0156) associated with all six tosses being heads. Thus, if we toss a fair coin six times, we would expect two heads approximately 23% of the time.

Unfortunately Pascal's triangle works only for dichotomous outcomes, which represent a 50/50 chance of occurring (each outcome has a probability of 0.50). The binomial equation, which follows Pascal's triangle, is based on the experiments of Jacob Bernoulli in the late 1600s (Bernstein, 1996, p.123). This can be used to calculate the likelihood associated with any number of successful outcomes regardless of the probability associated with that success, providing the probabilities of the independent events are known. The probability for each individual outcome can be calculated using the following formula:

$$p(x) = \binom{n}{x} p^x q^{n-x} \qquad \text{Eq. 2.12}$$

where n is the number of possible outcomes, x is number of successful outcomes, p is probability of success and q is the probability of failure (or not success, $1 - p$). For example, what is the probability of having two heads out of six coin tosses?

$$p(x) = \binom{n}{x} p^x q^{n-x} = p(2) = \binom{6}{2} (.5)^2 (.5)^{6-2}$$

$$p(2) = \frac{6!}{2!4!} (.5)^2 (.5)^4 = 15(0.25)(0.0625) = 0.2344$$

Here we produce the exact same results as seen with Pascal's triangle.

Four conditions must be met in order to calculate a binomial equation: 1) there must be a fixed number of trials (n); 2) each trial can result in only one of two possible outcomes that are defined as a success or failure; 3) the probability of success (p) is constant; and 4) each of the trials produces independent results, unaffected by any previous trial.

Using the binomial equation we can create a probability table to represent the associated probabilities. Again, let us use the example of coin tossing. The possible outcomes for heads based on ten tosses of a fair coin (or tossing ten separate fair coins at one time) would result in the distribution presented in Table 2.4. Using a binomial

Table 2.4 Possible Results from Tossing a Fair Coin Ten Times

Outcome - f(x) (number of heads)	p(f(x))	Cumulative p(f(x))
0	0.001	0.001
1	0.010	0.011
2	0.044	0.055
3	0.117	0.172
4	0.205	0.377
5	0.246	0.623
6	0.205	0.828
7	0.117	0.945
8	0.044	0.989
9	0.010	0.999
10	0.001	1.000

table it is possible to answer all types of probability questions by referring to the individual probabilities or the cumulative probabilities. For example, what is the probability of one head in ten tosses of a fair coin?

$$p(1) = 0.010$$

What is the probability of less than three heads in ten tosses?

$$p(0,1,2) = p(0) + p(1) + p(2) = 0.001 + 0.010 + 0.044 = 0.055$$

Because of the addition theorem we can sum all the probabilities for events less than three heads. Alternatively, we could read the results off the cumulative table, $p(<3) = 0.055$. What is the probability of seven or more heads in ten tosses?

$$p(7,8,9,10) = 0.117 + 0.044 + 0.010 + 0.001 = 0.172$$

Or, to read off the cumulative table for $1 - p(<7) = 1 - 0.828 = 0.172$. What is the probability of four to six heads in ten tosses?

$$p(6 \text{ or less}) - p(< 4) = 0.828 - 0.172 = 0.656$$

$$p(4,5,6) = 0.205 + 0.246 + 0.205 = 0.656$$

The binomial distribution can be applied to much of the data that is encountered in pharmacy research. For example:

- LD50 determination (animals live or die after dosing; used to determine the dose that kills 50% of the animals).
- ED50 determination (drug is effective or not effective; used to determine the dose that is effective in 50% of the animals).
- Sampling for defects (in quality control; product is sampled for defects and tablets are acceptable or unacceptable).
- Clinical trials (treatment is successful or not successful).
- Formulation modification (palpability preference for old and new formulation) (Bolton, 1984).

Excel can be used to calculate the binomial probability distribution by selecting the functions **BINOMDIST** or **BINOM.DIST** and entering the number of successes (*Number_s*), number of trials (*Trials*), the probability of success (*Probability_s*) and whether you want actual probability or cumulative probability (*Cumulative:* TRUE for cumulative or FALSE for actual probability for the number of successes (Figure 2.10). For example, consider results in Table 2.4 for the probability of two heads (success) out of ten coin tosses. Excel function commands would result in the following:

BINOM.DIST (2,10,0.50,FALSE) = 0.043945

If one were interested in the cumulative probability (zero, one, or two heads in six tosses) the commands and results would be:

BINOM.DIST (2,10,0.50,TRUE) = 0.054688

Poisson Distribution

Another discrete probability distribution is the Poisson distribution. As will be discussed in Chapter 6, the binomial distribution tends to be bell-shaped as n increases for any fixed value of p. However, dichotomous outcomes in which one of the two results is rare or has a very small probability of occurrence (e.g., 0.02 or 0.05), the binomial distribution will more than likely not produce a desired bell-shaped distribution. A process first introduced by Siméon Poisson in 1837 can be used to calculate probabilities associated with various events when p is relatively small:

$$p(x) = \frac{\mu^x}{x!} e^{(-\mu)} \qquad \text{Eq. 2.13}$$

where e is the constant 2.7183, the base of natural logarithms. In this case the best estimate of μ is np (the symbol μ will be discussed later in Chapter 5) or the symbol lambda (λ). Therefore, the formula can be rewritten:

$$p(x) = \frac{(np)^x}{x!} e^{(-np)} \quad or \quad \frac{\lambda^x}{x!} e^{-x} \qquad \text{Eq. 2.14}$$

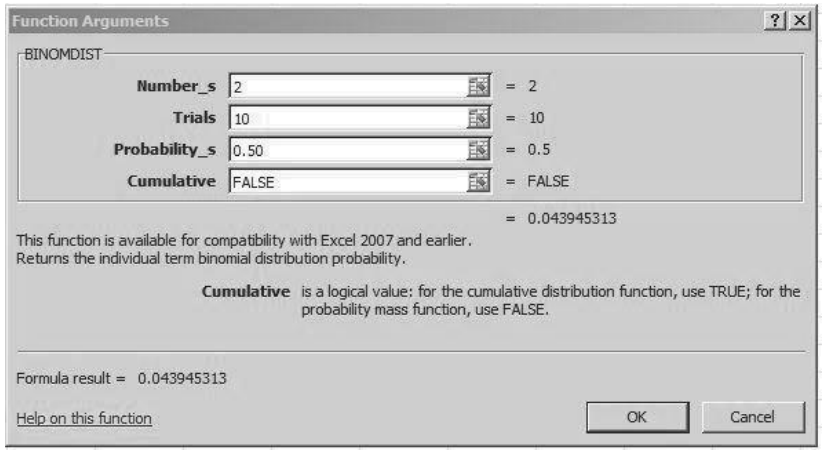


Figure 2.10 BINOMDIST function using Excel.

It can be shown, for every x, that $p(x)$ is equal to or greater than zero and that the sum of all the $p(x)$ equals 1.0, thus satisfying the requirements for a probability distribution. This produces a slightly more conservative distribution, with larger p-values associated with 0 and smaller numbers of outcomes. Because the two events of the Poisson distribution are mutually exclusive they can be summed similar to our discussion of a probability distribution.

For example, during production of a dosage form, the pharmaceutical company normally expects to have 0.5% of the tablets in a batch to have less than 95% of the labeled amount of a drug. These are defined as sub-potent tablets. If 30 tablets are randomly sampled from a batch, what is the probability of finding three sub-potent tablets? In this example: $p = 0.005$, the probability of a sub-potent tablet; n is 30 for the total sample size and x is 3 for the outcome of interest and $np = 30(0.005) = 0.15$.

$$p(3) = \frac{[0.15]^3}{3!} e^{(-0.15)} = (0.0005625)(.0.86078) = 0.000484$$

There is less than a 0.1% likelihood of randomly sampling and finding three sub-potent tablets out of 30. What is the probability of finding one defective tablet?

$$p(1) = \frac{[0.15]^1}{1!} e^{(-0.15)} = (0.15)(.0.86078) = 0.129106$$

We have a roughly 13% chance of randomly sampling 30 tablets and having one sub-potent tablet. Listed below is a comparison of the difference between results using the binomial and Poisson processes:

Number of defective tablets	Poisson p(f(x))	Binomial p(f(x))
0	0.860708	0.860384
1	0.129106	0.129706
2	0.009682	0.009451
3	0.000484	0.000443
4	0.000018	0.000015

It is possible to take this one step further and create a binomial distribution table for the probability of defective tablets and criteria for batch acceptance or rejection. Based on a sample of 20 tablets:

Defective tablets	Poisson p(f(x))	Cumulative p(f(x))
0	0.860708	0.860708
1	0.129106	0.989814
2	0.009682	0.999497
3	0.000484	0.999981
4	0.000018	0.999999

Thus, there is a 99% chance (0.989814) of finding one or no sub-potent tablets in 30 samples if there is an expected 0.5% rate. Finding more than one sub-potent tablet is a rare occurrence and can serve as a basis for rejecting a production batch, depending upon the manufacturer's specifications.

Excel can be used to calculate the Poisson probability distribution by selecting the Function **POISSON** or **POISSON.DIST** (both do the same calculations and require the same input) and entering the number of successes (*X*), the np or λ value (*Mean*), and whether you want actually probability or cumulative probability (*Cumulative:* TRUE for cumulative or FALSE for actual probability for the number of successes; see Figure 2.11). For example, consider results above for the probability of one sub-potent tablet out of 30 tablets sampled. The Excel function commands would be as follows:

POISSON (1,0.15,FALSE) = 0.129106

If you were interested in the cumulative probability (zero or one sub-potent tablet) the entry and results would be:

POISSON (1,0.15,TRUE) = 0.989814

References

Bernstein, P.L. (1996). *Against the Gods: The Remarkable Story of Risk*, John Wiley and Sons, New York.

Bolton, S. and Bon, C. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Marcel Dekker, Inc., New York, p. 55.

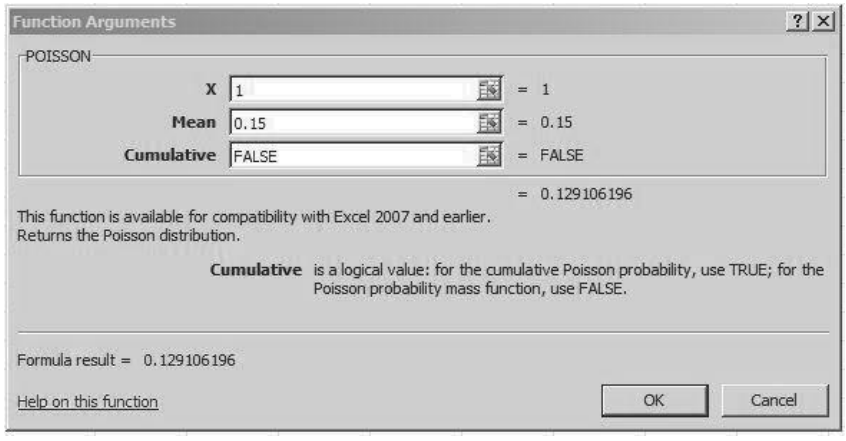


Figure 2.11 POISSON function using Excel.

Dawson, B. and Trapp, R.G. (2001), *Basic and Clinical Biostatistics*, Third edition, Lange Medical Books, New York, p. 74.

Kachigan, S.A. (1991). *Multivariate Statistical Analysis*, Second edition, Radius Press, New York, p. 59.

Suggested Supplemental Readings

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Seventh edition, John Wiley and Sons, New York, pp. 59-85.

Forthofer, R.N. and Lee, E.S. (1995). *Introduction to Biostatistics: A Guide to Design, Analysis and Discovery*, Academic Press, San Diego, pp. 93-102, 125-141.

Example Problems (Answers are provided in Appendix D)

1. A total of 150 healthy females volunteered to take part in a multicenter study of a new urine testing kit to determine pregnancy. One-half of the volunteers were pregnant, in their first trimester. Urinary pHs were recorded and 62 of the volunteers were found to have a urine pH less than 7.0 (acidic) at the time of the study. Also, 36 of these women with acidic urine were also pregnant.

   If one volunteer is selected at random:

   a. What is the probability the person is pregnant?

   b. What is the probability the person has urine that is acidic (less than pH 7)?

   c. What is the probability the person has a urine that is basic (pH equal to or greater than 7)?

d. What is the probability that the person is both pregnant and has urine that is acidic (less than pH 7)?

e. What is the probability that the person is either pregnant or has urine that is acidic (or less than pH 7)?

f. If one volunteer is selected at random from only those women with acidic urinary pHs, what is the probability that the person is also pregnant?

g. If one volunteer is selected at random from only the pregnant women, what is the probability that the person has a urine pH of 7.0 or greater?

2. Three laboratory technicians work in a quality control laboratory with five different pieces of analytical equipment. Each technician is qualified to operate each piece of equipment. How many different ways can each piece of the equipment be assigned to each technician?

3. Ten tablets are available for analysis, but because of time restrictions the scientist will only be able to sample five tablets. How many possible ways can these tablets be sampled?

4. With early detection, the probability of surviving a certain type of cancer is 0.60. During a mass screening effort eight individuals were diagnosed to have early manifestations of this cancer.

   a. What is the probability that all eight patients will survive their cancer?

   b. What is the probability that half will die of the cancer?

5. Newly designed shipping containers for ampules were compared to the existing one to determine if the number of broken units could be reduced. One hundred shipping containers of each design (old and new) were subjected to identical rigorous abuse. The containers were evaluated and failures were defined as containers with more than 1% of the ampules broken. A total of 15 failures were observed and 12 of those failures were with the old container. If one container was selected at random:

   a. What is the probability that the container will be of the new design?

   b. What is the probability that the container will be a “failure”?

   c. What is the probability that the container will be a “success”?

   d. What is the probability that the container will be both an old container design and a “failure”?

e. What is the probability that the container will be either of the old design or a "failure"?

f. If one container is selected at random from only the new containers, what is the probability that the container will be a "failure"?

g. If one container is selected at random from only the old container design, what is the probability that the container will be a "success"?

6. An in-service director for Galaxy Drugs is preparing a program for new employees. She has eight topics to cover and they may be covered in any order.

a. How many different programs (variations on eight topics) is it possible for her to prepare?

b. At the last minute she finds that she has time for only six topics. How many different programs is it possible for her to present if all are equally important?

If order is important?

If order is not important?

7. Calculate the following:

a. $\binom{6}{2}$ b. $\binom{9}{5}$ c. $\binom{30}{3}$

3

Sampling

Samples from a population represent the best estimate we have of the true parameters of that population. Two underlining assumptions for all statistical tests are that: 1) the samples are randomly selected or assigned at random to the different levels of the independent variable and 2) observations are measured independently of each other. Therefore, ensuring that samples are randomly selected from the study population is critical for all statistical procedures.

The **target population** is that population about which the researcher desires information. However, the population from which actual information is extracted is the **sampled population**. For example, assume a Phase III study is being designed to assess the effects of a new drug on patients with congestive heart failure (CHF). The study protocol will identify inclusion and exclusion criteria to carefully define "congestive heart failure." It would be impossible to sample all the people in the world who meet the definition and make up the target population. Instead the researchers focus on a multicenter, worldwide study where local principal investigators recruit volunteers who meet the criteria for the study. This sampled population (which is similar to the target population, at least with respect to the characteristics under investigation) is then tested with the drug. Based on the design of the study, the volunteers will be further divided into two groups: the first receiving the new drug for CHF, the second group receiving the current gold standard for treating CHF. The decision of which therapy is received would be based on simple random assignment of the volunteers to one of the two therapies.

Random Sampling

As mentioned in Chapter 1, as researchers we will be interested in identifying characteristics of a population (parameters). In most cases it will not be possible to obtain all the information about that particular characteristic. Instead, a sample will be obtained that will hopefully represent a suitably selected subset of the population. The probability theories presented in Chapter 2, and upon which statistics is based, require randomness.

In order to be a random sample, all elements of the population must have an equal chance (probability) of being included in the sample. In other words, each of the 50,000 tablets coming off a scale-up production run should have an equal likelihood

of being selected for analysis. If the manufacturer in the above example sampled tablets only at the beginning or the end of the production run, the results may not be representative of all the tablets. A procedure should be developed to ensure periodic sampling, for example, every 30 minutes during the production run.

Simple random sampling may be accomplished for a smaller number of units by using a random numbers table or by numbers generated at random using a calculator or computer. For example, assume that we are in a quality control department and want to analyze a batch of ointments. Samples have been collected during the production run and 250 tubes are available in the quality control department (these tubes are numbered in order from the first sample to the 250th tube). Because of time and expense, we are only able to analyze 10 tubes. The 10 samples would be our best guess of the target population (the production) represented by the 250 tubes of ointment (the sampling population).

The best way to select the 10 ointment tubes is through the use of a **random numbers table** (Table B1, Appendix B). Random numbers tables, usually generated by computers, are such that each digit (1, 2, 3, etc.) has the probability of occurring (0.10) and theoretically each pair of numbers (21, 22, 23, etc.) or triplicate (111, 112, 113, etc.) would have the same probability of occurrence, 0.01 and 0.001, respectively. We would begin using a random numbers table by dropping a pencil or pen point on the table to find an arbitrary starting point. To illustrate the use of this table, assume the pencil lands at the beginning of the sixth column, eighth row, in our Table B1:

23616

We have decided *a priori* (before the fact; before the dropping of the pencil point) to select numbers moving to the right of the point. We could have also decided to move to the left, up or down the table. Because we are sampling tubes between 001 and 250, the number would be selected in groupings of three digits.

236<u>16</u>

Thus, the first number would be 236 or the 236th ointment sample would be selected. The next grouping of three digits to the right would include the last two digits of this column and the first digit of the next column to the right.

23616 45170

The 164th ointment tube would be the second sample. Note there is nothing significant about the placements of the vertical and horizontal breaks in Table B1, they are simply included to make the table easier to read and use. The third set of three digits (517) exceeds the largest number (250) and would be ignored. Continuing to the right in groups of three digits, the third sample would be the 78th tube.

23616 45170 78646

To this point the first three samples would be ointment tubes 236, 164, and 078. The next three groupings all exceed 250 and would be ignored.

23616 45170 78646 77552 01582
23616 45170 78646 77552 01582
23616 45170 78646 77552 01582

The next sample that can be selected from this row is 158. The fourth sample is the 158th ointment tube.

23616 45170 78646 77552 01582

The researcher has decided to continue down the page (the individual could have used the same procedure moving up the page). Therefore, the last digit in the eighth row is combined with the first two digits in the ninth row to create the next sampling possibility.

…… 23616 45170 78646 77552 01582

11004 06949 40228 ….

With the 211th tube as the fifth sample, the remaining samples are selected moving across the row and ignoring numbers in excess of 250.

11004 06949 40228 95804 06583 10471 83884 27164 50516 89635
11004 06949 40228 95804 06583 10471 83884 27164 50516 89635
11004 06949 40228 95804 06583 10471 83884 27164 50516 89635
11004 06949 40228 95804 06583 10471 83884 27164 50516 89635
11004 06949 40228 95804 06583 10471 83884 27164 50516 89635

If any of the three digit number combinations had already been selected, it would also be ignored and the researcher would continue to the right until ten numbers were randomly selected. In this particular random sampling example, the ten tubes selected to be analyzed were:

Tubes	
004	078
022	158
047	164
065	211
069	236

Dropping the pencil at another location would have created an entirely different set of numbers. Thus, using this procedure, all of the ointment tubes have an equal likelihood of being selected.

Using Minitab® or Excel® to Generate a Random Sample

Minitab can be used for generating a random sample, if data have already been collected and there is a reason for a random sample from that "sampled population." The steps in Minitab are

Calc ➤ Random Data ➤ Sample from Columns

The sample size is "Number of rows to sample:", the location of the original sampled population is "From Columns:" and the location for the sample results is "Store samples in:". Seen Figure 3.1, ten random samples were selected from the first column (250 observations) and the values for those samples were placed in the third column. Note that Minitab does not indicate which samples are actually selected, only the value associated with each sample. Do not initiate the "Sample with replacement" option. Excel can produce similar results using *Sampling* under the *Data Analysis* option (Figure 3.2), where the "Input range:" is the sampled population, the sample size in "Number of Samples:" and the sample results begin at the "Output Range:" location.

Excel can be used to generate random numbers for sampling purposes or to create a random numbers table using the function **RAND** which will create a decimal. This could be multiplied in the function window by 100 for a two digit random number or 1000 to create a three digit number (Figure 3.3). A limitation with **RAND** is that it is a *volatile* function and will change every time a new action is taken. In Figure 3.4, the screen to the left in the original number randomly generated a new number in cell A1. When a new random number is created for cell A2, the number in A1 changes.

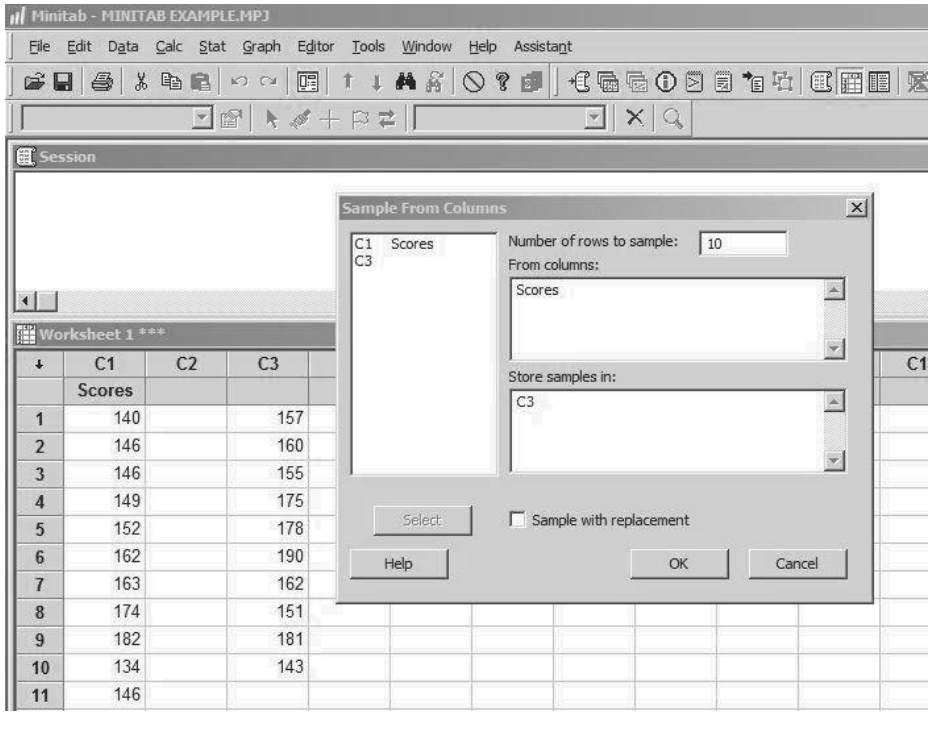


Figure 3.1 Illustration of random sampling using Minitab.

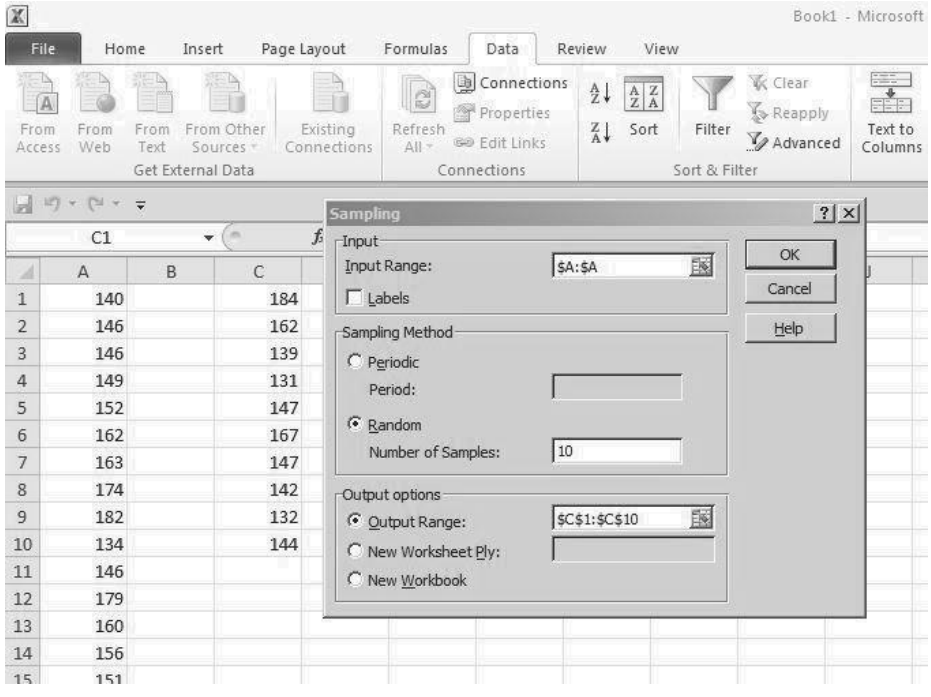


Figure 3.2 Illustration of random sampling using Excel.

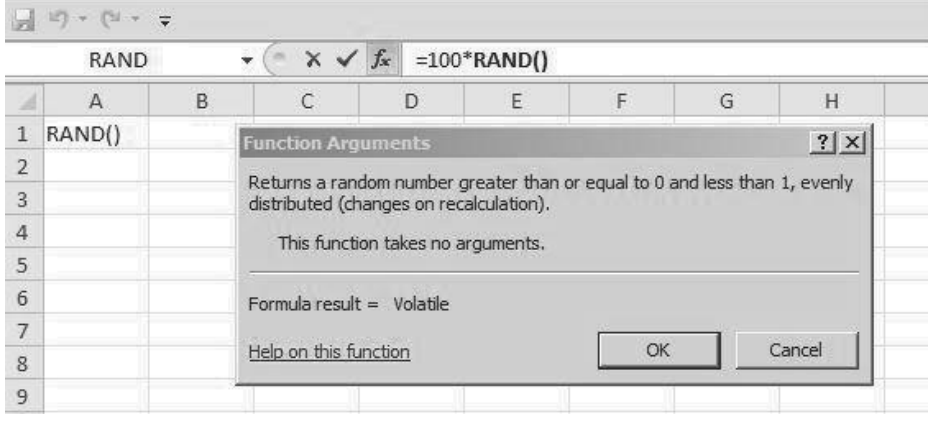


Figure 3.3 RAND function using Excel.

Every time a new random number is generated all previous ones will be changed. In Figure 3.4, the two random numbers would be 31 and 46 (with conventional rounding). There is also a **RANDBETWEEN** function in Excel where the upper and lower limits of the population can be specified (Figure 3.5). The statement in the

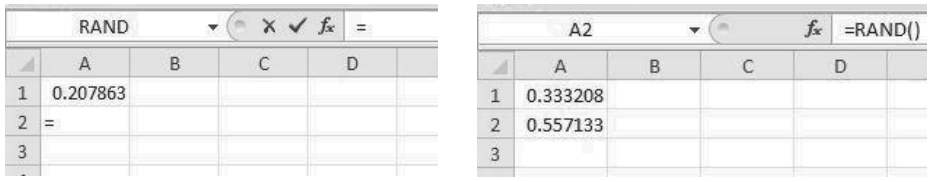


Figure 3.4 Volatile nature of the RAND function.

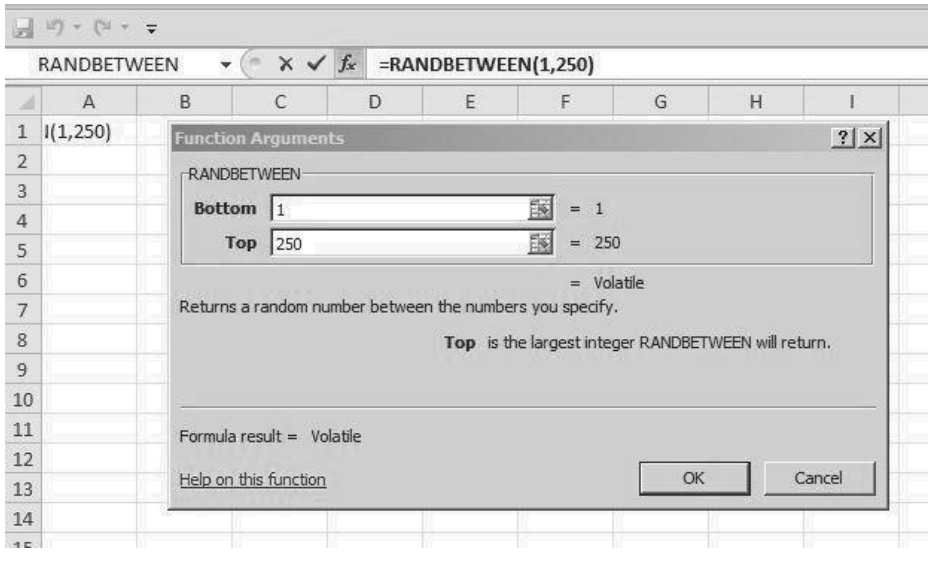


Figure 3.5 RANDBETWEEN function using Excel.

A1 cell can then be copied and duplicated in multiple cells to generate Table 3.1 (where the "bottom" is one and the "top" is 30). Note once again that these cells are volatile and change each time a new action is taken. Also, Excel allows replacements, so it is possible to get the same number multiple times, so sampling need to continue until 15 unique numbers are generated to create the experimental group in Table 3.1 and all other remaining numbers (30 or less) automatically become the control group.

Other Probability Sampling Procedures

The best representation of a given target population comes from a **probability sample** of either the target population or the sampled population. Examples of a probability sample include a simple random sample, as well as systematic, stratified, and cluster samples. These types of sampling are often used because they are convenient, relatively easy to accomplish, and often more realistic than pure random sampling. **Selective sampling** offers a practical means for producing a sample that is representative of all the units in the population. A sample is biased when it is not representative and every attempt should be made to avoid this situation.

Table 3.1 Table of Volunteers Meeting Inclusion and Exclusion Criteria

Experimental Group Volunteer Number				Control Group Volunteer Number			
2	11	20	28	1	9	16	23
3	12	21	29	5	10	18	25
4	14	24	30	6	13	19	26
7	17	27		8	15	22	

Systematic sampling is a process by which every *nth* object is selected. Consider a mailing list for a survey. The list is too large for us to mail to everyone in this population. Therefore, we select every 6th or 10th name from the list to reduce the size of the mailing while still sampling across the entire list (A-Z). The limitation is that certain combinations may be eliminated as possible samples (i.e., spouses or identical twins with the same last names); therefore, producing a situation where everyone on the mailing does not have an equal chance of being selected. In the pharmaceutical industry this might be done during the production run of a certain tablet, where at selected time periods (every 30 or 60 minutes) tablets are randomly selected as they come off the tablet press and weighed to ensure the process is within control specifications. In this production example, the time selected during the hour can be randomly chosen in an attempt to detect any periodicity (regular pattern) in the production run.

In **stratified sampling** the population is divided into groups (strata) with similar characteristics and then individuals or objects can be randomly selected from each group. For example, in another study we may wish to ensure a certain percentage of smokers (25%) are represented in both the control and experimental groups in a clinical trial (n = 100 per group). First the volunteers meeting the inclusion and exclusion criteria are stratified into smokers and nonsmokers. Then, 25 smokers are randomly selected for the experimental group and an additional 25 smokers are randomly selected as controls. Similarly two groups of 75 nonsmoking volunteers are randomly selected to complete the study design. Stratified sampling is recommended when the strata are very different from each other and all of the objects or individuals within each stratum are similar.

Also known as "multistage" sampling, **cluster sampling** is employed when there are many individual "primary" units that are clustered together in "secondary" larger units that can be subsampled. For example, individual tablets (primary) are contained in bottles (secondary) sampled at the end of a production run. Assume that 150 containers of a bulk powder chemical arrive at a pharmaceutical manufacturer and the quality control laboratory needs to sample these for the accuracy of the chemical or lack of contaminants. Rather than sampling each container they randomly select ten containers. Then within each of the ten containers they further extract random samples (from the top, middle, or bottom) to be assayed.

In the final analysis, the probability sampling procedure that is chosen by the investigator depends on the experimental situation. There are several factors to be considered when choosing a sampling technique. They include: 1) cost of sampling, both associated expense and labor; 2) practicality, using a random number table for one million tablets during a production run would be unrealistic if not impossible to accomplish; 3) the nature of the population the sample is taken from: periodicity, unique strata or clustering of smaller units within larger ones; and 4) the desired

accuracy and precision of the sample.

Nonprobability Sampling Procedures

Other methods of sampling do not meet the criteria for probability sampling (each unit or member having an equal probability of being selected). Results from nonprobability sampling have limited value and should not be used to make statistical inferences, but only as generalizations about the populations. Examples of nonprobability samples would include: case study samples, convenience samples, judgmental samples mechanical samples, quote samples, or snowball samples.

Case study sampling is a sampling technique used where the researcher is limited to a single patient or small group of patients, often with similar characteristics.

Convenience sampling involves items, objects or persons arbitrarily selected at the convenience of the researcher. Usually the researcher makes limited effort to ensure that the sample accurately represents the population from which the subset is supposedly sampled. Examples include co-workers, friends, people in close proximity or samples already collected and available in a convenient location. This is commonly seen in clinical trials because of the advantage of logistics and costs.

Judgmental sampling is nonprobability sampling where the researchers, based on personal experience or familiarity, choose the sample they feel are most suitable for the study. This method (also called purposive or selective sampling) is used when there are very few people or objects with the characteristics for the area being researched.

Mechanical sampling is typically used for collecting samples of solids, liquids or gases where devices such as thief probes are used to collect samples. Care is taken to ensure that the sample is representative, but specific locations may be sampled where there are potential hot spots or sources that may indicate lack of homogeneity. This is a nonprobability version of cluster sampling.

Quota sampling is used where quotas are established prior to sample collection (i.e., 50% females) and the researchers choose volunteers until the quotas are met. Sometimes referred to as ad hoc quota sampling, it is a nonprobability version of stratified sampling.

Referral sampling occurs where current volunteers in the study are used to recruit more subjects for the same study. Synonyms include chain, chain-referral or snowball sampling. The latter term is derived from an analogy with the growth of a rolling snowball. This could be seen with volunteers for a clinical trial, especially those that provide financial incentives for early volunteers to recruit peers.

Random Assignment to Two or More Experimental Levels

Even if the initial pool of objects or people selected by probability or non-probability sampling procedures, it is critically important to randomly divide them into the different experimental levels involved in the study. Let us assume we are involved in a clinical trial comparing a new oral anticoagulant to warfarin. We want to follow the patients for a set period of time to determine changes in their clotting time. Even if we use a sample of convenience by recruiting these volunteers (patients visiting our anticoagulation clinic over a four week period) every effort should be

Table 3.2 Results of Weights for Tablets (mg)

Sample	Weight	Sample	Weight	Sample	Weight	Sample	Weight
1	649	14	653	27	645	40	650
2	654	15	646	28	650	41	651
3	644	16	644	29	656	42	639
4	648	17	649	30	649	43	648
5	650	18	647	31	649	44	652
6	636	19	650	32	657	45	648
7	652	20	652	33	643	46	669
8	662	21	646	34	653	47	647
9	646	22	648	35	645	48	664
10	650	23	655	36	650	49	649
11	648	24	651	37	647	50	653
12	651	25	642	38	651		
13	660	26	647	39	654		

made to ensure that each individual has an equal probability of being assigned to each treatment option. This can be accomplished by simple randomization, using a random numbers table to assign volunteers to one of the two treatment levels. Let's assume 30 patients meet the inclusion and exclusion criteria and are willing to take part in the study. Patients would be assigned numbers, possibly in the order they were enrolled. Then a random numbers table (Table B2 in Appendix B) would be used to create a table similar to Table 3.2, where the experimental group would receive the new oral anticoagulant and the control group would receive the more traditional warfarin therapy.

Precision, Accuracy, and Bias

As mentioned, it is desirable that sample data be representative of the true population from which it is sampled and every effort should be made to ensure this is accomplished.

Precision refers to how closely data are grouped together or the compactness of the sample data. Illustrated in Figure 3.6 are data that are less scattered or closely clustered data, which have greater precision (Samples A and C). Also included is Sample B with a great deal of scatter and which does not have good precision. Precision measures the variability of a group of measurements. A precise set of measurements is compact and, as discussed in Chapter 5, is reflected by a small standard deviation or a small relative standard deviation.

However, assume that the boxes for Samples A, B, and C represent the true value for the population from which the samples were taken. In this example, even though Samples A and C have good precision, Sample C provided the only accurate predictor of the population. **Accuracy** is concerned with "correctness" of the results and how closely the sample data represents the true value of the population. It is desirable to have data that is both accurate and precise.

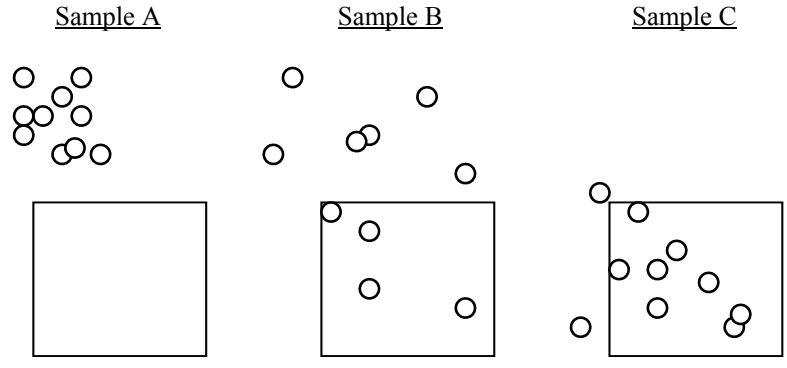


Figure 3.6 Samples comparing precision and accuracy.

An analogy for precision and accuracy is to consider Figure 3.6 as an example of target shooting with the box representing bull's-eyes. Sample C is desired because all of the shots are compacted near or within the bull's-eye of the target. Sample B is less precise, yet some shots reach the center of the target. Sample A is probably the most precise, but it lacks accuracy. This lack of accuracy is bias. **Bias** can be thought of as **systematic error** that causes some type of constant error in the measurement or idiosyncrasy with the measurement system. In the example of target shooting, the system error might be improper adjustment of the aiming apparatus or failure to account for wind velocity, either of which would cause a constant error. In a laboratory environment, systematic errors could be caused by contamination, calibration errors, losses or degradation of the product, sampling errors, unsuitable methods, or through operator incompetence. Ideally, investigators should use random sampling to avoid selection bias. **Selection bias** occurs when certain characteristics make potential observations more (or less) likely to be included in the study. For example, always sampling from the top of storage drums may bias the results based on particle size, assuming smaller particles settle to the lower regions of the drums. Bias can result from incorrect sampling, inappropriate experimental design, inadequate blinding, or mistakes (blunders) in observing or recording the data.

Even random samples of the same pool of objects (i.e., tablets of a particular batch) are very unlikely to be exactly the same. For example, the average weights of ten tablets will vary from sample to sample. Multiple samples from the same pool or population will result in a distribution of possible outcomes, which is called the sampling distribution (Chapter 6). All data points in a set of data are subject to two different types of error: systematic and random errors. **Random errors**, or chance errors, are unpredictable and will vary in sign (+ or –) and magnitude; but systematic errors always have the same sign and magnitude, and produce biases.

Reliability and Validity

Closely related to the accuracy of the sample data are its reliability and validity. **Reliability** is a collection of factors and judgments that, when taken together, are a measure of reproducibility. Reliability is the consistency of measures and deals with the amount of error associated with the measured values. In order for data to be reliable, all sources of error and their magnitude should be known, including both constant errors (bias) and random (chance) errors. With respect to this measure of reproducibility, if subjects are tested twice and there is a strong relationship between successive measurements (correlation, Chapter 13) this is referred to as **test-retest reliability**. It is a method of pairing the scores on the first test and the retest to determine the reliability. A second type of reliability measure, in the case of a knowledge test, is to divide the test into two portions: one score on the odd items and one score on the even items (or first half and second half of the test). If there is a strong relationship between the scores on the two halves it is called **split-half reliability**. Reliability is basic to every measurement situation and interpretation that we place on our sample data (see Chapter 17).

Validity refers to the fact that the data represents a true measurement. A valid piece of data describes or measures what it is supposed to represent. It is possible for a sample to be reliable without being valid, but it cannot be valid without being reliable. Therefore, the degree of validity for a set of measurements is limited by its degree of reliability. Also, if randomness is removed from the sampling technique used to collect data, it potentially removes the validity of our estimation of a population parameter.

Suggested Supplemental Readings

Bolton, S. (1997). *Pharmaceutical Statistics: Practical and Clinical Applications*, Third edition, Marcel Dekker, New York, pp. 102-109.

Forthofer, R.N. and Lee, E.S. (1995). *Introduction to Biostatistics: A Guide to Design, Analysis and Discovery*, Academic Press, San Diego, pp. 23-35.

Example Problems (Answers are provided in Appendix D)

1. Using the random numbers table presented as Table B1 in Appendix B, randomly sample five tablets from Table 3.2. Calculate the average for the three values obtained by the sample (add the five numbers and divide by five).

2. Repeat the above sampling exercise five times and record the average for each sample. Are these averages identical?

3. From the discussion in Chapter 2, how many possible samples (n = 5) could be randomly selected from the data in Table 3.2?

4

Presentation Modes

Data can be communicated in one of four different methods: 1) verbal; 2) written descriptions; 3) tables; or 4) graphic presentations. This chapter will focus on the latter two methods for presenting **descriptive statistics**. As will be seen in subsequent chapters, a primary reason for using statistics is to estimate some unknown property of a population. In this chapter and the next, descriptor graphs and statistics are discussed and will used as the best estimates or estimators of the true population from which they have been sampled. Often the graphic representation of data may be beneficial for describing and/or explaining research data. The main purpose in using a graph is to present a visual representation of the data and the distribution of observations.

The old adage "a picture is worth a thousand words" can be especially appropriate with respect to graphic representation of statistical data. Visualizing data can be useful when reviewing preliminary data, for interpreting the results of inferential statistics, and for detecting possible extreme or erroneous data (outliers). A variety of graphic displays exist and a few of the most common are presented in this chapter.

Tabulation of Data

The simplest and least informative way to present experimental results is to list the observations (raw scores or raw data). For example, working in a quality control laboratory we are requested to sample 30 tetracycline capsules during a production run and to report to the supervisor the results of this sample. Assume the information in Table 4.1 represents the assay results for the random sample of 30 capsules. Data presented in this format is relatively useless other than to merely provide the individual results.

We could arrange the results of the 30 samples in order from the smallest assay result to the largest (Table 4.2). With this ordinal ranking of the data we begin to see certain characteristics of our data: 1) most of the observations cluster near the middle of the distribution (e.g., 250 mg) and 2) the spread of outcomes varies from as small as 245 mg to as large as 254 mg.

The purpose of descriptive statistics is to organize and summarize information; therefore, tables and graphics can be used to present this data in a more useful format.

Table 4.1 Results from the Assay of 30 Tetracycline Capsules

Capsule #	mg	Capsule #	mg	Capsule #	mg
1	251	11	250	21	250
2	250	12	253	22	254
3	253	13	251	23	248
4	249	14	250	24	252
5	250	15	249	25	251
6	252	16	252	26	248
7	247	17	251	27	250
8	248	18	249	28	247
9	254	19	246	29	251
10	245	20	250	30	249

Table 4.2 Rank Ordering from Smallest to Largest

Rank	mg	Rank	mg	Rank	mg
1	245	11	249	21	251
2	246	12	250	22	251
3	247	13	250	23	251
4	247	14	250	24	252
5	248	15	250	25	252
6	248	16	250	26	252
7	248	17	250	27	253
8	249	18	250	28	253
9	249	19	251	29	254
10	249	20	251	30	254

What we are doing is called the process of **data reduction**: trying to take data and reduce it to more manageable information. The assay results seen in Tables 4.1 and 4.2 represent a continuous variable (mg of drug present); however, as mentioned in Chapter 1, continuous data can be grouped together to form categories and then handled as a discrete variable. Assume that the desired amount (labeled amount) of tetracycline is 250 mg per capsule. The data can be summarized to report results: 1) focusing on those capsules which meet or exceed the labeled amount:

Outcome	n	%
<250 mg	11	36.7
≥250 mg	19	63.3
Total	30	100.0

(*n* representing the number of occurrences in a given level of this now discrete variable); 2) showing capsules that do not exceed the labeled amount:

Outcome	f	%
≤250 mg	18	60.0
>250 mg	12	40.0
Total	30	100.0

(the number of occurrences can also be listed as *f* or the frequency of the outcomes,); or 3) listing those capsules that exactly meet the label claim and those which fall above or below the desired amount:

Outcome	f	cf	%	cum. %
<250 mg	11	11	36.7	36.7
=250 mg	7	18	23.3	60.0
>250 mg	12	30	40.0	100.0

In this last table on which the cumulative frequencies *(cf)* and cumulative percentages (*cum. %*) are reported, in addition to the frequency and percentage, the **frequency** or number of observations for each discrete level appears in the second column. Since the three categories are in an ascending or ordinal arrangement (smallest to largest) the third column represents the **cumulative frequency,** which is obtained by summing the frequencies for the level of interest plus each preceding discrete level. The last two columns report the percentages associated with each level of the discrete variable. The fourth column, also called the **relative frequency** (*rf*), is the frequency converted to the percentage of the total number of observations. The last column shows the cumulative outcomes expressed as **cumulative percent** or proportion of the observations. One of the problems associated with converting a continuous, quantitative variable into a categorical, discrete variable is a loss of information. Notice in the last table that 10 different values (ranging from 245 to 254 mg) have been collapsed into only three discrete intervals. Also notice in all three tables above, we have created mutually exclusive and exhaustive categories.

Visual Displays for Discrete Variables

Often simple data, such as the previous example, can be presented in a graphic form. **Bar graphs** are appropriate for visualizing the frequencies associated with different levels of a discrete variable. Also referred to as **block diagrams**, they are drawn with spaces between the bars symbolizing the discontinuity among the levels of the discrete variable (this is in contrast to histograms for continuous data that will be discussed later). In Figure 4.1, information is presented using the three mutually exclusive and exhaustive levels created for the data in Table 4.2. In preparing bar graphs, the horizontal plane (x-axis or **abscissa**) usually represents observed values or the discrete levels of the variable (in this case <250, =250, or >250 mg.). The vertical axis (y-axis or **ordinate**) represents the frequency or proportion of observations (in this case the frequency). Bar charts can be rotated 90 degrees and presented as a horizontal orientation and well as vertical one presented in Figure 4.1.

A **line chart** is similar to a bar chart except that thin lines, instead of thicker bars,

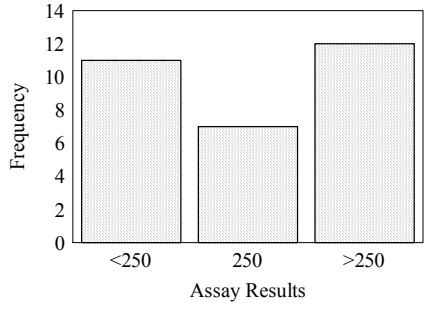


Figure 4.1 Example of a bar graph.

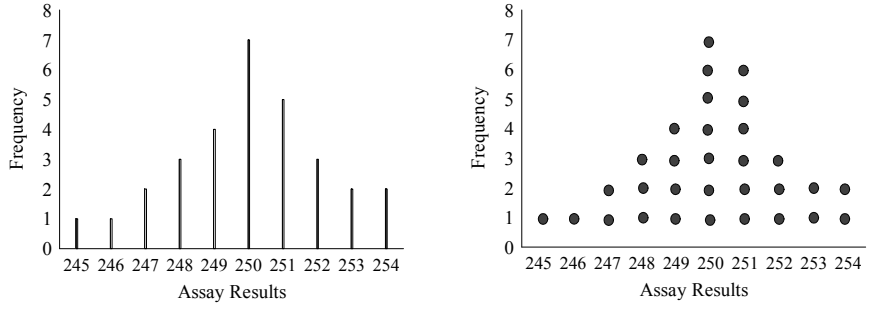


Figure 4.2 Examples of a line chart and point plot.

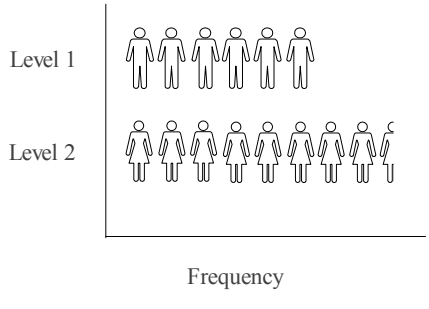


Figure 4.3 Example of a pictogram.

are used to represent the frequency associated with each level of the discrete variable. **Point plots** are identical to line charts; however, instead of a line a number of points or dots equivalent to the frequency are stacked vertically for each value of the horizontal axis. Also referred to as **dot diagrams**, point plots are useful for small data sets. Using the data presented in Table 4.2, a corresponding line chart and dot diagram are presented in Figure 4.2.

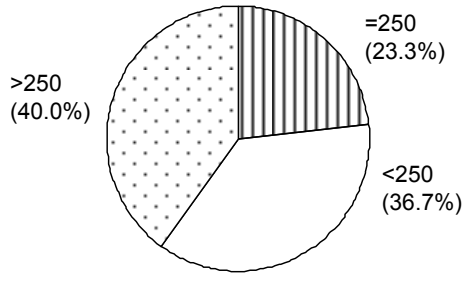


Figure 4.4 Example of a pie chart using Minitab.

Pictograms are similar to bar charts. They present the same type of information, but the bars are replaced with a representative number of icons. This type of presentation for descriptive statistics dates back to the beginning of civilization when pictorial images were used to record numbers of people, animals, or objects (Figure 4.3).

Pie charts provide a method for viewing and comparing levels of a discrete variable in relationship to a variable as a whole. Whenever a data set can be divided into parts, a pie chart may provide the most convenient and effective method for presenting the data (Figure 4.4).

Visual Displays for Continuous Variables

The **stem-and-leaf plot** is a visual presentation for continuous data. Also referred to as a **stemplot**, it contains features common to both the frequency distribution and dot diagrams. Digits, instead of bars, are used to illustrate the spread and shape of the distribution. Each piece of data is divided into "leading" and "trailing" digits. For example, based on the range of data points, the value *125* could be divided into either *12* and *5*, or *1* and *25*, as the leading and trailing digits. All the leading digits are sorted from lowest to highest and listed to the left of a vertical line. These digits become the stem. The trailing digits are then written in the appropriate row to the right of the vertical line. These become the leaves. The frequency or "depth" of the number of leaves at each value in the lead digit of the stem are listed on the left side and can be used to calculate the median, quartiles, or percentiles. An *M* and *Q* are placed on the vertical line to identify the **median** and **quartiles**. These measures of central tendency will be discussed in the next chapter. For the present, the median represents that value below which 50% of the observations fall. The quartiles are the values below which 25% and 75% of the data would be located. For example, the data presented for 125 patients in Table 4.3 can be graphically represented by the stemplot in Figure 4.5. The appearance of the stemplot is similar to a horizontal bar graph (rotated 90 degrees from the previous example of a bar graph); however, individual data values are retained. Also shown are the maximum and minimum scores, and also the range (distance from the largest to smallest observation) can be easily calculated. The stem-and-leaf plot also could be expanded to provide more information about the distribution. In the above example, if each stem unit was divided into halves (upper

Table 4.3 C_{max} Calculations for Bigomycin in Micrograms (mcg)

739	775	765	751	761	738	759	761	764	765	749	767
764	743	739	759	752	762	730	734	759	745	743	745
751	760	768	766	756	741	741	774	756	749	760	765
743	752	729	735	725	750	745	745	738	763	752	737
706	769	760	755	767	750	728	778	740	741	771	752
756	746	788	743	725	765	754	766	755	772	758	763
734	728	755	778	785	718	730	731	714	752	770	732
770	755	720	754	764	731	790	793	753	780	732	751
766	751	762	734	755	761	740	767	775	755	766	736
755	755	770	741	751	774	780	724	720	746	754	766
743	743	775	732	762							

Frequency	Stem		Leaves
1	70		6
2	71		48
8	72		00455889
18	73		001122244456788999
19	74	Q	0011113333355556699
31	75	M	00111112222234445555555668999
28	76	Q	0001112223344455556666677789
12	77		000124455588
4	78		0058
2	79		03
125			

Figure 4.5 Example of a stem-and-leaf plot.

and lower), then the leaves would be established for 70.0 to 70.4, 70.5 to 70.9, 80.0 to 80.4, etc.

A **back-to-back stemplot** could be used to visually compare two sets of data. For example the information in Table 4.4 is plotted in Figure 4.6. Visually the data obtained for the two formulations appear to be different. In Chapter 9, we will reevaluate these data to determine if there is a statistically significant difference or if the difference could be due to some type of random difference.

Similar to the back-to-back stemplot, the **cross diagram** is a simple graphic representation for two or more levels of a discrete independent variable and a dependent continuous variable. The values for the dependent variable are represented on a horizontal or vertical line (Figure 4.7). Data are plotted on each side of the line based on which level of the independent variable they represent.

One simple plot that displays a great deal of information about a continuous variable is the **box-and-whisker plot** (Figure 4.8). The box plot illustrates the bulk of the data as a rectangular box in which the upper and lower lines represent the third

Table 4.4 C_{max} Values for Two Formulations of the Same Drug

Formulation A						Formulation B					
125	130	135	126	140	135	130	128	127	149	151	130
128	121	123	126	121	133	141	145	132	132	141	129
131	129	120	117	126	127	133	136	138	142	130	122
119	133	125	120	136	122	129	150	148	136	138	140

Formulation A		Formulation B
97	11	
98766655321100	12	27899
6553310	13	0002236688
0	14	0112589
	15	01

Figure 4.6 Example of a back-to-back stem-and-leaf plot.

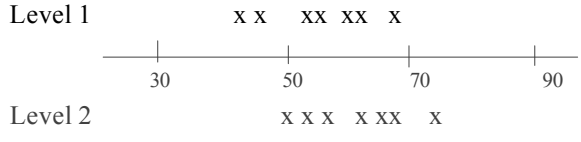


Figure 4.7 Example of a cross diagram.

quartile (75% of observations below Q_3) and first quartile (25% of observations below Q_1), respectively. The second quartile (50% of the observations below this point) is depicted as a horizontal line through the box. The arithmetic average may or may not be shown as an *x*. Vertical lines (whiskers) extend from the top and bottom lines of the box to an upper and lower **adjacent value**. The adjacent values equal three **semi-interquartile ranges** (SIQRs) above and below the median. The SIQR is the distance between the upper or lower quartile and the median, or:

$$SIQR = \frac{(Q_3 - Q_1)}{2} \qquad \text{Eq. 4.1}$$

Observations that fall above or below the adjacent values can be identified as potential outliers (Chapter 23).

Both the stem-and-leaf plots and the box-and-whisker plots are examples of **exploratory data analysis (EDA)** techniques. These procedures were developed by John Tukey and colleagues in the 1960's (Tukey, 1977). They provide the researcher

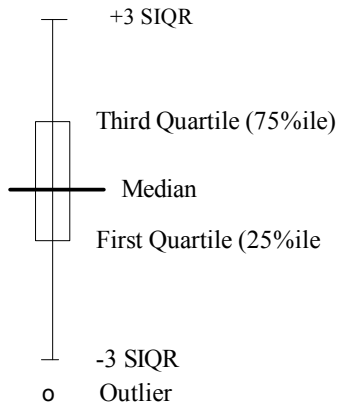


Figure 4.8 Example of a box and whisker plot.

with a visual method for identifying trends, relationships, or unexpected patterns in sample data.

Similar to bar charts and point plots, **histograms** are useful for displaying the distribution for a continuous variable, especially as sample sizes become larger or if it becomes impractical to plot each of the different values observed in the data. The vertical bars are connected and reflect the continuous nature of the observed values. Each bar represents a single value or a range of values within the width of that bar. For example, a histogram representing the 30 tetracycline capsules listed in Table 4.1 is presented in Figure 4.9. Each value represents a continuous variable and the equipment used had precision to measure to only the whole mg (e.g., 248 or 251 mg). If more exact instruments were available, the measurements might be in tenths or hundredths of a milligram. Therefore, the value of 248 really represents an infinite number of possible outcomes between 0.5 mg below and 0.5 mg above that particular measure (247.5 to 248.5 mg). Similarly, the value 250 represents all possible results between 249.5 and 250.5 mg. The histogram representing this continuum is presented in Figure 4.10.

The data in Figure 4.10 represent an **ungrouped frequency distribution**, which is a visual representation of each possible outcome and its associated frequency (e.g., for the interval 246.5 to 247.5 the frequency is two). Such a distribution shows the extremes of the outcomes, as well as how they are distributed and if they tend to concentrate in the center or to one end of the scale. Unfortunately, with large data sets or where there is increased precision in the measurement, ungrouped frequency distributions may become cumbersome and produce a histogram with many points on the abscissa with frequency counts of only one or two per level. A more practical approach would be to group observed values or outcomes into **class intervals**. In a **grouped frequency distribution**: 1) all class intervals must be the same width, or size; 2) the intervals are mutually exclusive and exhaustive; and 3) the interval widths should be assigned so the lowest interval includes the smallest observed outcome and the top interval includes the largest observed outcome. The number of class intervals

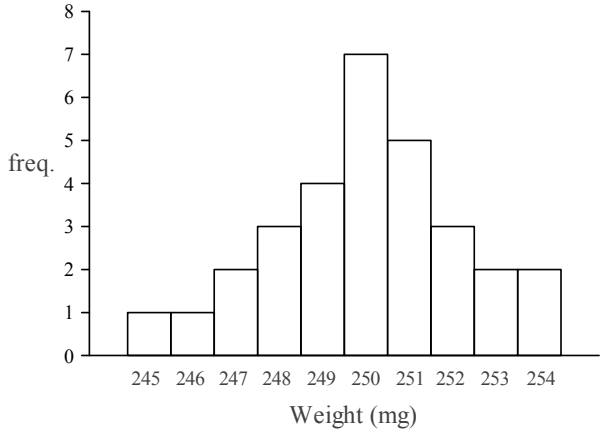


Figure 4.9 Example of a histogram.

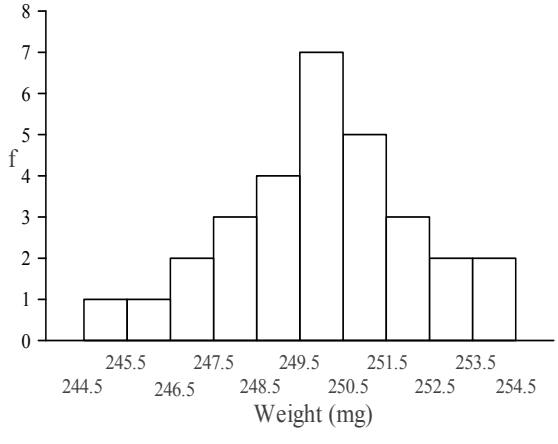


Figure 4.10 Example of a histogram with correction for continuity.

and their size (boundaries) must be specified. Two questions exist regarding these intervals: how many intervals should be used and what should be the width for each interval? To illustrate this process, consider the pharmacokinetic data presented in Table 4.3, representing a sample of patients (n = 125) receiving the fictitious drug bigomycin. As mentioned previously, the range is the difference between the largest and smallest value in a set of observations and represents the simplest method for reporting the dispersion of the data. In this example the largest observation is 792 mcg and the smallest is 706 mcg. The difference represents the **range** of the observations:

$$792\ mcg - 706\ mcg = 86\ mcg$$

Table 4.5 Number of Intervals for Various Sample Sizes Using Sturges' Rule

Sample Size	K Intervals
23-45	6
46-90	7
91-181	8
182-363	9
364-726	10
727-1454	11
1455-2909	12

But into how many class intervals should this data be divided? Some authors provide approximations such as 10 to 20 (Snedecor and Cochran, 1989), 8 to 12 (Bolton, 2004), or 5 to 15 intervals (Forthofer and Lee, 1995). However, **Sturges' rule** (Sturges, 1926) provides a less arbitrary guide to determine the number of intervals based on the sample size (n):

$$K_{intervals} = 1 + 3.32 \log_{10}(n) \qquad \text{Eq. 4.2}$$

A quick reference on the number of intervals for various sample sizes based on Sturges' rule is presented in Table 4.5. The interval width is found by dividing the range by the prescribed number of intervals:

$$width\ (w) = \frac{range}{K} \qquad \text{Eq. 4.3}$$

In our current example, for a sample size of 125 and a range of 86, the number of intervals and width of those intervals would be:

$$K = 1 + 3.32 \log_{10}(125) = 1 + 3.32(2.10) = 7.97 \approx 8\ intervals$$

$$w = \frac{range}{K} = \frac{86}{8} = 10.75 \approx 11\ mcg$$

Thus, the most representative histogram would consist of eight intervals, each with a width of 11. In order to include the smallest and largest values the sections of the histogram would be divided as seen in the first column of Table 4.6. However, the values represent a continuous variable; therefore, correcting for continuity, the true boundaries (**interval boundary values**) of each interval of the histogram and their associated frequencies would be the second column of Table 4.6.

Note that the distribution represents eight intervals that are mutually exclusive and exhaust all possible outcomes. The histogram would appear as presented in Figure 4.11. The center of this distribution can be calculated, as well as a measure of dispersion and these will be discussed in the following chapter.

Table 4.6 Example of Intervals Created Using Sturges' Rule

Interval	Interval Boundary Values	Midpoint (m_i)	Frequency
706-716	705.5-716.5	711	2
717-727	716.5-727.5	722	6
728-738	727.5-738.5	733	18
739-749	738.5-749.5	744	22
750-760	749.5-760.5	755	35
761-771	760.5-771.5	766	28
772-782	771.5-782.5	777	10
783-793	782.5-793.5	788	4

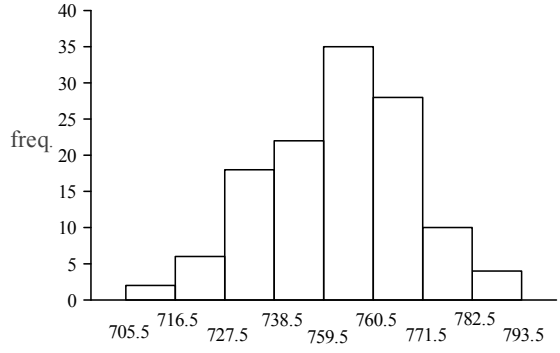


Figure 4.11 Histogram for data presented in Table 4.3 using Sturges' rule.

A **frequency polygon** can be constructed by placing a dot at the midpoint for each class interval in the histogram and then these dots are connected by straight lines. This frequency polygon gives a better concept of the shape of the distribution. The **class interval midpoint** for a section in a histogram is calculated as follows:

$$Midpoint = \frac{highest + lowest\ point}{2} \qquad \text{Eq. 4.4}$$

For class interval 705.5 to 716.6 the midpoint would be:

$$Midpoint = \frac{highest + lowest\ point}{2} = \frac{705.5 + 716.5}{2} = 711$$

The midpoints for the above histogram are also presented in Table 4.6. The frequency polygon is then created by listing the midpoints on the x-axis, frequencies on the y-axis, and drawing lines to connect the midpoints for each interval as presented in Figure 4.12 for the previous data. The midpoint of the class interval represents all the values within that interval and will be used in drawing frequency polygons and in the

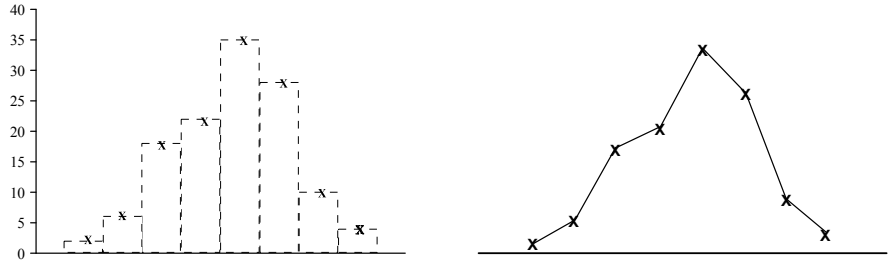


Figure 4.12 Example of a frequency polygon.

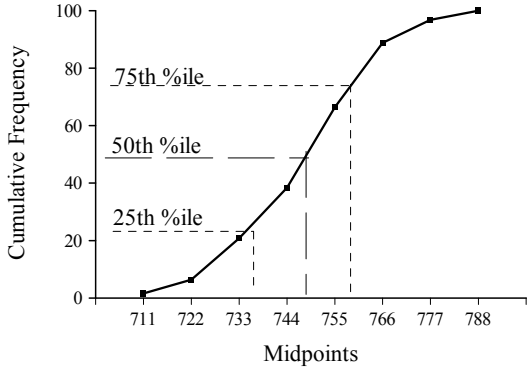


Figure 4.13 Example of a cumulative frequency polygon.

calculation of measures of central tendency (Chapter 5). Unfortunately there is some loss of precision with grouped frequency distribution because only one value (the midpoint) represents all the various data points within the class interval.

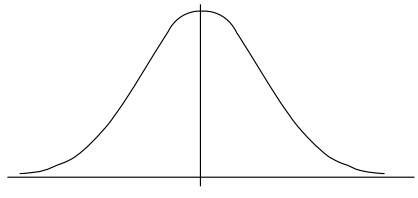
At times it may be desirable to prepare graphs that show how the values accumulate from lowest class intervals to highest. These **cumulative frequency polygons** display the frequency or percentage of the observed values falling below each interval. By using such a drawing it is possible to establish certain percentiles. Figure 4.13 shows a cumulative frequency polygon for the same data presented in the above frequency polygon (data from Table 4.3). Note that lines are drawn from the 25th (*Q1*), 50th (*Q2*), and 75th (*Q3*) percentile on the y-axis (point at which 25, 50, and 75% of the results fall below) and where they cross the polygon is the approximation of each percentile. If the population from which the sample approximates a normal or bell-shaped distribution, the cumulative distribution is usually S-shaped or **ogive**.

If there were an infinite number of midpoints (the interval width in both the histogram and frequency polygon approaches zero), it would be represented by a smooth curve. The skewness of a distribution describes the direction of the stringing out of the tail of the curve (Figure 4.14). In a **positively skewed distribution** most of

Figure 4.14 Examples of skewed distributions.

the values in the frequency distribution are at the left end of the distribution with a few high values causing a tapering of the curve to the right side of the distribution. In contrast, a **negatively skewed distribution** has most of the values at the right side of the distribution with a few low values causing a tapering of the curve to the left side of the distribution. Another way to think of skewness is the amount of tilt or lack of tilt in a distribution.

If a sample were normally distributed it would not be skewed to the left or right, but would be symmetrical in shape. The normal distribution and its characteristics will be discussed at great length in Chapter 6.



Kurtosis is a property associated with a frequency distribution and refers to the shape of the distribution of values regarding its relative flatness and peakedness. **Mesokurtic** is a frequency distribution that has the characteristics of a normal bell-shaped distribution. If the normal distribution is more peaked than a traditional bell-shaped curve is it termed **leptokurtic** and **platykurtic** refers to a shape that is less peaked or flatter than the normal bell-shaped curve.

Measures of skew and kurtosis will be discussed in Chapter 6.

Visual Displays for Two or More Continuous Variables

A **scatter diagram** or **scatter plot** is an extremely useful graphic presentation for showing the relationship between two continuous variables. The two-dimensional plot has both horizontal and vertical axes that cover the range of values for the two variables. Plotted data points represent paired observations for both the x and y variables (Figure 4.15). These types of plots are valuable for correlation and regression inferential tests (Chapters 13 and 14).

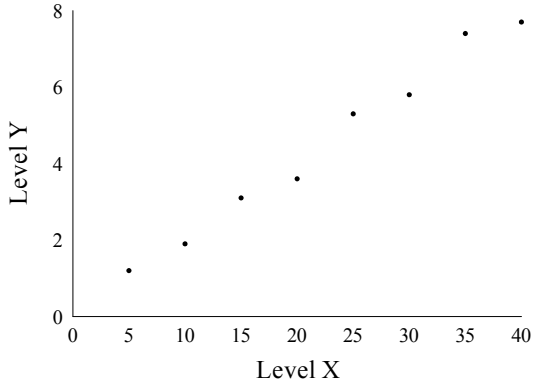


Figure 4.15 Example of a scatter diagram.

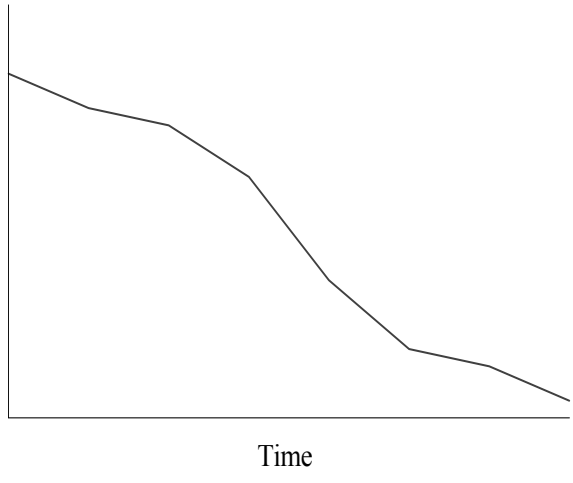


Figure 4.16 Example of a sequence plot.

A **sequence plot** is a plot where the horizontal axis represents a logical or physical sequencing of data. An example might be a measurement of successive lots of a particular product, where the vertical axis is a continuous variable and the horizontal axis represents the first lot, followed by the second, then the third, etc. If time is considered, then data is arranged chronologically on the horizontal axis. This **time series graph** is a visual representation of changes in data over time, in which the dependent variable is placed on the y-axis (Figure 4.16).

Further data reduction techniques for continuous data will be presented in the next chapter where we will explore methods for defining the center of distributions and how data are distributed around that center. However, visual or graphic techniques should be considered as a possible alternative to obtain a "feel" for the shape of the statistical descriptive data.

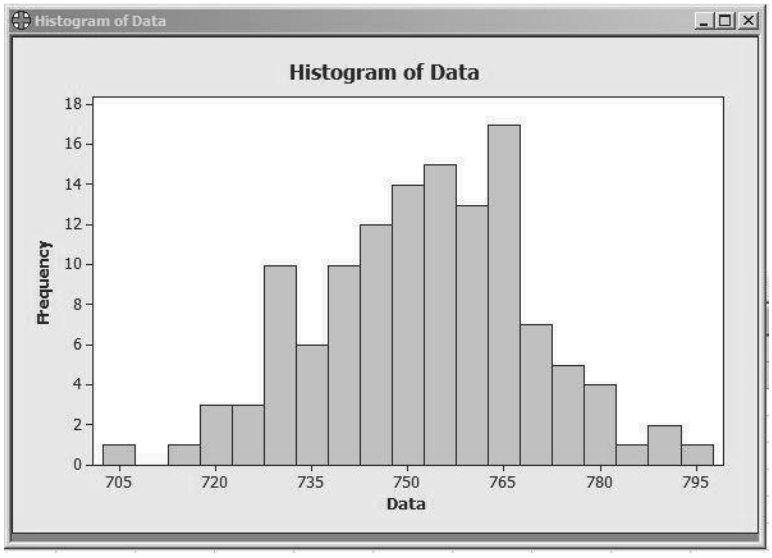


Figure 4.17 Histogram of data in Table 4.3 using Minitab.

Using Excel® or Minitab® for Visual Displays

Excel has several graph options. Data for each variable should be arranged by column or row. Graphic options are found under "Insert" on the title bar and listed under the "Chart" options. Options for discrete variables include: 1) for a vertical bar graph, select "column"; 2) for a horizontal bar graph, use "bar"; and 3) for a pie chart select "pie". For continuous variables choices include "line" and "scatter" for two continuous variables. All graphics offer a variety of presentation styles.

Minitab provides more graphic options. Data must be recorded as one column per variable and the column number (C#) will be requested for each operation. All visual displays are listed under "Graph" title bar. For discrete variables they include "Bar Chart…" (vertical), "Pie Chart…", and "Dotplot…". For continuous variables there are "Histogram…", "Stem-and-Leaf…", "Boxplot…" for box-and-whisker plot, and a "Time Series Plot…"; as well a "Scatterplot…" for two continuous variables. With the histogram option, Minitab selects the number of intervals using an algorithm different from Sturges' rule. In most cases Minitab will create more intervals than calculated using Sturges' rule (compare Figures 4.11 and 4.17).

References

Snedecor, G.W. and Cochran, W.G. (1989). *Statistical Methods*. Iowa State University Press, Ames, p. 18.

Bolton, S. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*. Third edition, Marcel Dekker, Inc., New York, p. 33.

Forthofer, R.N. and Lee, E.S. (1995). *Introduction to Biostatistics*. Academic Press, San Diego, p. 52.

Sturges, H.A. (1926). "The choice of a class interval," *Journal of the American Statistical Association* 21:65-66.

Suggested Supplemental readings

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Seventh edition, John Wiley and Sons, New York, pp. 15-27.

Mason, R.L., Gunst, R.F., and Hess, J.L. (1989). *Statistical Design and Analysis of Experiments*, John Wiley and Sons, New York, pp. 44-62.

Fisher, L.D. and van Belle, G. (1993). *Biostatistics: A Methodology for the Health Sciences*, John Wiley and Sons, New York, pp. 35-52.

Tukey, J.W. (1977). *Exploratory Data Analysis*, Addison-Wesley, Reading, MA.

Example Problems (Answers are provided in Appendix D)

1. During clinical trials, observed adverse effects are often classified by the following scale:

Mild:	Experience was trivial and did not cause any real problem.
Moderate:	Experience was a problem but did not interfere significantly with patient's daily activities or clinical status.
Severe:	Experience interfered significantly with the normal daily activities or clinical status.

   Based on 1109 patients involved in the Phase I and II clinical trials for bigomycin, it was observed that 810 experienced no adverse effects, while 215, 72, and 12 subjects suffered from mild, moderate, and severe adverse effects, respectively. Prepare visual and tabular presentations for this data.

2. The following assay results (percentage of label claim) were observed in 50 random samples during a production run.

102	100	96	99	101	102	100	105	97	100
92	103	101	100	99	102	96	100	101	98
107	95	98	100	100	99	97	104	101	103
98	101	100	105	99	101	102	100	87	98
101	103	93	99	101	97	100	102	99	104

Report these results as a box-and-whisker plot, stemplot, and histogram.

3. During a study of particle sizes for a blended powder mixture, the results of percent of powder retained on the various sizes were 50.1%, 27.2%, 10.4%, 6.0%, and 5.1% in sieve mesh sizes of 425, 180, 150, 90, and 75 μM, respectively. Only 1.2% was captured on the pan (<75 μM). Prepare visual and tabular presentations for these data.

4. Comparison of two methods for measuring anxiety in patients is listed below:

Method A	Method B	Method A	Method B
55	90	52	97
66	117	61	110
46	94	44	84
63	124	55	112
57	105	53	102
59	115	67	112
70	125	72	130
57	97		

Prepare a scatter plot to display the relationship between these two variables.

5

Measures of Central Tendency

Central tendency involves description statistics for the observed results of a continuous variable and takes into consideration two important aspects: 1) the center of that distribution and 2) how the observations are dispersed within the distribution. Three points are associated with the center (mode, median, and mean) and three other measures are concerned with the dispersion (range, variance, and standard deviation).

Measures of central tendency can be used when dealing with ordinal, interval, or ratio scales. It would seem logical, with any of these continuous scales, to be interested in where the center of the distribution is located and how observations tend to cluster around or disperse from this center. Many inferential statistical tests involve continuous variables (see Appendix A) and all require information about the central tendency of associated sample data.

Centers of a Continuous Distribution

The **sample mode** is simply that value with the greatest frequency of occurrence. In other words, the value that is most "popular" in a continuous distribution of scores. For example, what is the mode for the following group of observations?

2, 6, 7, 5, 3, 8, 7, 6, 5, 3, 2, 5, 4, 6, 8, 3, 4, 4, 7, 6, 5, 1, 5

Graphically the distribution would look as presented in Figure 5.1. In this distribution of observations, the **modal value** is 5 because it has the greatest frequency. The mode is the simplest, but least useful measure of the center for a distribution. The mode is most useful when continuous data has been divided into categories (e.g., a histogram) or where it represents the category with the greatest frequency.

A distribution may be multimodal and have several different values that have the same greatest relative frequency. Such a distribution may have several peaks. An example of a **bimodal distribution** appears below with slow and fast metabolizers of isoniazid (Figure 5.2). The first peak (to the left) represents a central point for the rapid metabolizers (a lower concentration of drug after six hours) and the second peak depicts the slow metabolizers where higher concentrations are seen at the same point in time.

The **sample median** is the center point for any distribution of scores. It

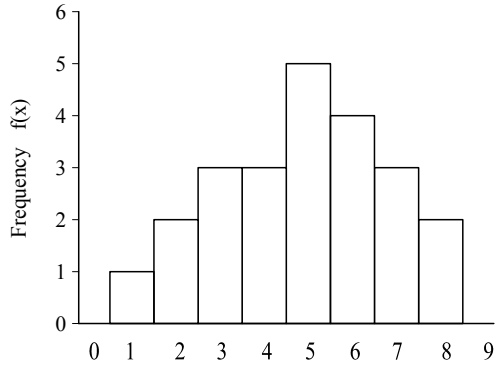


Figure 5.1 Histogram of sample data.

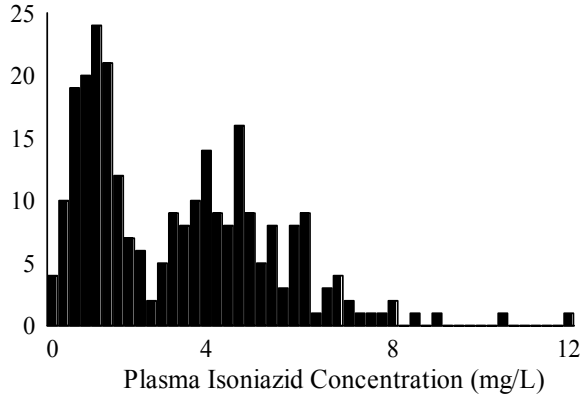


Figure 5.2 Bimodal distribution (Evans, 1960).

represents that value below which 50% of all scores are located. The median (from the Latin word *medianus* or "middle") divides the distribution into two equal parts (the 50th **percentile**). For example, using the same data as the previous example for the mode, a rank ordering of the scores from lowest to highest would produce the following:

Example 5A: 1, 2, 2, 3, 3, 3, 4, 4, 4, 5, 5, 5, 5, 5, 6, 6, 6, 6, 7, 7, 7, 8, 8

In this case 5 is the median, which is the value that falls in the exact center of the distribution. If there is an even number of observations, the 50th percentile is between the two most central values and the median would be the average of those two central scores. For example, in the following set of numbers the median (represented by an underlined area) is located between the two center values (ten data points are above and ten data points are below this center):

Example 5B: 20, 22, 23, 24, 24, 24, 25, 25, 25, 25, __
26, 26, 26, 27, 27, 28, 28, 28, 29, 30

The calculation of the median would be:

$$\frac{25+26}{2} = 25.5$$

The median value is a better estimate of the center of a distribution than the mode. However, it is neither affected by, nor representative, of extreme values in the sample distribution. For example, consider the following two samples:

Example 5C - Table weights in milligrams:

Sample 1	36, 45, 48, 50, 50, 51, 51, 53, 54
Sample 2	47, 48, 49, 50, 50, 51, 52, 57, 68

Even though both samples have the same median (50 mg), Sample 1 appears to have more observations that are relatively smaller and Sample 2 has more samples that are larger. The two samples appear to be different, yet both produce the same median. If possible, a measure of the center for a given distribution should consider all extreme data points (e.g., 36 and 68). However, at the same time, this inability to be affected by extreme values also represents one of the advantages of using the median as a measure of the center. The median is a robust statistic and not affected by any one observation. As will be seen in Chapter 23, an outlier or atypical data point, can strongly affect the arithmetic center of the distribution, especially in small sample sizes. The median is insensitive to these extreme values.

The median is a relative measure, in that it is defined by its position in relation to the other ordered values for a set of data points. In certain cases it may be desirable to describe a particular value with respect to its position related to other values. The most effective way to do this is in terms of its **percentile location** (the percent of observations that data point exceeds):

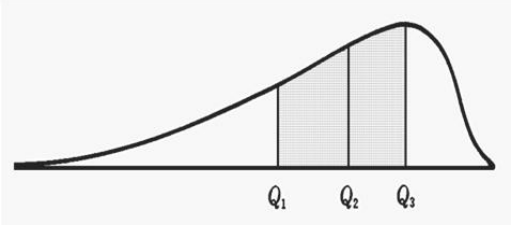
$$percentile = \frac{number\ of\ values\ less\ than\ the\ given\ value}{total\ number\ of\ values} \times 100 \qquad \text{Eq. 5.1}$$

For example consider Table 4.2 where 30 tetracycline capsules were placed in ranked order from smallest to largest. If one were interested in the percentile for 252 mg (the 24th largest value) the calculation would be

$$percentile = \frac{23}{30} \times 100 = 77\ percentile$$

Thus, 252 mg represents the 77th percentile for the data presented in Table 4.2. At the same time, when using percentiles, it is possible to calculate variability in a distribution, especially a distribution that is skewed in one direction. In this case, the measure would be the **interquartile range (IQR)** or **interrange** (the distance

between the 25th and the 75th percentiles).



The **sample mean** is what is commonly referred to as the **average**. It is the weighted center point of a distribution, and is computed by summing all the observations and dividing by the total number of observations.

$$\overline{X} = \frac{x_1 + x_2 + x_3 + \ldots + x_n}{n}$$

The character $\overline{X}$ (x-bar) will be used to symbolize the sample mean. The observed values of a given variable are designated with the same letter (usually x) and each individual value is distinguished with a subscript number or letter. For example x_i indicates the ith observation in a set of data. The symbol sigma ($\sum$) indicates the addition (summation) of all variable observations. Also referred to as the **arithmetic mean**, the formula for this equation is written as follows:

$$\overline{X} = \frac{\sum_{i=1}^{n} x_i}{n} \qquad \text{Eq. 5.2}$$

In this equation, all observations (x_i) for variable x are added together from the first (i=1) to the last (n) observation and divided by the total number of sample observations (n). Equation 5.2 can be simplified as follows:

$$\overline{X} = \frac{\sum x}{n}$$

The advantage in using the mean over the other measures of central tendency is that it takes into consideration how far each observation differs from the center and allows for extreme scores to impact this measure of center. Other measures do not account for this consideration. The mean can be thought of as a balancing point or center of gravity for our distribution. For the above Example 5A the mean would be:

$$\overline{X} = \frac{2 + 6 + 7 + \ldots 5}{23} = 4.9$$

We typically calculate a mean (and other measures of central tendency) to one decimal point beyond the precision of the observed data. In this case the precison of the data in Example 5A is to the whole number, thus the mean is expressed in tenths. Other authors have established more definitive rules for rounding and significant figures (Torbeck, 2004).

In the third example (Example 5C – tablet weights), the two medians were identical, and the means for the two samples differ because the extreme measures (i.e., 36 and 68 mg) were considered in this weighted measure of central tendency:

$$\text{Sample 1:} \quad \overline{X}_1 = 48.7 \text{ mg}$$
$$\text{Sample 2:} \quad \overline{X}_2 = 52.4 \text{ mg}$$

The relative positioning of the three measures of a continuous variable's center can give a quick, rough estimate of the shape of the distribution. As will be discussed in the next chapter, in a normal (bell-shaped) distribution the mode = median = mean; in the case of a positively skewed distribution the mode < median < mean and for a negatively skewed distribution the mode > median > mean.

If data is normally distributed, or the sample is assumed to be drawn from a normally distributed population, the mean and standard deviation are the best measures of central tendency. The median is the preferred measure of central tendency in skewed distributions where there are a few extreme values (either small or large). In such cases the interquartile range is the appropriate measure of dispersion.

Dispersion within a Continuous Distribution

The mean is only one dimension in the measure of central tendency, namely, the weighted middle of the sampling distribution. Seen in Figure 5.3 are two distributions that have the exact same median (5) and the same mean (4.9). However, the dispersions of data around the center of these two distributions are considerably different. Thus, measures of central tendency should also be concerned with the spread or concentration of data points around the center of the distribution.

The **sample range** is the simplest method for reporting a distribution of observations and represents the difference between the largest and smallest value in a set of outcomes. In Example 5A the largest observation is 8 and the smallest is 1. The range for these observations is 7. Similarly, the ranges for the two sample batches of tablet weights in Example 5C are:

$$\text{Sample 1:} \quad 54 - 36 = 18 \text{ mg} \qquad \text{Sample 2:} \quad 68 - 47 = 21 \text{ mg}$$

Some texts and statisticians prefer to correct for continuity (due to the fact that the continuous variable actually extends to one decimal smaller and larger than the measure). In Sample 1 listed above, 54 to 36, would be 54 to 36 inclusive or 54.5 to 35.5. In this case the range would be:

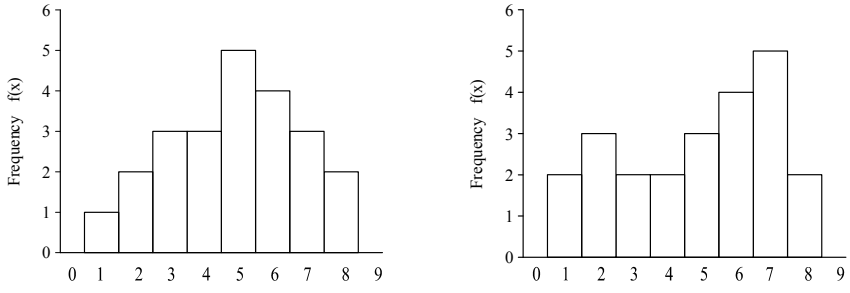


Figure 5.3 Example of distributions with the same mean and median.

$$R = (largest\ observation - smallest\ observation) + 1$$

$$R = 54 - 36 + 1 = 19\ mg$$

If the range were measured in tenths, then 0.1 would be added; if the range is in hundredths, then add an additional 0.01, and so on.

A second measure of dispersion already discussed is the interquartile range. Even though the range and interquartile range are quick and easy measures of dispersion, they possess a limitation similar to the median; specifically they do not account for the actual numerical value of every individual observation. Much like the mean, a measure is needed to account for how *each* observation varies from the center of the distribution.

One possible measure would be to determine the distance between each value and the center (Figure 5.4). Unfortunately, because the distances to the left and to the right of the mean are equal (since the mean is the weighted center), the sum of all the individual differences ($\sum x_i - \overline{X}$) equals zero and provides no useful information. Therefore, the sum of all the squared differences between the individual observations

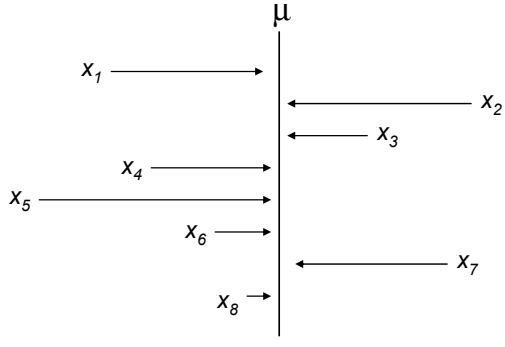


Figure 5.4 Distribution of observations around the mean.

and the mean is computed, and divided by the number of degrees of freedom (n – 1). This average of the squared deviations produces an intermediate measure known as the **sample variance**:

$$S^2 = \frac{(x_1 - \overline{X})^2 + (x_2 - \overline{X})^2 + (x_3 - \overline{X})^2 + \ldots + (x_n - \overline{X})^2}{n - 1}$$

Degrees of freedom (*df*) is used to correct for bias in the results that would occur if just the number of observations (*n*) was used in the denominator. If the **average squared deviation** is calculated by dividing the summed squared differences by *n* observations, it tends to underestimate the variance. The term "degrees of freedom" is best examined by considering the example where the sum of all the deviations ($x_i - \overline{X}$) equals zero. All but one number has the "freedom" to vary. Once we know all but the last data point ($n - 1$), we can predict the last value because the sum of all the deviations must equal zero. Therefore, to prevent bias, most statistical analyses involve degrees of freedom ($n - 1$) rather than the total sample size (*n*). The sample variance formula can be written:

$$S^2 = \frac{\sum(x_i - \overline{X})^2}{n - 1} \qquad \text{Eq. 5.3}$$

Obviously as the size of our sample of data increases, the effect of dividing by *n* or *n*-1 becomes negligible. However, for theoretical purposes, degrees of freedom will continually appear in descriptive as well as inferential equations.

The variance, using the data from Example 5A (with a mean of 4.9), is calculated as follows:

$$S^2 = \frac{(2 - 4.9)^2 + (6 - 4.9)^2 + (7 - 4.9)^2 + \ldots (5 - 4.9)^2}{23 - 1} = 3.8$$

An easier method for calculating the variance (especially for computers) would be as follows:

$$S^2 = \frac{n(\sum x^2) - (\sum x)^2}{n(n - 1)} \qquad \text{Eq. 5.4}$$

where each observation is squared and both the sum of the observation and the sum of the observations squared are entered into the formula. Algebraically, this produces the exact same results as the original variance formula (Eq. 5.3). Once again, using data from Example 5A, this method produces the same variance:

x_i	x_i^2
2	4
6	36
...	...
5	25
112	628

$$S^2 = \frac{n(\Sigma x^2) - (\Sigma x)^2}{n(n-1)} = \frac{23(628) - (112)^2}{23(22)}$$

$$S^2 = \frac{14444 - 12544}{506} = \frac{1900}{506} = 3.8$$

The two previous equations represent different ways to calculate the same value. The first (Eq. 5.3) is a **definitional formula** because it defines how the variance term is calculated (the average of the squared deviants). The second (Eq. 5.4) is a **computational formula** because it represents an easier formula to compute using computer software or a hand calculator. Throughout this book there will be examples of formulas using either the definitional or computational approaches or both.

Variance is only an intermediate measure of dispersion. Each difference ($x_i - \overline{X}$) was squared to produce the variance term. The square root of the variance is needed to return the results to the same measurement scale used for the mean (for example, if the mean is expressed in milligrams, then this new measure, called the standard deviation, will also be expressed as milligrams).

The **sample standard deviation** (*S* or *SD*) is the square root of the variance. It also measures variability about the mean and is most commonly used to express the dispersion of the observations.

$$S = \sqrt{S^2} \qquad \text{Eq. 5.5}$$

Using the previous set of data as an example:

$$S = \sqrt{3.8} = 1.9$$

Since the standard deviation can be thought of as the square root of the mean of the squared deviations, some textbooks refer to variance as the **root mean square**, or **RMS** value.

It is important to note that the variance has no relevant term of measurement, but the standard deviation is expressed in the same units as the mean. For the sake of illustration, consider the observations for the two samples of tablet weights in Example 5C:

	$\overline{X}$	S^2	S	n
Sample 1	48.7	29.5	5.4	9
Sample 2	54.4	42.3	6.5	9

In this case the average weights of the tablets in Sample 1 would be 48.7 mg with a standard deviation of 5.4 mg. The variance is simply 29.5, not 29.5 mg or mg squared.

To illustrate the use of central tendency measurements, thirty bottles of a cough syrup are randomly sampled from a production line and the results are reported in Table 5.1. The descriptive statistics reporting the measures of central tendency for the sample would be:

Mode: 120.1 ml (largest frequency with 3 outcomes)

Median: The average of the center two values (15th and 16th ranks in the right columns)

$$\frac{120.0 + 120.1}{2} = 120.05\ ml$$

Mean: Weighted average of all 30 samples

$$\overline{X} = \frac{120.7 + 120.2 + ...119.7}{30} = 120.05\ ml$$

Table 5.1. Data for Samples of Bottles of Cough Syrup

Original Samples (volume in ml)				Samples Ordered Smallest to Largest	
Sample	Volume	Sample	Volume		
1	120.7	16	119.0	118.3	120.1
2	120.2	17	121.1	118.5	120.1
3	119.6	18	121.7	118.9	120.1
4	120.1	19	119.2	119.0	120.2
5	121.3	20	120.0	119.0	120.2
6	120.7	21	120.8	119.2	120.4
7	121.0	22	119.9	119.6	120.5
8	119.7	23	119.8	119.7	120.7
9	118.3	24	119.9	119.7	120.7
10	118.9	25	120.2	119.8	120.8
11	120.5	26	120.0	119.8	121.0
12	121.4	27	120.1	119.9	121.1
13	120.4	28	119.0	119.9	121.3
14	118.5	29	120.1	120.0	121.4
15	119.8	30	119.7	120.0	121.7

Range: 121.7 – 118.3 = 3.4 ml
(or 3.5 = 121.75 – 118.25 if an **inclusive range**)

Variance (definitional formula):

$$S^2 = \frac{(120.7 - 120.05)^2 + ...(119.7 - 120.05)^2}{30(29)} = 0.70$$

Standard deviation:

$$S = \sqrt{0.70} = 0.84\ ml$$

Summary data, presented in reports, posters or journal articles, usually report only the mean, standard deviation and sometimes the number of observations. The mean and standard deviation are usually presented as *mean* ± *standard deviation*. However, the reader should be cautious if the author does not clearly indicate what is represented on the right of the ± sign. As discussed in Chapter 7 it could represent a measure which is not the standard deviation.

Population versus Sample Measures of Central Tendency

The statistics presented thus far have represented means, variances, and standard deviations calculated for sample data and not an entire population. The major reason for conducting a statistical analysis is to use sample data as an estimate of the parameters for the entire population of events. For example, it is impractical, and impossible if destructive methods are used, to sample all the tablets in a particular batch. Therefore, compendia or in-house standards for content uniformity testing might consist of a sample of 30 tablets randomly selected from a batch of many thousands or millions of tablets.

Parameters are to populations as **statistics** are to samples. As seen in Table 5.2, the observed statistics (mean and standard deviation from the sample) are the best estimates of the true population parameters (the population mean and population standard deviation). Note in Table 5.2 that the Greek symbols μ (mu) and σ (sigma) are used to represent the population mean and population standard deviation, respectively. Also, in the formulas that follow, *N* replaces *n* for the total observations in a population. These symbols will be used throughout the book with Greek symbols referring to population parameters.

The **population mean** is calculated using a formula identical to the sample mean:

$$\mu = \frac{\sum_{i=1}^{N} X_i}{N} \qquad \text{Eq. 5.6}$$

The formula for the **population variance** is similar to that of the sample estimate, except that the numerator is divided by the number of all observations (*N*). If all the

Table 5.2 Symbols Used for Sample and Population Measures of Central Tendency

	Sample Statistic	Population Parameter
Mean	$\overline{X}$	μ
Variance	S^2	σ^2
Standard deviation	S	σ
Number of observations	n	N

data is known about the population, it is not necessary to use degrees of freedom to correct for bias.

$$\sigma^2 = \frac{\sum_{i=1}^{N} (x_i - \mu)^2}{N} \qquad \text{Eq. 5.7}$$

Similar to the sample standard deviation, the **population standard deviation** is the square root of the population variance.

$$\sigma = \sqrt{\sigma^2} \qquad \text{Eq. 5.8}$$

One should be cautious using scientific or programmable calculators when computing the standard deviation. Some calculators may compute the population standard deviation, some the sample standard deviations and others can display both measures. It is important to know which measure of dispersion is calculated by your calculator, especially when dealing with smaller sample sizes. A quick and simple check to determine the type of standard deviation(s) displayed on a calculator is to enter the three values 1, 2, and 3. The mean is obviously 2.0. If a sample standard deviation (S) is calculated the result will be 1.0; whereas the population standard deviation (σ) is 0.8165.

Measurements Related to the Sample Standard Deviation

The variability of data may often be better described as a relative variation rather than as an absolute variation (e.g., the standard deviation). This can be accomplished by calculating the **coefficient of variation** (CV) that is the ratio of the standard deviation to the mean.

$$CV = \frac{standard\ deviation}{mean} \qquad \text{Eq. 5.9}$$

Table 5.3 Examples with Relative Standard Deviations

	Assayed Amount of Drug			% Labeled Claim
	9.96	99.6	996	99.6
	10.05	100.5	1005	100.5
	9.92	99.2	992	99.2
	9.92	99.2	992	99.2
	9.86	98.6	986	98.6
	9.85	98.5	985	98.5
	10.01	100.1	1001	100.1
	9.90	99.0	990	99.0
	9.96	99.6	996	99.6
	9.86	98.6	986	98.6
Mean =	9.929	99.29	992.9	99.29
S.D. =	0.067	0.666	6.657	0.666
RSD =	0.67%	0.67%	0.67%	0.67%

The *CV* is usually expressed as a percentage **(relative standard deviation** or ***RSD*)** and can be useful in many instances because it places variability in perspective to the distribution center.

$$RSD = CV \times 100 \text{ (percent)} \qquad \text{Eq. 5.10}$$

In the previous Example 5A ($CV = 1.94/4.87 = 0.398$ and $RSD = 0.398 \times 100 = 39.8$), the standard deviation is 40% of the mean. In the previous example of the liquid volumes (Table 5.1), the coefficient of variation and *RSD* would be:

$$CV = \frac{0.835}{120.05} = 0.007$$

$$RSD = 0.007 \times 100 = 0.7\%$$

Thus, relative standard deviations present an additional method of expressing this variability, which takes into account its relative magnitude (expressed as the ratio of the standard deviation to the mean). Table 5.3 illustrates the amount of assayed drug and the second and third columns represent 10- and 100-fold increases in the original values. These increases also result in a 10- and 100-fold increase in both the mean and standard deviation, but the relative standard deviation remains constant. In the pharmaceutical industry, this can be used as a measure of precision between various batches of a drug, if measures are based on percent label claim (column 4 in Table 5.3).

A second example illustrating the relative standard deviation would be the peak area on an HPLC reading:

	HPLC Peak (x)	x^2
Run 1	59.45	3534.30
Run 2	59.50	3540.25
Run 3	58.70	3445.69
Run 4	59.25	3510.56
	236.90	14030.80

Measures of central tendency are as follows:

Mean:

$$\overline{X} = \frac{236.9}{4} = 59.23\ ml$$

Variance (computational formula):

$$S^2 = \frac{4(14030.8) - (236.9)^2}{4(3)} = 0.13$$

Standard deviation:

$$S = \sqrt{0.13} = 0.36\ ml$$

Coefficient of variation:

$$C.V. = \frac{0.36}{59.23} = 0.0061$$

Relative standard deviation:

$$RSD = 0.0061 \times 100 = 0.61\%$$

Trimmed Mean

In certain software statistical packages a trimmed mean is reported in the descriptive results along with the algebraic mean. This measure represents the weighted center for the majority of the data, but "trims" the extreme values, usually 5%, from each end of the distribution. The remaining 90% of the data is then used to compute the mean. This offers two advantages over the arithmetic mean: 1) it eliminates outliers (Chapter 23); and 2) for positively or negatively skewed distributions it approximates the median and gives a better estimate of center. The disadvantages are that it could greatly decrease the variance for the sample data and may eliminate important information provided by outliers. In the case of a normal, bell-shaped distribution (Chapter 6), removal of 5% of the upper end of the

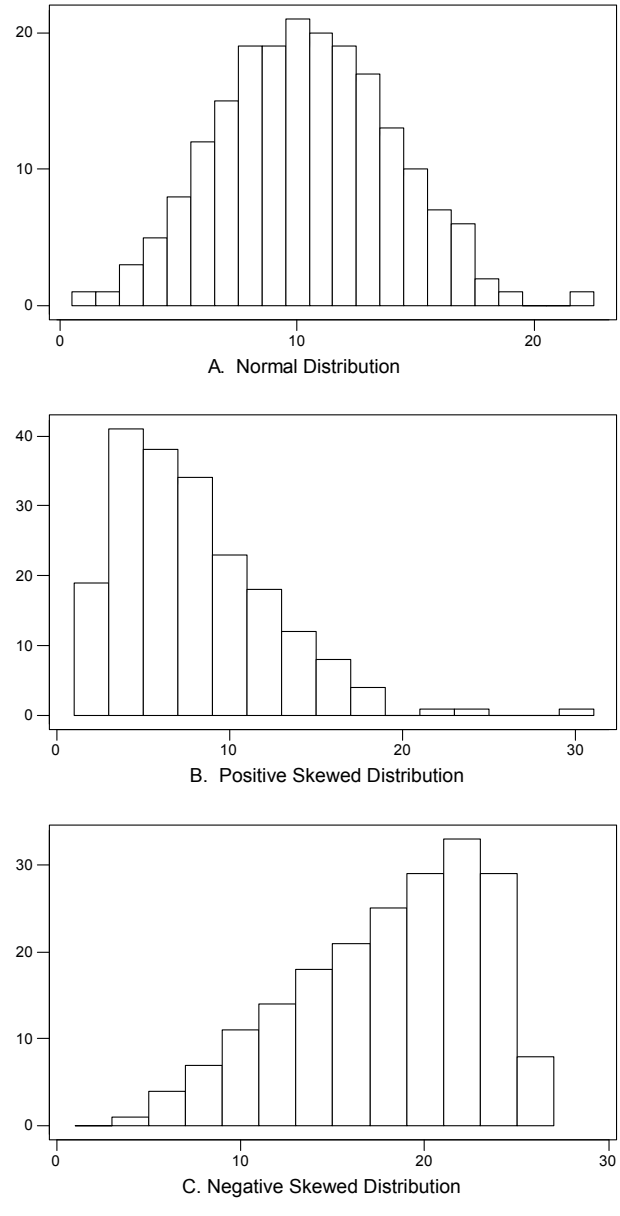


Figure 5.5 Various distributions prior to trimming the mean.

distribution and removal of 5% of the lower end will not affect the mean because of the symmetry of the distribution. To illustrate this, consider three distributions (Figure 5.5) each representing a sample of 200 observations: 1) *A* is approximately normally distributed; 2) *B* is positively skewed; and 3) *C* is negatively skewed. Measures of center would be as follows:

Distribution	Mean	Trimmed Mean	Median
A. Normal distribution	10.37	10.35	10.0
B. Positive skew	7.470	7.144	7.0
C. Negative skew	17.545	17.767	18.0

The problem rests with the reduced spread when the data is trimmed:

	Standard Deviation	
Distribution	Untrimmed	Trimmed
A. Normal distribution	3.665	2.963
B. Positive skew	4.527	3.472
C. Negative skew	5.125	4.337

As seen in these examples, the standard deviation is decreased from 15.3 to 23.3% by simply removing the extreme 10% of the distribution.

Using Excel® or Minitab® for Measures of Central Tendency

Excel has several function options that produce descriptive statistics. These are listed in Table 5.4. Also under the "data analysis" command, multiple descriptive statistics can be created with one command using the "Descriptive Statistics" option and the "Summary Statistics" command (Figure 5.6).

Data ➤ Data Analysis ➤ Descriptive Statistics ➤ Summary Statistics

All these test require that you indicate a range of cells where the data is located ("Input Range") and where the output should be reported, either starting at a specific cell ("Output Range") or creating a new worksheet. Figure 5.7 show a typical Excel output for the "descriptive statistics" procedure (data from Table 4.3). The variance represents the sample statistic. Skew and kurtosis will be discussed in Chapter 6 and standard error will be addressed in Chapter 7.

Minitab offers a similar descriptive statistics option, but with more choices. It is accessed by choosing "Stat" on the title bar and "Basic Statistics" and "Display Description Statistics" from the two dropdown menus:

Stat ➤ Basic Statistics ➤ Display Description Statistics

Pick the column(s) of data you wish to evaluate and select "Statistics" to choose from the available options (Figure 5.8). All or part of the options can be selected.

Table 5.4 Excel Function Commands for Descriptive Statistics

Statistics	Excel Function
Mode	MODE
Median	MEDIAN
Mean	AVERAGE
Smallest Data Point	MIN
Largest Data Point	MAX
Range	=MAX-MIN
Variance (sample)	VAR
Variance (population)	VAR.P
Standard Deviation (sample)	STDEV
Standard Deviation (population)	STDEV.P
Coefficient of Variation	=STDEV/AVERAGE

Additional options with Minitab include: sum x, sum x^2, coefficient of variance (without needing to do a separate calculation), trimmed mean, first and third quartile and IQR. Note that the Minitab's "coefficient of variation" is the relative standard deviation (CV x 100%) and the trimmed mean represents the result of removing the extreme 10% of the values (5% from each end of the distribution of observation). MSSD stands for the mean of the squared successive differences, is used primarily for

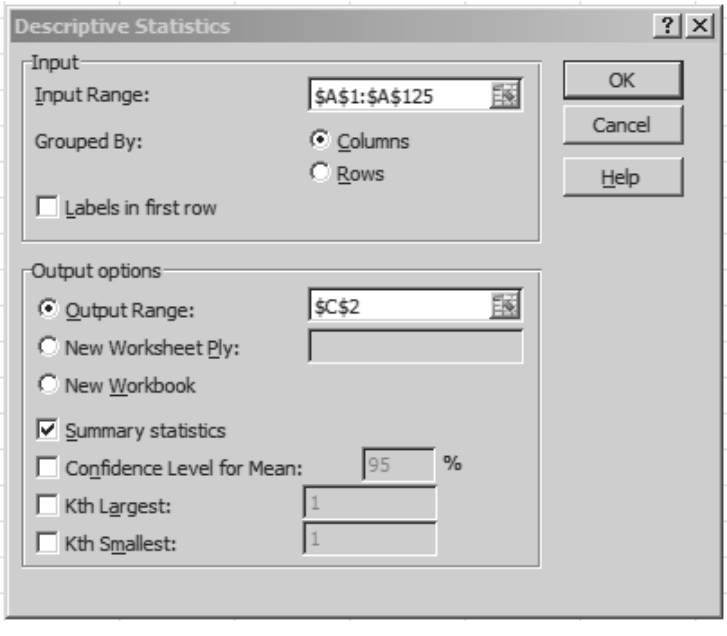


Figure 5.6 Descriptive Statistic options.

Column1	
Mean	752.456
Standard Error	1.50395196
Median	754
Mode	755
Standard Deviation	16.814694
Sample Variance	282.733935
Kurtosis	-0.16409249
Skewness	-0.15736529
Range	87
Minimum	706
Maximum	793
Sum	94057
Count	125

Figure 5.7 Output from Excel descriptive statistics command.

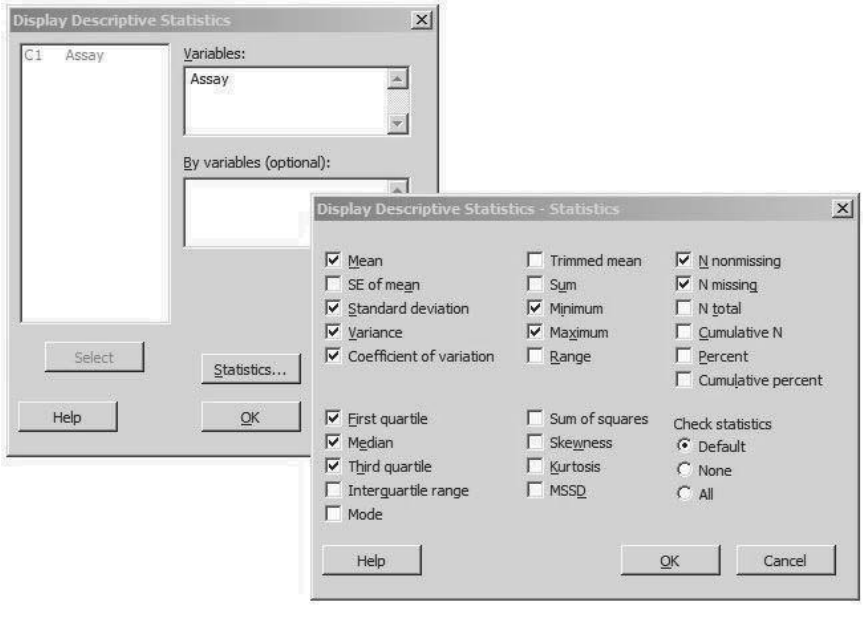


Figure 5.8 Options for descriptive statistics available with Minitab.

Descriptive Statistics: Assay

Variable	N	N*	Mean	SE Mean	TrMean	StDev	Variance	CoefVar	Sum
Assay	125	0	752.46	1.50	752.56	16.81	282.73	2.23	94057.00

Variable	Sum of Squares	Minimum	Q1	Median	Q3	Maximum	Range
Assay	70808813.00	706.00	741.00	754.00	765.00	793.00	87.00

Variable	IQR	Mode	N for Mode	Skewness	Kurtosis
Assay	24.00	755	8	-0.16	-0.16

Figure 5.9 Output from Minitab descriptive statistics command.

quality control statistics, and will be discussed in Chapter 7. Once again, skew, kurtosis and standard error will be discussed in following chapters. Minitab offers also a dropdown menu where several individual items in Figure 5.8 can be calculated, but only one descriptive statistic at a time:

Calc ➤ Column Statistics

It is much easier to select the "Basic Statistics" option to select the required information. Results for the data presented in Table 4.3 using Minitab are seen in Figure 5.9. In most cases you would select only those options of interest for your research and produce a less complicated output.

Alternative Computational Methods for Calculating Central Tendency

Various other methods can be used to determine sample means and standard deviations. They include calculations from binomial distributions, probability distributions, and frequency distributions.

Binomial Distribution. As mentioned in Chapter 2, the binomial distribution is concerned with two mutually exclusive outcomes. If the probability of one of the outcomes is known, the mean (or **expected value**, *E(x)*) and standard deviation for the distribution can be calculated. In this case the measure of central tendency represents the mean and standard deviation of the values taken by the variable in many repeated binomial experiments. For example, if we flipped a fair coin 1000 times, we would expect on the average to have 500 heads (defined as success with $p = 0.50$). The mean is

$$\overline{X} \text{ or } E(x) = n \cdot p \qquad \text{Eq. 5.11}$$

with a variance of

$$S^2 \text{ or } Var(x) = n \cdot p \cdot q \qquad \text{Eq. 5.12}$$

and standard deviation of

$$S = \sqrt{n \cdot p \cdot q} \qquad \text{Eq. 5.13}$$

where p is the probability of success, q the probability of failure (1.00 – p) and n is total number of outcomes or observations. The binomial distribution tends to the normal distribution as n increases, however, in small samples the distribution may be noticeably skewed. For that reason, np should be greater than 5 to use this approximation.

As an example, the probability of rolling a six on one toss of the die is 0.1667. If a single die is tossed 50 times what is the expected number of times a six will appear? What is the variability of this expected outcome? In this case $p(6) = 1/6 = 0.1667$; $q(\text{not } 6) = 1 - 0.1667 = 0.8333$; and n is 50 for the sample size.

$$E(x) = np = (50)(0.1667) = 8.3$$

The average number of times six would appear in 50 rolls is 8.3 times. The standard deviation would be:

$$S = \sqrt{npq} = \sqrt{(50)(0.1667)(0.833)} = 2.6$$

Probability Distribution. If the probabilities of all possible outcomes are known, the mean and standard deviation for the distribution can be calculated by first creating a table:

$\underline{x}$	$\underline{p(x)}$	$\underline{x \cdot p(x)}$	$\underline{x^2 \cdot p(x)}$
x_1	$p(x_1)$	$x_1 \cdot p(x_1)$	$x_1 \cdot x_1 \cdot p(x_1)$
x_2	$p(x_2)$	$x_2 \cdot p(x_2)$	$x_2 \cdot x_2 \cdot p(x_2)$
...	...	...	...
x_n	$\underline{p(x_n)}$	$\underline{x_n \cdot p(x_n)}$	$\underline{x_n \cdot x_n \cdot p(x_n)}$
$\Sigma =$	1.00	$\Sigma(x \cdot p(x))$	$\Sigma(x^2 \cdot p(x))$

where x_i is the occurrence and $p(x_i)$ is the probability of that occurrence. The mean is represented by the sum of the third column:

$$\overline{X} = \sum(x \cdot p(x)) \qquad \text{Eq. 5.14}$$

The variance and standard deviations involve the sums of the third and fourth columns:

$$S^2 = [\sum(x^2 \cdot p(x))] - [\sum(x \cdot p(x))]^2 \qquad \text{Eq. 5.15}$$

$$S = \sqrt{[\Sigma(x^2 \cdot p(x))] - [\Sigma(x \cdot p(x))]^2} \qquad \text{Eq. 5.16}$$

To illustrate the process, what is the mean and standard deviation for the following values and their respective probabilities of occurrence?

Value	Probability	Value	Probability	Value	Probability
0	0.07776	4	0.11646	8	0.00017
1	0.22680	5	0.04042	9	0.00001
2	0.29700	6	0.00971	10	0.00000
3	0.22995	7	0.00150		

x	$p(x)$	$x \cdot p(x)$	$x^2 \cdot p(x)$
1	0.22680	0.22680	0.22680
2	0.29700	0.59400	1.18800
3	0.22995	0.68985	2.06955
4	0.11646	0.46584	1.86336
5	0.04042	0.20210	1.01050
6	0.00971	0.05826	0.34956
7	0.00150	0.01050	0.07350
8	0.00017	0.00136	0.01088
9	0.00001	0.00009	0.00081
10	0.00000	0.00000	0.00000
$\Sigma =$	1.00000	2.24880	6.79296

$$\overline{X} = \Sigma(x \cdot p(x)) = 2.2488 = 2.25$$

$$S^2 = [\Sigma(x^2 \cdot p(x))] - [\Sigma(x \cdot p(x))]^2 = 6.79296 - (2.24880)^2 = 1.74$$

$$S = \sqrt{S^2} = \sqrt{1.74} = 1.32$$

Frequency Distribution. If data is presented that reports the frequency of each occurrence (for example, the frequency of response to a Likert-type scale), the mean and standard deviation can be calculated as follows where x_i is the event and $f(x_i)$ is the frequency associated with that event.

x	$f(x)$	$x \cdot f(x)$	$x^2 \cdot f(x)$
x_1	$f(x_1)$	$x_1 \cdot f(x_1)$	$x_1 \cdot x_1 \cdot f(x_1)$
x_2	$f(x_2)$	$x_2 \cdot f(x_2)$	$x_2 \cdot x_2 \cdot f(x_2)$
x_3	$f(x_3)$	$x_3 \cdot f(x_3)$	$x_3 \cdot x_3 \cdot f(x_3)$
...	...	...	...
x_n	$f(x_n)$	$x_n \cdot f(x_n)$	$x_n \cdot x_n \cdot f(x_n)$
$\Sigma =$	$\Sigma f(x) = N$	$\Sigma(x \cdot f(x))$	$\Sigma(x^2 \cdot f(x))$

The mean is:

$$\overline{X} = \frac{\Sigma(x \cdot f(x))}{N} \qquad \text{Eq. 5.17}$$

In this case N represents the sum of all the sample frequencies ($n_1 + n_2 ... + n_i$) and not a population N. The variance is:

$$S^2 = \frac{N\,[\Sigma(x^2 \cdot f\,(x))] - [\Sigma(x \cdot f\,(x))]^2}{N(N-1)} \qquad \text{Eq. 5.18}$$

with a standard deviation of:

$$S = \sqrt{\frac{N[\Sigma(x^2 \cdot f\,(x))] - [\Sigma(x \cdot f\,(x))]^2}{N(N-1)}} \qquad \text{Eq. 5.19}$$

For an example using a frequency distribution, consider a final examination in which 12 pharmacy students scored 10 points, 28 scored 9, 35 scored 8, 26 scored 7, 15 scored 6, 8 scored 5, and one student scored 4. What are the mean and standard deviation on this final examination?

x	$f(x)$	$x \cdot f(x)$	$x^2 \cdot f(x)$
10	12	120	1200
9	28	252	2268
8	35	280	2240
7	26	182	1274
6	15	90	540
5	8	40	200
4	1	4	16
$\Sigma =$	125	968	7738

$$\overline{X} = \frac{\Sigma(x \cdot f(x))}{N} = \frac{968}{125} = 7.74$$

$$S^2 = \frac{N\,[\Sigma(x^2 \cdot f\,(x))] - [\Sigma(x \cdot f\,(x))]^2}{N(N-1)} = \frac{125(7738) - (968)^2}{125(124)} = 1.95$$

$$S = \sqrt{S^2} = \sqrt{1.95} = 1.40$$

Table 5.5 Intervals Created Using Sturges' Rule for Table 4.3

Interval Range	Midpoint	Frequency	$m_i f_i$	$m_i^2 f_i$
705.5-716.5	711	2	1,422	1,011,042
716.5-727.5	722	6	4,332	3,127,704
727.5-738.5	733	18	13,194	9,671,202
738.5-749.5	744	22	16,368	12,177,792
749.5-760.5	755	35	26,425	19,950,875
760.5-771.5	766	28	21,448	16,429,168
771.5-782.5	777	10	7,770	6,037,290
782.5-793.5	788	4	3,152	2,483,776
	$\sum =$	125	94,111	70,888,849

Central Tendency from a Histogram. An estimate of the mean and standard deviation involves using the frequency distribution and the midpoint of each interval. For example, for the first interval in Table 5.5, the midpoint would be:

$$Midpoint = \frac{highest + lowest\ points}{2} = \frac{705.5 + 716.5}{2} = 711 \qquad \text{Eq. 5.20}$$

A table can be prepared by tabulating the midpoints and their associated frequencies:

Interval Range	Midpoint (m_i)	Frequency (f_i)	$m_i f_i$	$m_i^2 f_i$
Interval 1	m_1	f_1	$m_1 f_1$	$m_1^2 f_1$
Interval 2	m_2	f_2	$m_2 f_2$	$m_2^2 f_2$
Interval 3	m_3	f_3	$m_3 f_3$	$m_3^2 f_3$
...	...	...	...	...
Interval n	m_n	f_n	$m_n f_n$	$m_n^2 f_n$
	$\sum =$	n	$\sum m_i f_i$	$\sum m_i^2 f_i$

The midpoint of each class interval, m_i, was weighted by its corresponding frequency of occurrence f_i. The computation of the mean and standard deviation involves a table similar to that used for frequency distributions discussed under central tendency. With a mean of

$$\overline{X} = \frac{\sum m_i f_i}{N} \qquad \text{Eq. 5.21}$$

a variance of

$$S^2 = \frac{n(\sum m_i^2 f_i) - (\sum m_i f_i)^2}{n(n-1)} \qquad \text{Eq. 5.22}$$

and a standard deviation as the square root of the variance of

$$S = \sqrt{\frac{n(\sum m_i^2 f_i) - (\sum m_i f_i)^2}{n(n-1)}} \qquad \text{Eq. 5.23}$$

Using the pharmacokinetic example in Chapter 4 (Table 4.3), the mean and standard deviation are calculated below. How accurate is the measure of the mean and standard deviation using Sturges' Rule to create the histogram? The data is presented in Table 5.4 and the calculations of the mean and standard deviation using the above formulas are presented below. The sample statistics for all the observations presented in the original table of C_{max} is: Mean = 752.4 mcg and S.D. = 16.8 mcg. In this particular case, there is less than 5% difference between the means and standard deviations, which were calculated from the raw data and calculated from the intervals created by Sturges' rule.

$$\overline{X} = \frac{94,111}{125} = 752.9 \text{ mcg}$$

$$S^2 = \frac{125(70,888,849) - (94,111)^2}{125(124)}$$

$$S^2 = \frac{4,225.804}{15,500} = 272.63$$

$$S = \sqrt{S^2} = \sqrt{272.63} = 16.51 \text{ mcg}$$

If one were interested in calculating the median for a histogram the following formula could be used:

$$Median = L + w \cdot \left(\frac{\frac{n}{2} - F}{f} \right) \qquad \text{Eq. 5.24}$$

where L is the lower limit of the interval that contains the median, w is the width of the class interval for each interval, n is the total sample size, F is the cumulative frequency corresponding to the lower limit of the interval (cumulative frequency for all the intervals in the histogram below the one containing the median), and f is the number of observations in the interval that contains the median. Using the example in Table 5.5, the interval with the median is 749.5 to 760.5, because 48 observations fall below 749.5 mcg (38.4%) and 83 observations are below 760.5 mcg (66.4%). With a width of 11 mcg, the median is:

$$Median = 749.5 + 11 \cdot \left(\frac{\frac{125}{2} - 48}{35} \right) = 754.1$$

References

Evans, D.A.P., et al. (1960). "Genetic control of isoniazid metabolism in man," *British Medical Journal* 2:489.

Torbeck, L.D. (2004). "Significant digits and rounding," *Pharmacopeial Forum* 30(3):1090-1095.

Suggested Supplemental Readings

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Seventh edition, John Wiley and Sons, New York, pp. 35-47.

Forthofer, R.N. and Lee, E.S. (1995). *Introduction to Biostatistics: A Guide to Design, Analysis and Discovery*, Academic Press, San Diego, pp. 61-67, 71-77.

Snedecor, G.W. and Cochran W.G. (1989). *Statistical Methods*, Iowa State University Press, Ames, IA, pp. 26-36.

Example Problems (Answers are provided in Appendix D)

1. Pharmacy students completing the final examination for a pharmacokinetics course received the following scores. Report the range, median, mean, variance, and standard deviation for these results.

Student	%	Student	%	Student	%	Student	%
001	85	009	78	017	77	025	97
002	79	010	85	018	83	026	76
003	98	011	77	019	87	027	69
004	84	012	86	020	78	028	86
005	72	013	90	021	60	029	80
006	84	014	84	022	88	030	92
007	70	015	75	023	87	031	85
008	90	016	96	024	82	032	80

2. Calculate the measures of central tendency for noradrenaline levels (nmol/L) obtained during a clinical trial involving 15 subjects.

2.5	2.6	2.5	2.4	2.4
2.5	2.5	2.6	2.5	2.6
2.3	2.7	2.3	2.8	2.2

3. Calculate the measures of central tendency for prolactin levels (ng/L) obtained during a clinical trial involving 10 subjects.

9.4	7.0	7.6	6.3	6.7
8.6	6.8	10.6	8.9	9.4

4. In a study designed to measure the effectiveness of a new analgesic agent, 8 mg of drug was administered to 15 laboratory animals. The animals were subjected to the Randall-Selitto paw pressure test and the following results (in grams) were observed.

Number	Response	Number	Response	Number	Response
1	240	6	260	11	265
2	295	7	275	12	240
3	225	8	245	13	260
4	250	9	225	14	275
5	245	10	260	15	250

Calculate the mean, median, variance, and standard deviation for this data.

5. Using Excel or Minitab, report the various measures for central tendency for the 30 samples of tetracycline capsules presented in Table 4.1.

6. Listed below are the results of a first time in human clinical trial of a new agent with 90 mg/tablet administered to six healthy male volunteers. Use Excel or Minitab to report the measures of central tendency for these C_{max} results.

C_{max} for Initial Pharmacokinetic with New Agent

Subject Number	C_{max} (ng/ml)
001	60
002	71
003	111
004	46
005	81
006	96

7. A Midwestern CRO runs a series of IgA analysis for setting specifications. Analytical results from eight different batches are as follows. Report the descriptive statistics for the analyses.

Lot	IgA (mcg/ml)	Lot	IgA (mcg/ml)
1	150	5	117
2	135	6	147
3	141	7	162
4	144	8	141

8. In a Phase I study 40 mg of the active ingredient was administered to twelve healthy male volunteers. The AUC results are as follows:

AUC for Phase I Pharmacokinetic Study

Subject Number	AUC (mg/L·H)
01	1.37
02	1.33
03	1.89
04	1.48
05	1.65
06	1.40
07	1.31
08	1.26
09	1.44
10	1.53
11	1.70
12	1.30

Assuming the data is from a normally distributed population, calculate the measures of central tendency.

6

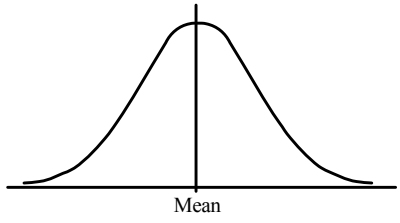
The Normal Distribution and Data Transformation

Described as a "bell-shaped" curve, the normal distribution is a symmetrical distribution that is one of the most commonly occurring outcomes in nature and its presence is assumed in several of the most commonly used statistical tests. Properties of the normal distribution have a very important role in the statistical theory of drawing inferences about population parameters (estimating confidence intervals) based on samples drawn from that population.

There are ways to transform initial data to produce distributions approximating a normal distribution. Various graphic and mathematical methods are available to test for normality.

The Normal Distribution

The normal distribution is the most important distribution in statistics. This curve is a special frequency distribution that describes the population distribution of many continuously distributed biological traits. The normal distribution is often referred to as the **Gaussian distribution**, after the mathematician Carl Friedrich Gauss, even though a formula to calculate a normal distribution was first reported by the French mathematician Abraham DeMoivre in the mid-eighteenth century (Porter, 1986).



It is critical at this point to realize that we are focusing our initial discussion on the *total population, not a sample*. As mentioned in the previous chapter, in the population, the mean is expressed as μ and standard deviation as σ. Sample data ($\overline{X}$ and S) are the best estimates of these population parameters and the distribution of the

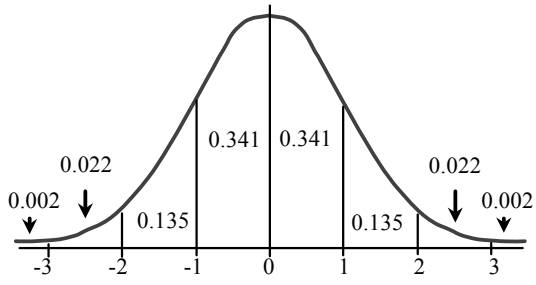


Figure 6.1 Proportions between various standard deviations under a normal distribution.

sample data provides the best estimator of the population distribution.

The characteristics of a normal distribution are as follows. First, the normal distribution is continuous and the curve is symmetrical about the mean. Second, the mode, median, and mean are equal and represent the middle of the distribution. Third, since the mean and median are the same, the 50th percentile is at the mean with an equal amount of area under the curve, above and below the mean. Fourth, the probability of all possible outcomes is equal to 1.0, therefore, the total area under the curve is equal to 1.0. Since the mean is the 50th percentile, the area to left or right of the mean equals 0.5. Fifth, by definition, the area under the curve between one standard deviation above and one standard deviation below the mean contains an area equal to approximately 68% of the total area under the curve. At two standard deviations this area is approximately 95%. Sixth, as distance from the mean (in the positive or negative direction) approaches infinity, the frequency of occurrences approaches zero. This last point illustrates the fact that most observations cluster around the center of the distribution and very few data points occur at the extremes of the distribution. Also, if the curve is infinite in its bounds we cannot set absolute external limits on the distribution.

The frequency distribution (curve) for a normal distribution is defined as follows:

$$f_i = \frac{1}{\sigma\sqrt{2\pi}} e^{-(x_i-\mu)^2/2\sigma^2} \qquad \text{Eq. 6.1}$$

where: π (pi) = 3.14159 and e = 2.71828 (the base of natural logarithms).

In a normal distribution, the area under the curve between the mean and one standard deviation is approximately 34%. Because of the symmetry of the distribution, 68% of the curve would be divided equally above and below the mean. Why 34%? Why not a nice round number like 35%, 30%, or even better, 25%? The standard deviation is that point of inflection on the normal curve where the frequency distribution stops its descent to the baseline and begins to pull parallel with the *x*-axis. Areas or proportions of the normal distribution associated with various standard deviations are seen in Figure 6.1.

The term "*the* bell-shaped curve" is a misnomer since there are many bell-shaped

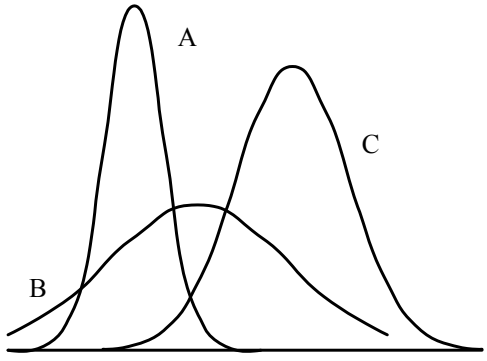


Figure 6.2 Example of three normal distributions with different means and different standard deviations.

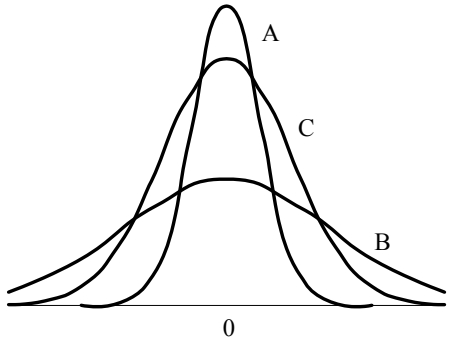


Figure 6.3 Example of three normal distributions with the same mean and different standard deviations.

curves, ranging from those that are extremely peaked with very small ranges to those that are much flatter with wide distributions (Figure 6.2). A normal distribution is completely dependent on its parameters of μ and σ. A standardized normal distribution has been created to compare and compute variations in such a distribution regardless of center or spread from the center. In this standard normal distribution the mean equals 0 (Figure 6.3). The spread of the distribution is also standardized by setting one standard deviation equal to +1 or –1, and two standard deviations equal to +2 or –2 (Figure 6.4).

As seen previously, the area between +2 and –2 is approximately 95%. Additionally, fractions of a standard deviation are calculated and their equivalent areas presented. If such a distribution can be created (with a mean equal to 0 and standard deviation equal to 1) then the equation for the frequency distribution (Eq. 6.1) can be simplified to:

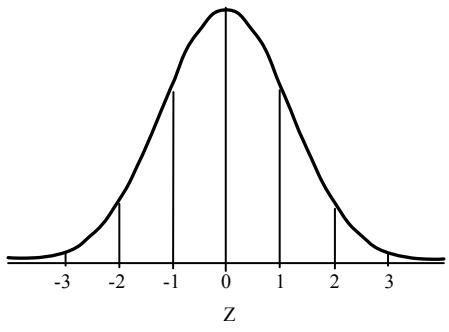


Figure 6.4 Standard normal distribution.

$$f_i = \frac{1}{\sqrt{2\pi}} e^{-(x_i)^2/2}$$

$$f_i = \frac{1}{2.5066272} 2.71828^{-(x_i)^2/2}$$

$$f_i = 1.0844371^{-(x_i)^2/2} \qquad \text{Eq. 6.2}$$

Table 6.1 is an abbreviation of a **standard normal distribution** (a more complete distribution is presented in Table B2 in Appendix B, where every hundredth of the *z*-distribution is defined between 0.01 to 3.69). An important feature of the standard normal distribution is that the number of standard deviations away from the population mean can be expressed as a given percent or proportion of the area of the curve. The symbol *z*, by convention, symbolizes the number of standard deviations away from the population mean. The numbers in these tables represent the area of the curve that falls between the mean (z = 0) and that point on the distribution above the mean (e.g., *z* = +1.5, would be the point at 1.5 standard deviations above the mean). Since the mean is the 50th percentile, the area of the curve that falls below the mean (or below zero) is 0.5000. Because a normal distribution is symmetrical, this table could also represent the various areas below the mean. For example, for *z* = –1.5 (or 1.5 standard deviations below the mean), *z* represents the same area from 0 to –1.5, as the area from 0 to +1.5. A *z*-value tells us how far above and below the mean any given score is in units of the standard deviation.

Using the information in Table 6.1, the area under the curve that falls below +2 would be the area between +2 and 0, plus the area below 0.

Area (< +2) = Area (between 0 and +2) + Area (below 0)
Area (< +2) = 0.4772 + 0.5000 = 0.9772

These probabilities can be summed because of the addition theorem discussed in Chapter 2.

Table 6.1 Selected Areas of a Normal Standardized Distribution (Proportion of the Curve between 0 and *z*)

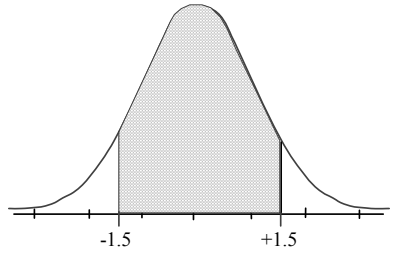
z	Area	z	Area	z	Area
0.00	0.0000	1.00	0.3413	2.00	0.4772
0.05	0.0199	1.05	0.3531	2.05	0.4798
0.10	0.0398	1.10	0.3543	2.10	0.4821
0.15	0.0596	1.15	0.3749	2.15	0.4842
0.20	0.0793	1.20	0.3849	2.20	0.4861
0.25	0.0987	1.25	0.3944	2.25	0.4878
0.30	0.1179	1.30	0.4032	2.30	0.4893
0.35	0.1368	1.35	0.4115	2.35	0.4906
0.40	0.1554	1.40	0.4192	2.40	0.4918
0.45	0.1736	1.45	0.4265	2.45	0.4929
0.50	0.1915	1.50	0.4332	2.50	0.4938
0.55	0.2088	1.55	0.4394	2.55	0.4946
0.60	0.2257	1.60	0.4452	2.60	0.4953
0.65	0.2422	1.65	0.4505	2.65	0.4960
0.70	0.2580	1.70	0.4554	2.70	0.4965
0.75	0.2734	1.75	0.4599	2.75	0.4970
0.80	0.2881	1.80	0.4641	2.80	0.4974
0.85	0.3023	1.85	0.4678	2.85	0.4978
0.90	0.3159	1.90	0.4713	2.90	0.4981
0.95	0.3289	1.95	0.4744	2.95	0.4984

All possible events would fall within this standard normal distribution ($p\sum(x) = 1.00$). Since the probability of all events equals 1.00 and the total area under the curve equals 1.00, then various areas within a normalized standard distribution can also represent probabilities of certain outcomes. In the above example, the area under the curve below two standard deviations (represented as +2) was 0.9972. This can also be thought of as the probability of an outcome being less than two standard deviations above the mean. Conversely, the probability of being two or more standard deviations above the mean would be 1.0000 – 0.9772 or 0.0228.

Between three standard deviations above and below the mean, approximately 99.8% of the observations will occur. Therefore, assuming a normal distribution, a quick method for roughly approximating the standard deviation is to divide the range of the observations by six, since almost all observations will fall within these six intervals. For example, consider the data in Table 4.3. The true standard deviation for this data is 16.8 mcg. The range of 86 mcg, divided by six would give a rough approximation of 14.3 mcg for the standard deviation (the actual *S* is 16.8 mcg as seen in Figure 5.7).

It is possible to calculate the probability of any particular outcome within a normal distribution. The areas within specified portions of our curve represent the probability of the values of interest lying between the vertical lines. To illustrate this, consider a large container of tablets (representing a total population) that is expected to be normally distributed with respect to the tablet weight. What is the probability of

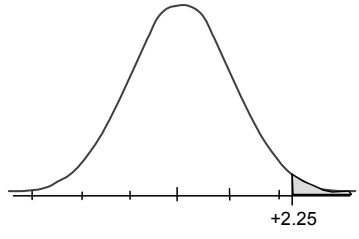
randomly sampling a tablet that weighs within 1.5 standard deviations of the mean?



Because weight is a continuous variable, we are concerned with p(> −1.5 or < +1.5). From Table 6.1:

p(z < +1.5) = Area between 0 and +1.5 =	0.4332
p(z > −1.5) = Area between 0 and −1.5 =	0.4332
p(z −1.5 to +1.5) =	0.8664

There is a probability of 0.8664 (or 87% chance) of sampling a tablet within 1.5 standard deviations of the mean. What is the probability of sampling a tablet greater than 2.25 standard deviations above the mean?



First, we know that the total area above the mean is 0.5000. By reading Table 6.1, the area between 2.25 standard deviations (z = 2.25) and the mean is 0.4878 (the area between 0 and +2.25). Therefore the probability of sampling a tablet weighing more than 2.25 standard deviations above the mean weight is:

$$p(z > +2.25) = 0.5000 - 0.4878 = 0.0122$$

If we wish to know the probability of a tablet being less than 2.25 standard deviations above the mean, the complement probability of being less than a *z*-value of +2.25 is:

$$p(z < +2.25) = 1 - p(z > +2.25) = 1.000 - 0.0122 = 0.9878$$

Also calculated as:

$$p(z < +2.25) = p(z < 0) + p(z < 2.25) = 0.5000 + 0.4878 = 0.9878$$

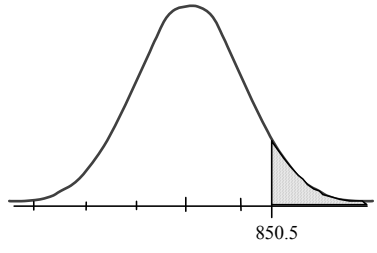
If the mean and standard deviation of a population are known, the exact location (z above or below the mean) for any observation can be calculated using the following formula:

$$z = \frac{x - \mu}{\sigma} \qquad \text{Eq. 6.3}$$

Because values in a normal distribution are on a continuous scale and are handled as continuous variables, we must correct for continuity. Values for x would be as follows for some arbitrary measures in mg:

Likelihood of being: greater then 185 mg =	p(>185.5)
less than 200 mg =	p(<199.5)
200 mg or greater =	p(>199.5)
between and including 185 and 200 mg =	p(>184.5 and <200.5)

To examine this, consider a sample from a known population with expected population parameters (previous estimates of the population mean and standard deviation, for example based on prior production runs for a specific hard shell capsule). With an expected population mean assay of 750 mg and a population standard deviation of 60 mg, what is the probability of sampling a capsule with an assay greater than 850 mg?

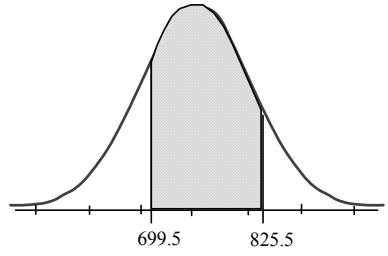


As a continuous variable, the p(>850 mg) is actually p(>850.5 mg) when corrected for continuity.

$$z = \frac{x - \mu}{\sigma} = \frac{850.5 - 750}{60} = \frac{100.5}{60} = +1.68$$

$$p(z > +1.68) = 0.5000 - p(z < 1.68) = 0.5000 - 0.4535 = 0.0465$$

Given the same population as above, what is the probability of randomly sampling a capsule with an assay between 700 and 825 mg?



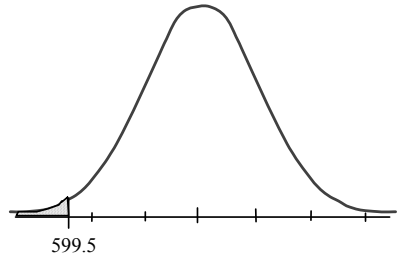
Once again correcting for continuity, p(<825 mg) is rewritten as p(<825.5 mg) and p(>700 mg) is really p(>699.5 mg).

$$z = \frac{x - \mu}{\sigma} = \frac{825.5 - 750}{60} = \frac{75.5}{60} = +1.26$$

$$z = \frac{x - \mu}{\sigma} = \frac{699.5 - 750}{60} = \frac{-50.5}{60} = -0.84$$

p(between 699.5 and 825.5) = p(z < +1.26) + p(z > –0.84)
= 0.3962 + 0.2995 = 0.6957

Given the same population, what is the probability of randomly sampling a capsule with an assay less than 600 mg?



As a continuous variable, p(<600 mg) is p(>599.5 mg):

$$z = \frac{x - \mu}{\sigma} = \frac{599.5 - 750}{60} = \frac{-150.5}{60} = -2.5$$

p(z < –2.5) = 0.5000 – p(z < 2.5) = 0.5000 – 0.4938 = 0.0062

Thus, in these examples with the given population mean and standard deviation, the likelihood of randomly sampling a capsule greater than 850 mg is approximately 5%, a capsule less than 600 mg is less than 1%, and a capsule between 700 and 825 mg is almost 70%.

Lastly, the probability of obtaining any one particular value is zero, but we can determine probabilities for specific ranges. Correcting for continuity, the value 750 (the mean) actually represents an infinite number of possible values between 749.5 and 750.5 mg. The area under the curve between the center and the upper limit would be

$$z = \frac{x - \mu}{\sigma} = \frac{750.5 - 750}{60} = \frac{0.5}{60} = +0.01$$

$$p(z < 0.01) = 0.004$$

Since there would be an identical area between 749.5 and the mean, the total proportion associated with 750 mg would be

$$p(750 \text{ mg}) = 0.008$$

In the previous examples we knew both the population mean (μ) and the population standard deviation (σ). However, in most statistical investigations this information is not available and formulas must be employed that use estimates of these parameters based on the sample results.

Important *z*-values related to other areas under the curve for a normal distribution include:

$$90\% \; -1.64 < z < +1.64$$
$$95\% \; -1.96 < z < +1.96$$
$$99\% \; -2.57 < z < +2.57$$

Determining if the Distribution is Normal

The sample used in a study is our best guess of the characteristics of the population: the center, the dispersion, and the shape of the distribution. Therefore, the appearance of the sample is our best estimate whether or not the population is normally distributed. In the absence of any information that would disprove normality, it is assumed that a normal distribution exists (e.g., initial sample does not look extremely skewed or bimodal).

One quick method to determine if the population is normally distributed is to determine if the sample mean and median are approximately equal. If they are about the same (similar in value) then the population probably has a normal distribution. If the mean is substantially greater than the median, the population is probably positively skewed and if the mean is substantially less than the median, a negatively skewed population probably exists.

Normality can be estimated visually by looking at a histogram or box plot of the sample data, or by plotting data on graph paper or probability paper. Using a **box-and-whisker plot** (described in Chapter 4), it would reflect a normally distributed population if the top and bottom lines of the box plot (25th and 75th percentiles) were approximately equidistant from the center line (the median). Visually inspecting a

histogram can indicate if the distribution for sample data is approximately normal, with most of the results clustering near the center and few observations at each end of the distribution. A **scatter plot** (also described in Chapter 4) can use sample data to plot a theoretical cumulative distribution function (cdf) on the *x*-axis against actual cumulative distribution functions on the *y*-axis. If normality exists a straight line will be produced. Finally a scatter plot reflecting a **quantile-by-quantile plot,** with the expected cumulative distributions by quintiles for a normal distribution on one axis and of the quantiles for the observed sample data on the other axis, should create a 45 degree line. The quantile-by-quantile plot could be used in a similar manner for other well-defined distributions.

Two other similar visual methods to determine if sample data is consistent with expectations for a normal distribution are to plot a **cumulative frequency curve** using normal graph paper or a **normal probability plot** using special graph paper known as **probability paper.** In a cumulative frequency curve, data is arranged in order of increasing size and plotted on normal graph paper:

$$\%\, cumulative\ \ frequency = \frac{cumulative\ \ frequency}{n} \times 100$$

If the data came from a normally distributed population, the result will be an S-shaped curve.

Probability paper (e.g., National #12-083 or Keuffel and Esser #46-8000) has a unique nonlinear scale on the cumulative frequency axis that will convert the S-shaped curve to a straight line. The normal probability plot or P-P plot will produce a 45 degree straight line that can be drawn through the percent cumulative frequency data points if the estimated population is normally distributed. If a curvilinear relationship exists, the population distribution is skewed. Using the distribution presented in Table 6.2, Figure 6.5 illustrates the data presented as a: a) box-and-whisker plot; b) histogram; 3) cumulative frequency curve; and 4) probability plot.

The skew of a distribution also can be estimated numerically using the formula:

$$Skew = \frac{n}{(n-1)(n-2)} \cdot \sum \left(\frac{x_i - \overline{X}}{s} \right)^3 \qquad 6.4$$

A value close to zero indicates symmetric data. If the results are negative it indicates a negative skew and positive a positive skew. The larger the value (negative or positive), the greater the skew. One rule of thumb for the significance of skew is provided by Bulmer (1967): highly skewed data if greater than +1 or less than –1; moderate skew if between 0.5 and 1.0 (positive or negative); and approximately symmetrical if less than +0.5 or more than –0.5. For the data presented in Table 6.2 the distribution would be considered approximately symmetrical.

$$Skew = \frac{100}{(99)(98)} \cdot \left(\left(\frac{112 - 124.95}{4.63} \right)^3 + \ldots \left(\frac{140 - 124.95}{4.63} \right)^3 \right) = 0.38$$

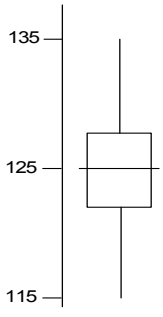
Table 6.2. Assay Results for 100 Randomly Sampled Tablets

Tablet Assay	f	cf
112	1	1
116	1	2
117	2	4
118	2	6
119	4	10
120	5	15
121	7	22
122	8	30
123	8	38
124	10	48
125	11	59
126	7	66
127	8	74
128	6	80
129	6	86
130	3	89
131	3	92
132	2	94
133	2	96
134	1	97
135	1	98
137	1	99
140	1	100

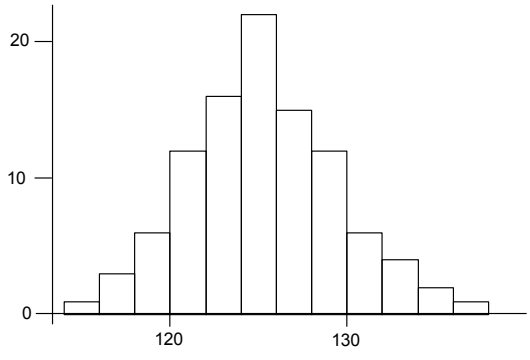
As mentioned in Chapter 4, **kurtosis** is the characteristic of a frequency distribution that refers to the shape of the distribution of values regarding its relative flatness and peakedness. It indicates the extent to which a distribution is more peaked or flat-topped than a normal distribution. For the normal distribution, the theoretical kurtosis value equals zero and the distribution is described as **mesokurtic**. If the distribution has long tails (relatively larger tails), the statistic will be greater than zero and called **leptokurtic**. One estimate of kurtosis from sample data is:

$$Kurtosis = \frac{n(n+1)}{(n-1)(n-2)(n-3)} \cdot \sum \left(\frac{x_i - \overline{X}}{s} \right)^4 - \frac{3(n-1)^2}{(n-2)(n-3)} \quad \text{Eq. 6.5}$$

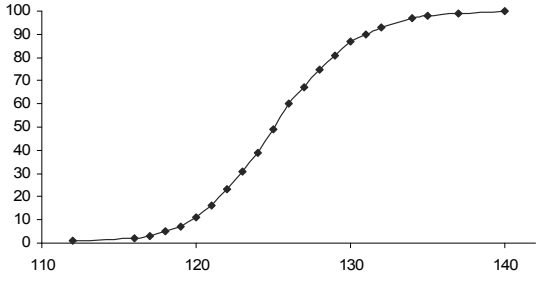
A rule-of-thumb kurtosis should be within the +3 to –3 range when the data are normally distributed. Negative kurtosis means there are too many cases in the tails of the distribution; whereas a positive kurtosis reflects too few cases in the tails. Using the data presented in Table 6.2, the mean is 124.95 with a standard deviation of 4.63 and the kurtosis is:



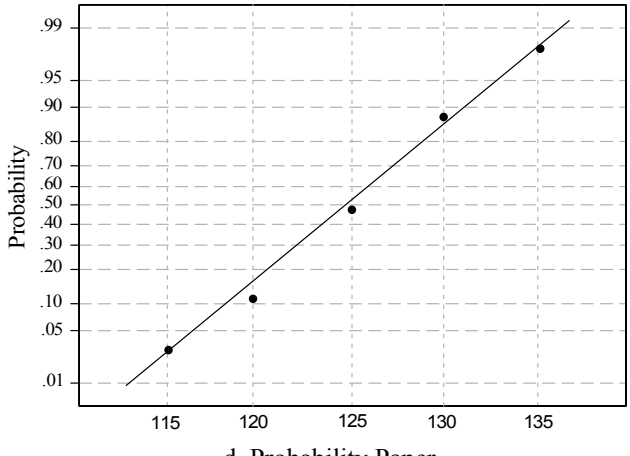
a. Box-and Whisker Plot



b. Histogram



c. Cumulative Frequency Curve



d. Probability Paper

Figure 6.5 Various visual methods for determining normality.

$$Kurtosis = \frac{100(99)}{(99)(98)(97)} \cdot \left(\left(\frac{112 - 124.95}{4.63} \right)^4 + \ldots \left(\frac{140 - 124.95}{4.63} \right)^4 \right) - \frac{3(`100 - 1)^2}{(98)(97)}$$

$$Kurtosis = 0.855$$

The result is well below a +3 and one can assume normality, which is similar to the visual results observed in Figure 6.5.

Several statistical procedures exist to test for population normality based on sample data. These tests include: 1) chi-square goodness-of-fit test; 2) the Kolmogorov-Smirnov D test; 4) Anderson-Darling test; 5) the Lilliefors test and 6) Shapiro-Wilk W test. Both the **chi-square goodness-of-fit test** and **Kolmogorov-Smirnov (K-S) test** will be described in Chapters 16 and 21, respectively, with fully worked out examples. The Anderson-Darling test is covered in detail in Chapter 21.

Both the **Anderson-Darling test** and **Lilliefors test** are modifications of the K-S test and the latter is sometimes referred to as the **Kolmogorov-Smirnov Lilliefors test**. Without the Lilliefors correction, the K-S test is more conservative (greater likelihood of rejecting a normality). The Anderson-Darling test gives more weight to the tallies of the distribution than the K-S test. When sample size is large, one should be cautious because even small deviations from normality can be significant with the K-S test or chi-square goodness-of-fit tests. Information about these tests can be found in D'Agostino and Stephens (1986).

The **Shapiro-Wilk W test** is another standard test for normality and is recommended for smaller sample sizes (2000 or less). It is conducted by regressing the quantiles of the observed data against that of the best-fit for the normal distribution. The Shapiro-Wilk *W*-statistic is calculated as follows.

$$W = \frac{\left(\sum a_i x_i\right)^2}{\sum \left(x_i - \overline{X}\right)^2} \qquad \text{Eq. 6.6}$$

where the x_i's are the ordered sample values ($x_{(1)}$ is the smallest) and the a_i's are coefficients from a table, based on the means and variances of the ordered statistics of a sample from a normal distribution (Shapiro and Wilk, 1965). The resultant W-statistic (ranging from 0 to 1) is then compared to a table of critical values. Computer software computes the W-statistic and corresponding p-value. A significant statistic (small values for W, with corresponding p-value <0.05) would result in rejecting the assumption that the sample comes from a normally distributed population.

All of the previously mentioned tests are classified as **empirical distribution function** statistics or EDF tests. They involve making a good guess of the "true distribution function" (in this case a normal distribution) and by using the observed results from a random sample. Graphs can be constructed, where the empirical distribution function, *S(x)*, is always a step function and each step has a height *1/n*. These graphs and/or the above statistics are computed to determine the amount of discrepancy between the theoretical, *F(x)* distribution and the experimental (empirical) results.

Data Transformations: An Overview

A normal distribution is defined by its mean (μ) and its standard deviation (σ). When a sample is taken from a normal distribution population, $\overline{X}$ and S summarize all of the information available in the sample about the parent distribution. Unfortunately, the ability to use $\overline{X}$ and S as summary statistics does not apply to nonnormal distributions. Certain inferential statistics, such as confidence intervals (Chapter 7), cannot be applied, and if applied, intervals tend to be wider and tests of hypothesis (Chapter 8) have less power. Also, as will be seen in future chapters, inferential statistical tests are based on assumptions and the validity of results obtained from these tests will depend on how well these assumptions were met. One assumption for several commonly used tests (e.g., t-test, F-test, correlation) is that the data is sampled from a normally distributed population. As seen in the previous section it is possible graphically or through statistical procedures to determine if the population (via sample results) is normally distributed. What if the population is assumed to be nonnormal in its distribution? Through the use of various transformation procedures, nonnormally distributed data can be altered to create a new distribution that approximates the symmetric bell-shaped curve of a normal distribution. We have already seen an example of data transformation when we created a standard normal distribution where a normal distribution was created with a mean of 0 and a standard deviation of 1. Distributions were standardized by changing the original data points to standard scores (z-scores) using Eq. 6.3.

Lognormal Transformation and the Geometric Mean

The most commonly encountered transformations are involved with populations that appear to be positively skewed. In this case, **logarithmic** or **lognormal transformations** are used when most of the values are to the left of the distribution (near zero) and few values are in the right side of the curve. This process involves converting each number to its logarithmic form:

$$x_i' = log(x_i) \qquad \text{Eq. 6.7}$$

Logarithms in base 10 are usually used, but any base would be satisfactory. Use of data transformations should make theoretical sense. Note that the log of zero is undefined and will lead to error messages. If zeros are present in the sample, one can pick an arbitrary small value (e.g., 0.0001) and replace all zeros with that value. Some statisticians prefer the following equation based on theoretical grounds and it is preferred when dealing with small numbers of observations. Also, this equation removes the problem of dealing with zeros:

$$x_i' = log(x_i + 1) \qquad \text{Eq. 6.8}$$

Either transformation can be used if the variable effects are multiplicative rather than additive.

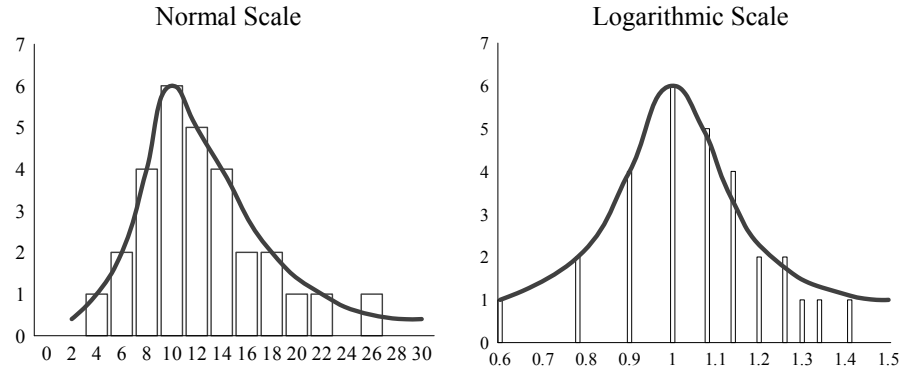


Figure 6.6 Comparisons of data on normal and logarithmic scales.

For calculating the center of the logarithmic transformation of data, the **geometric mean** is reported. Because of the few extreme values to the right of the curve, the arithmetic mean ($\overline{X}$) would be "pulled" to the right. Performing a logarithmic transformation would produce a distribution that is approximately a normal distribution and the final mean will be more to the "left" and thus closer to the median of the original distribution.

This process involves converting each number to its logarithmic form (Eq. 6.7 or Eq. 6.8). These values are summed and divided by the total number of observations to produce an average logarithmic value. This value is then converted back to real numbers (original units of measure) by taking the antilog, which represents the geometric mean.

$$\overline{X}_G = antilog\left(\frac{\sum log(x_i)}{n}\right) \qquad \text{Eq. 6.9}$$

Illustrated below are two sets of identical data, with the left on a normal scale and the right using a logarithmic scale (Figure 6.6). Notice the line on the normal scale appears to be skewed while on the logarithmic scale it appears to be more bell-shaped. A positively skewed distribution, as seen above, is often referred to as a **log-normal distribution**.

To illustrate this process, consider the T_{max} data observed in 12 health volunteers, which appears in Table 6.3. The arithmetic mean ($\overline{X}$) is 2.33 and the median is 1.65. The few extreme scores in this skewed distribution have pulled the mean to the right of the median. The conversion to a logarithmic transformation of scores is seen in the last column of Table 6.3. The mean for the logarithmic transformed scores is:

$$Average\ \ log = \frac{0.079 + 0.146 + ... 0.857}{12} = 0.304$$

Table 6.3 T_{max} Results in Ascending Order

Subject	T_{max}	Log Transformation
3	1.2	0.079
5	1.4	0.146
9	1.5	0.176
10	1.5	0.176
12	1.6	0.204
1	1.6	0.204
6	1.7	0.230
2	1.8	0.255
8	2.1	0.322
4	2.6	0.415
11	3.8	0.580
7	7.2	0.857
$\sum =$	28.0	3.644

Converted back to the antilog, the geometric mean is:

$$\overline{X}_G = antilog\ (0.304) = 2.01$$

If Eq. 6.8 were used to calculate the log values, the geometric mean would be reported as the antilog of the mean minus one. Notice that the geometric mean is much closer to the median of the original distribution of T_{max} data. An alternate formula for calculating the geometric mean is to take the *nth* root of the product of all the observations:

$$\overline{X}_G = \sqrt[n]{product\ of\ all\ data} \qquad \text{Eq. 6.10}$$

This gives the same result as the logarithmic transformation.

$$\overline{X}_G = \sqrt[12]{1.2 \times 1.4 \times 1.5 \times \ldots 7.2} = 2.01$$

If Eq. 6.9 were used, one would be added to each value and one subtracted from the final result.

Excel calculates the geometric mean by using the Function option **GEOMEAN**.

Other Types of Transformations

The **square-root transformation** is similar to the lognormal transformation and useful for more positively skewed data. The transformed data is the square root of each original measurement and is used when the data consist of counts.

$$x_i' = \sqrt{x_i} \qquad \text{Eq. 6.11}$$

or

$$x_i' = \sqrt{x_i + 0.5} \quad \text{Eq. 6.12}$$

The square-root transformation is useful when the sample means are approximately proportional to the variances of the samples. Using the second equation (Eq. 6.12) avoids potential problems with zeros in the data and is useful for small data sets. This can be helpful for Poisson distributions, for example, counts associated with rare events such as number of defects in a production run.

The **reciprocal transformation** is also for positively skewed data. Also called **inverse transformation**, this can be used when standard deviation is proportional to the square root of the mean and data is clustering near zero:

$$x_i' = \frac{1}{x_i} \quad \text{Eq. 6.13}$$

or

$$x_i' = \frac{1}{x_i + 1} \quad \text{Eq. 6.14}$$

For example, a few patients may take a very long time to respond to a given therapy and cause a skewed distribution. The reciprocal transformation helps make this type of data more symmetrical. Thus, there are three transformations that can be used to normalize positively skewed data (logarithmic, square root, and reciprocal transformations). The inverse (reciprocal) transformation can be used for the most extreme cases of positive skewing. For less severely skewed data the recommended transformation is logarithmic and the square root for more positively skewed distribution. For Poisson distributions it is recommended to normalize the distribution using the square root transformation.

Theoretically, proportions for binomial distribution are approximately normally distributed when p is near 0.50. However, as p goes to extremes (0 to 20% and 80 to 100%), normality is lost. If the square root of each proportion in a binomial distribution is transformed to its arcsine, the resultant proportions p' will have a distribution that is approximately normal.

$$p' = arcsin\sqrt{p} \quad \text{Eq. 6.15}$$

This transformation is referred to as the **arcsine transformation**, **arcsine square root transformation**, **angular transformation,** or **inverse sine tranformation**. This transformation is used only when the data are proportions or percents.

For data that is negatively skewed (tailing to the left), subtract each data point from the largest data point and add one to each resulting value. This will result in a positively skewed distribution. This positively skewed distribution, based on the severity of the skew, can be transformed using square root, logarithmic, or inverse transforms. If the data is negatively skewed for proportional data ($0 \leq p \leq 1$) a

different log transformation equation can be employed:

$$x_i' = log\left(\frac{x}{1-x}\right) \qquad \text{Eq. 6.16}$$

If transformations are used to modify the data to produce data that assumes the shape of a normal distribution, then mathematical manipulations for the subsequent statistical test are performed on the transformed data, not the original data.

Using Excel® or Minitab® for Evaluating Normality

Excel has function options for both kurtosis (**KURT**) and skew (**SKEW**) using Eq. 6.4 and 6.5. Both kurtosis and skew also are reported as part of the descriptive statistics option under data analysis (see Figure 5.7).

Data ➤ Data Analysis ➤ Descriptive Statistics ➤ Summary Statistics

For Minitab skew and kurtosis can both be evaluated as part of the display descriptive statistics options in Figure 5.8.

Stat ➤ Basic Statistics ➤ Display Description Statistics

For the data presented in Table 6.2 the Minitab summary would look as follows:

Descriptive Statistics: Assay

Variable	N	Mean	StDev	Skewness	Kurtosis
Assay	100	124.95	4.63	0.38	0.86

Minitab provides three tests to evaluate normality:1) Anderson Darling; 2) Kolmogorov-Smirnov and 3) the Ryan-Joiner test which is similar to the Shapiro-Wilk W test. These are located under "Normality Test" under the Basic Statistics menu:

Stat ➤ Basic Statistics ➤ Normality Test

All produce a graphic result and data are evaluated based on their proximity to a straight line on probability paper as presented in Figure 6.7 for the Anderson Darling evaluation of the data in Table 6.2. The important feature is the p-value to the right of the graph. An explanation of the meaning of the p-value will be discussed in the following chapter. For the current discussion, assume that the distribution is symmetrical if the $p > 0.05$.

In addition Minitab can perform the Anderson Darling normality test as part of the results when the "Graphical Summary" option is selected from the basic statistics menu:

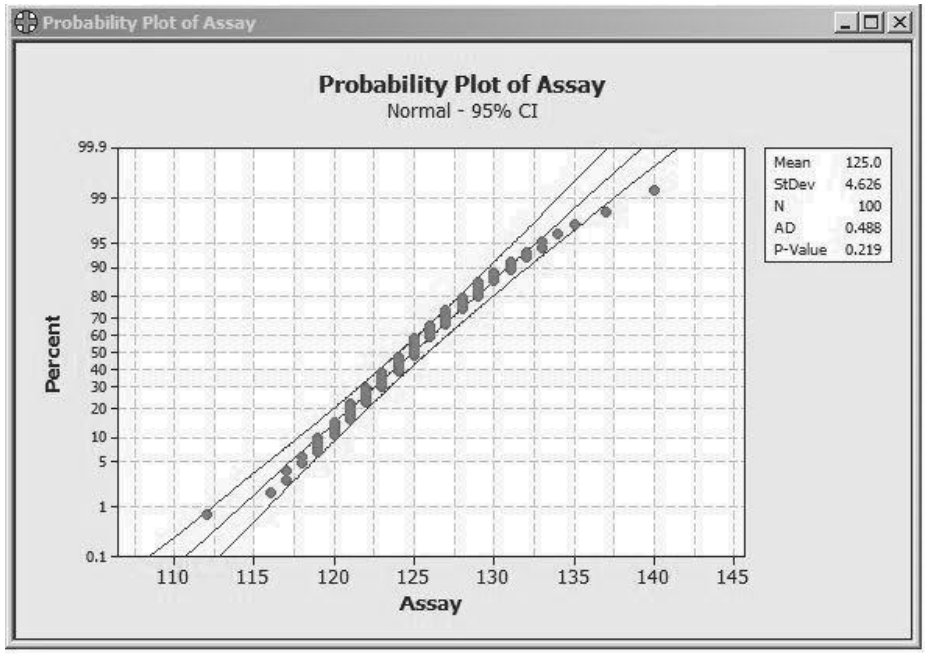


Figure 6.7 Anderson Darling graphic display with Minitab.

Stat ➤ Basic Statistics ➤ Graphical Summary

Figure 6.8 displays the results for the data in Table 6.2.

References

Bulmer, M. G. (1967), *Principles of Statistics*, MIT Press, Cambridge, MA, p. 63.

D'Agostino, R. and Stephens, M. (1986). *Goodness-of-Fit Techniques*. Marcel Dekker, New York, pp. 102-184, 372-373.

Kachigan, S.K. (1991). *Multivariate Statistical Analysis*, Second edition, Radius Press, New York, pp. 89-90.

Porter, T.M. (1986). *The Rise of Statistical Thinking*, Princeton University Press, Princeton, NJ, p. 93.

Shapiro, S.S. and Wilk, M.B. (1965). "An analysis of variance test for normality (complete samples)," *Biometrika* 52 (3 and 4): 591-611.

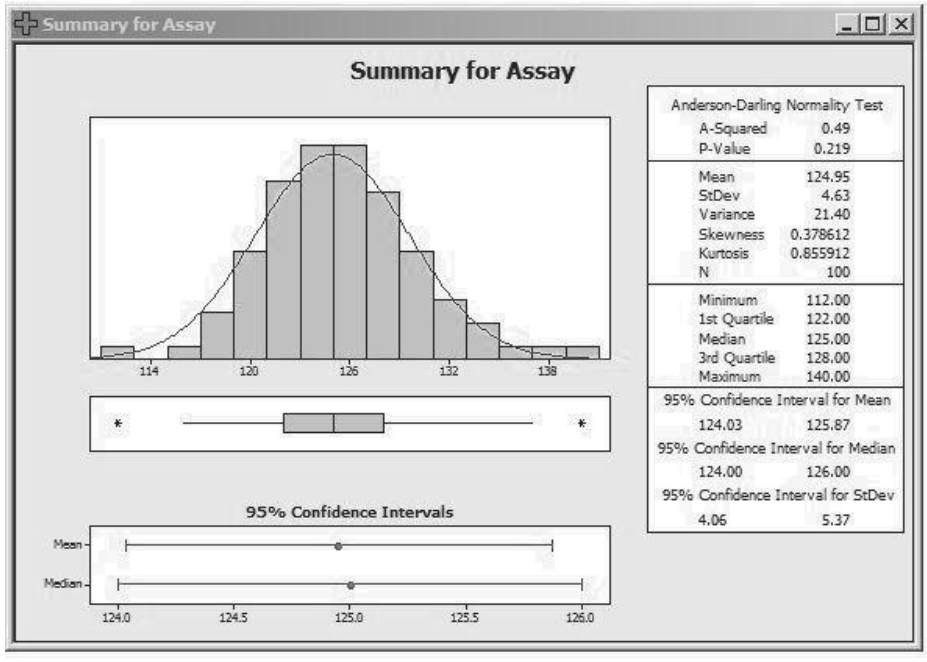


Figure 6.8 Results of a graphical summary with Minitab.

Suggested Supplemental Readings

Box, G.E.P. and Cox, D.R. (1964).“An analysis of transformations,” *Journal of the Royal Statistical Society*, B 26: 211-234.

Natrella, M.G. “The use of transformations,” *Experimental Statistics*, National Bureau of Standards Handbook 9, U.S. Department of Commerce, Washington, DC, 1963, pp. 20.1-20.13.

Stephens, M.A. (1974). “EDF statistics for goodness of fit and some comparisons,” *Journal of the American Statistical Association* 69(347): 730-737.

Example Problems (Answers are provided in Appendix D)

1. Listed in Table 6.4 are the times to maximum concentration observed during a clinical trial. It is believed that the data is positively skewed. Calculate the median, mean, and geometric mean. Based on the sample, does the population appear to be positively skewed?

2. Recalculate the data in Table 6.4 using the square root and reciprocal transformation methods. Calculate the transformed mean and then transform the mean back into the original units of measure.

Table 6.4 Clinical Trial Results - T_{max} (in hours)

Subject	t_{max}	Subject	t_{max}	Subject	t_{max}
A	1.41	F	1.96	K	1.62
B	1.81	G	0.78	L	1.15
C	3.25	H	1.51	M	2.03
D	1.37	I	1.18	N	2.21
E	1.09	J	2.56	O	0.91

3. Repeat question 8 in Chapter 5 assuming the data is positively skewed and calculate the geometric mean for the sample data.

7

Confidence Intervals and Tolerance Limits

Intervals can be created to estimate population characteristics based on sample data. A confidence interval estimate the true population mean based on the best estimator available, the sample means. Although we will never know the exact population mean we can create a range of possible values for the mean and know that the population mean is located within that interval. Similarly, we can use tolerance limits to once again use the sample mean and sample standard deviation to estimate range within which we would expect to find a certain percentage of the observations. In both cases, we can never be 100% certain of our results, but can assume we are correct with a certain amount of confidence in the intervals we create.

Sampling Distribution

If we have a population and withdraw a random sample of observations from that population, we could calculate a sample mean and a sample standard deviation. As mentioned previously, sample statistics would be our best estimates of the true population parameters.

$$\overline{X}_{\text{sample}} \approx \mu_{\text{population}}$$
$$S_{\text{sample}} \approx \sigma_{\text{population}}$$

The characteristics of dispersion or variability are not unique to samples alone. Individual samples can also vary around the population mean. Just by chance, or luck, we could have sampled from the upper or lower ends of the population distribution and calculated a sample mean that was too high or too low. Through no fault of our own, our estimate of the population mean would be erroneous.

To illustrate this point, let us return to the pharmacokinetic data used in Chapter 4. From this example, we will assume that the data in Table 4.3 represented the *entire population* of pharmacokinetic studies ever conducted on this drug. Due to budgetary restraints or time, we were only able to analyze five samples from this population. How many possible ways could five samples be randomly selected from this data? Based on the combination formula (Eq. 2.11) there would be

Table 7.1 Possible Samples from Population Presented as Table 4.3

	Sample A	Sample B	Sample C	Sample D
	706	731	724	778
	714	760	752	785
	718	752	762	788
	720	736	734	790
	724	785	775	793
Mean =	716.4	752.8	749.4	786.8
S.D. =	6.8	21.5	20.6	5.7

$$\binom{125}{5} = \frac{125!}{5!\,120!} = 234{,}531{,}275$$

possible ways. Thus, it is possible to sample these 125 values in over 234 million different ways and because they are sampled at random, each possible combination has an equal likelihood of being selected. Therefore, by chance alone we could sample the smallest five values in our population (Sample A) or the largest five (Sample D) or any combination between these extremes (Table 7.1). Samples B and C were generated using the Random Numbers Table B1 in Appendix B.

The mean is a more efficient estimate of the center, because with repeated samples of the same size from a given population, the mean will show less variation than either the mode or the median. Statisticians have defined this outcome as the central limit theorem and its derivation is beyond the scope of this book. However, there are three important characteristics that will be utilized in future statistical tests.

1. The mean of all possible sample means is equal to the mean of the original population from which they were sampled.

$$\overline{X}_{\overline{X}} = \mu \qquad \text{Eq. 7.1}$$

   If we averaged all 234,531,275 possible sample means, this grand mean or **mean of the mean** would equal the population mean (μ = 752.4 mcg for N = 125) from which they were sampled.

2. The standard deviation for all possible sample means is equal to the population standard deviation divided by the square root of the sample size.

$$\sigma_{\overline{X}} = \frac{\sigma}{\sqrt{n}} \qquad \text{Eq. 7.2}$$

   Similar to the mean of the sample means, the standard deviation for

all the possible means would equal the population standard deviation divided by the square root of the sample size. The standard deviation for the means is referred to as the **standard error of the mean** or **SEM**.

3. Regardless of whether the population is normally distributed or skewed, if we plot all the possible sample means, the frequency distribution will approximate that of a normal distribution, based on the **central limit theorem**. This theorem is critical to many statistical formulas because it justifies the assumption of normality. This will approximate a normal distribution, regardless of the distribution of the original population, when the sample size is relatively large. To demonstrate this point, Figure 7.1 illustrates the distribution of sample means (n = 3) resulting from a normal, skewed and rectangular distribution. All three resultant possible means take on an approximate normal distribution. As the sample size increases the distribution becomes even more Gaussian. In fact, a sample size as small as n = 30 will often result in a near-normal sampling distribution (Kachigan, 1991).

If all 234,531,275 possible means were plotted, they would produce a frequency distribution that is normally distributed. Because the sample means are normally distributed, values in the normal standardized distribution (z distribution) will also apply to the distribution of sample means. For example, of all the possible sample means:

68% fall within + or – 1.00 SEM
90% fall within + or – 1.64 SEM
95% fall within + or – 1.96 SEM
99% fall within + or – 2.57 SEM

The distribution of the mean will be a probability distribution, consisting of various values and their associated probabilities, and if we sample from any population, the resultant means will be distributed on a normal bell-shaped curve. Most will be near the center and 5% will be outside 1.96 standard errors of the distribution.

Standard Error of the Mean versus the Standard Deviation

As seen in the previous section, in a sampling distribution, the overall mean of the means would be equal to the population mean and the dispersion would depend on the amount of variance in the population. Obviously, the more we know about our population (the larger the sample size), the better our estimate of the population center. The best estimate of the population standard deviation is the sample standard deviation, which can be used to replace the σ in Eq. 7.2 to produce an estimate of the standard error of the mean based on sample data:

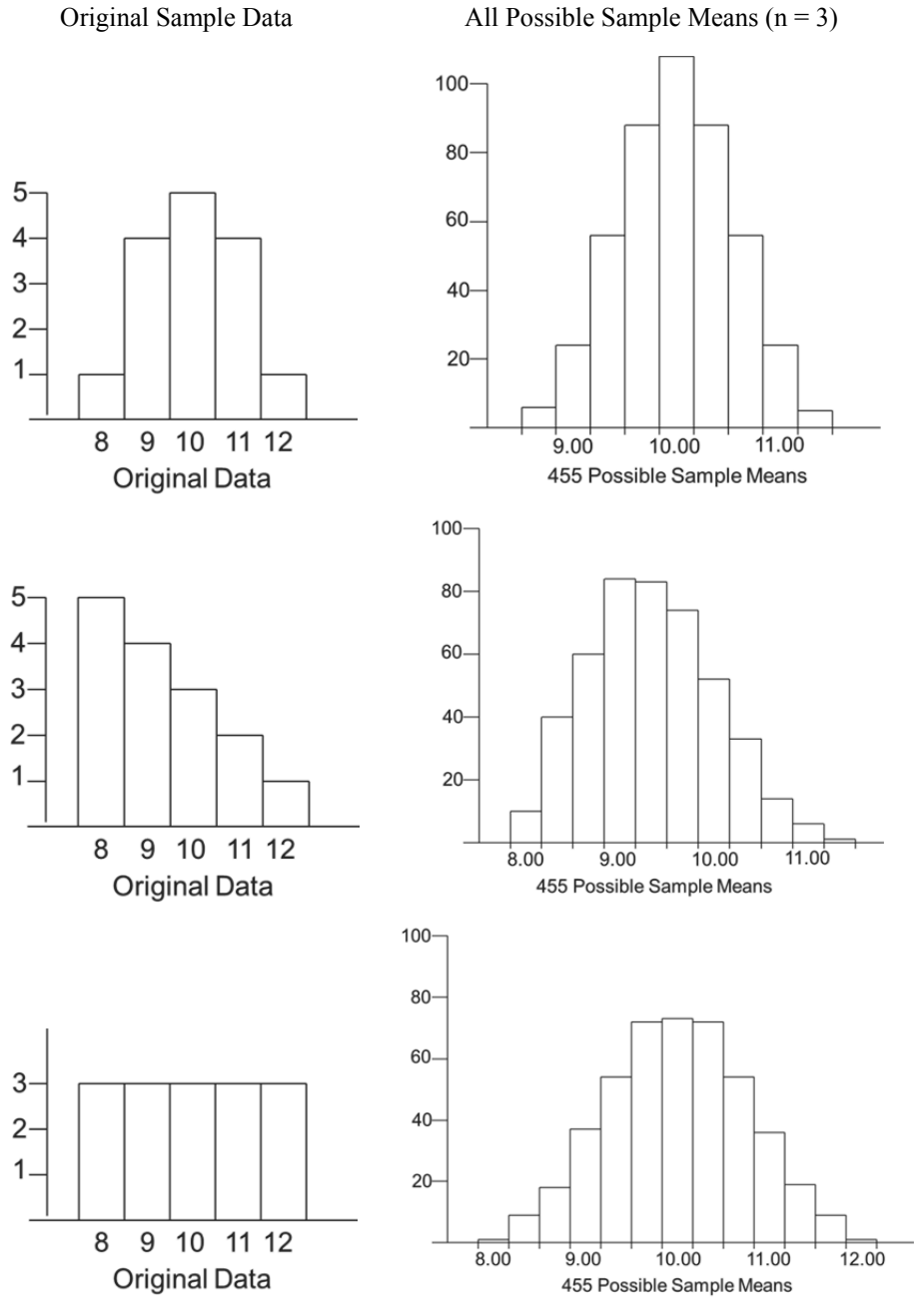


Figure 7.1 Examples of all possible sample means.

$$S_{\bar{x}} = \frac{S}{\sqrt{n}} = SEM \qquad \text{Eq. 7.3}$$

The standard deviation (*S* or SD) describes the variability within a sample; whereas the standard error of the mean (SEM) represents the possible variability of the mean itself. The SEM is sometimes referred to as the **standard error** (SE) and describes the variation of all possible sample means and equals the SD of the sample data divided by the square root of the sample size. As can be seen by the formula, the distribution of sample means (the standard error of the mean) will always be smaller than the dispersion of the sample (the standard deviation).

Authors may erroneously present the distribution of sample results by using the SEM to represent dispersion because there appears to be less variability. This may be misleading since the SEM has a different meaning from the SD. The SEM is smaller than the SD and the intentional presentation of the SEM instead of the larger SD is a manipulation to make data look more precise. The SEM is extremely important in the estimation of a true population mean, based on sample results. However, because it is disproportionately low, it should never be used as a measure of the distribution of sample results. For example, the SEM from our previous example of liquid fill volumes (Table 5.1) is much smaller (by a factor of almost six) than the calculated standard deviation:

$$SEM = \frac{0.835}{\sqrt{30}} = 0.152$$

By convention, the term standard error refers to the variability of a sampling distribution. However, authors still use the standard error of the mean to present sample distributions, because the SEM is much smaller than the SD and presents a much smaller variation of the results. An even more troublesome occurrence is the failure of authors to indicate in reports or publications whether a result represents an SD or an SEM. For example, a poster or report simply states "456.1 ± 1.3" with no indication of what the term to the right of the ± sign represents. Is this a very tight SD? Is it the SEM? Could it even be the RSD? Without proper labeling, the reader would never know what the dispersion term represents.

Standard error of the mean can be considered as a measure of precision. Obviously, the smaller the SEM, the more confident we can be that our sample mean is closer to the true population mean. However, at the same time, large increases in sample size produce relatively small changes in this measure of precision. For example, using a constant sample SD of 21.5 for sample B, presented above, the measure of SEM changes very little as sample sizes increase past 30 (Figure 7.2). A general rule of thumb is that with samples of 30 or more observations, it is safe to use the sample standard deviation as an estimate of population standard deviation.

Confidence Intervals

As discussed in Chapter 5, using a random sample and independent measures, one can calculate measures of central tendency ($\bar{X}$ and *S*). The result represents

n	SEM
3	12.40
5	9.62
10	6.80
15	5.55
20	4.81
25	4.30
30	3.93
50	3.04
100	2.15

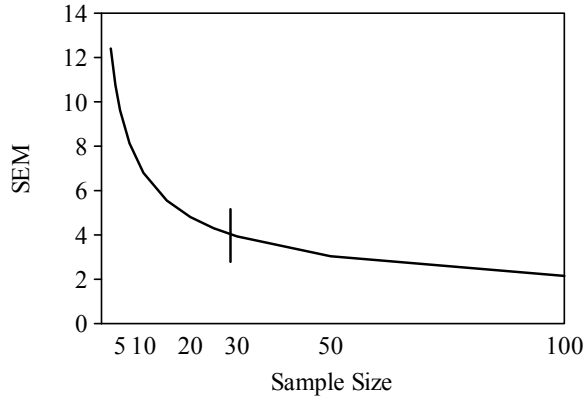


Figure 7.2 Variation in standard error of the means by sample size.

only one sample that belongs to a distribution of many possible sample means. Because we are dealing with a sample and in most cases do not know the true population parameters, we often must make a statistical "guess" at these parameters. For example, the previous samples A through D (Table 7.1) all have calculated means, any of which could be the true mean for the population from which they were randomly sampled. In order to define the true population mean, we need to allow for a range of possible means based on our estimate:

$$\begin{matrix}\textit{Population}\\ \textit{Mean}\end{matrix} = \begin{matrix}\textit{Estimate}\\ \textit{Sample Mean}\end{matrix} \pm \begin{matrix}\textit{"Fudge"}\\ \textit{Factor}\end{matrix}$$

This single estimate of the population mean (based on the sample) can be referred to as a **point estimate**. The result is a range of possible outcomes defined as **boundary values**, **interval estimators**, or **confidence limits**. At the same time, we would like to have a certain amount of confidence in our statement that the population mean falls within these boundary values. For example, we may want to be 95% certain that we are correct, or 99% certain. Note again that because it is a sample, not an entire population, we cannot be 100% certain of our prediction. The only way to be 100% certain would be to measure every item in the population and in most cases that is either impractical or impossible to accomplish. Therefore, in order to have a certain confidence in our decision (i.e., 95% or 99% certain) we need to add to our equation a factor to allow us this confidence:

$$\begin{matrix}\textit{Population}\\ \textit{Mean}\end{matrix} = \begin{matrix}\textit{Estimate}\\ \textit{Sample Mean}\end{matrix} \pm \begin{matrix}\textit{Reliability}\\ \textit{Coefficient}\end{matrix} \times \begin{matrix}\textit{Standard}\\ \textit{Error}\end{matrix} \qquad \text{Eq. 7.4}$$

This reliability coefficient (sometime referred to as the **confidence coefficient**) can be obtained from the normal standardized distribution. For example if we want to be certain 95% of the time, we will allow an error 5% of the time. We could err on the

high side or low side and if we wanted our error divided equally between the two extremes, we would allow a 2.5% error too high in our estimation and 2.5% too low in our estimate of the true population mean. In Table B2 of Appendix B we find that 95% of the area under the curve falls between −1.96 z and +1.96 z. This follows the theory of the normal distribution where 95% of the values, or in this case sample means, fall within 1.96 standard error of the mean units. The actual calculation for the 95% confidence interval would be:

$$\mu = \overline{X} \pm Z_{(1-\alpha/2)} \times \frac{\sigma}{\sqrt{n}} \qquad \text{Eq. 7.5}$$

The symbol $\alpha/2$ will be defined in the next chapter. For the time being, assume $\alpha/2$ is represented by 1.96 for the case of a 95% confidence interval and the equation would be:

$$\mu = \overline{X} \pm (1.96)\frac{\sigma}{\sqrt{n}}$$

The standard error term or standard error of the mean term is calculated based on the population standard deviation and specific sample size. If the confidence interval were to change to 99% or 90%, the reliability coefficient would change to 2.57 and 1.64, respectively (based on values in Table B1 where 0.99 and 0.90 of the area fall under the curve). In creating a range of possible outcomes instead of one specific measure, "it is better to be approximately correct, than precisely wrong" (Kachigan, 1991, p. 99).

Many of the following chapters will deal with the topic of confidence intervals and tests involved in this area. But at this point let us assume that we know the population standard deviation (σ), possibly through historical data or previous tests. In the case of the pharmacokinetic data (Table 4.3), the population standard deviation is known to be 16.8, based on the data in the table which represents the population, and was calculated using the formula to calculate a population standard deviation (Eqs. 5.7 and 5.8). Using the four samples from the population and presented in Table 7.1 it is possible to estimate the population mean based on data for each sample. For example, with Sample A:

$$\mu = 716.4 \pm 1.96\frac{16.8}{\sqrt{5}} = 716.4 \pm 14.7$$

$$701.7 < \mu < 731.1 \text{ mcg}$$

The best estimate of the population mean (for the researcher using Sample A) would be between 701.7 and 731.1 mcg. Note that the "fudge factor" will remain the same for all four samples since the reliability coefficient will remain constant (1.96) and the error term (the population standard deviation divided by square root of the sample

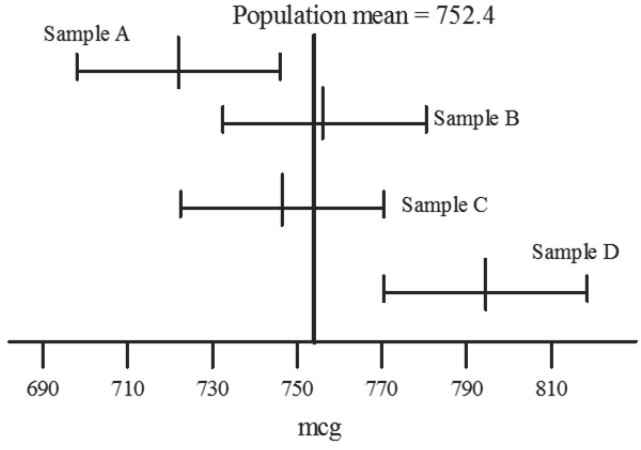


Figure 7.3 Sample results compared with the population mean.

size) does not change. Therefore the results for the other three samples would be:

Sample B: $\mu = 752.8 \pm 14.7$
$738.1 < \mu < 767.5$ mcg

Sample C: $\mu = 749.4 \pm 14.7$
$734.7 < \mu < 764.1$ mcg

Sample D: $\mu = 786.8 \pm 14.7$
$772.1 < \mu < 801.5$ mcg

From our previous discussion of presentation mode, the true population mean for these 125 data points is a C_{max} of 752.4 mcg. In the case of samples B and C, the true population mean did fall within the 95% confidence interval and we were correct in our prediction of this mean. However, with the extreme samples (A and D) the population mean falls outside the confidence interval (Figure 7.3). With over 234 million possible samples and using the reliability coefficient (95%), almost 12 million possible samples (5%) will give us erroneous results.

Adjusting the confidence interval can increase the likelihood of predicting the correct population mean. One more sample was drawn consisting of five outcomes and the calculated mean is 768.4. If a 95% confidence interval is calculated, the population mean falls outside the interval.

$$\mu = 768.4 \pm 1.96 \frac{16.8}{\sqrt{5}} = 768.4 \pm 14.7$$

$$753.7 < \mu < 783.1 \, mcg$$

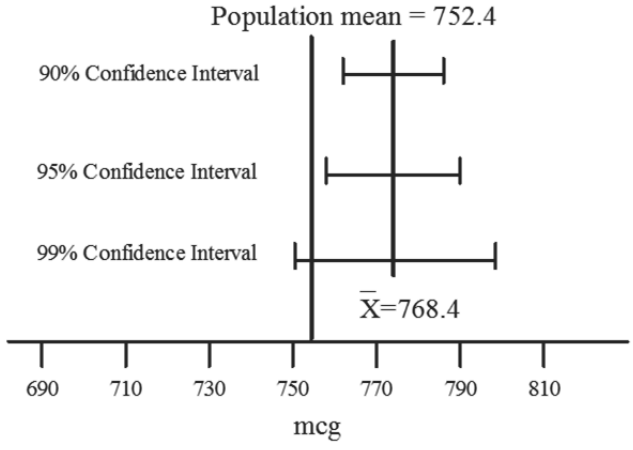


Figure 7.4 Sample results with different confidence levels compared with the true population mean.

However, if we decrease our confidence to 90%, the true population mean (μ = 752.4 mcg) falls even further outside the interval.

$$\mu = 768.4 \pm 1.64\frac{16.8}{\sqrt{5}} = 768.4 \pm 12.4$$

$$756.0 < \mu < 780.8 \text{ mcg}$$

Similarly, if we increase our confidence to 99%, the true population mean will be found within the predicted limits.

$$\mu = 768.4 \pm 2.57\frac{16.8}{\sqrt{5}} = 768.4 \pm 19.3$$

$$749.1 < \mu < 787.7 \text{ mcg}$$

As seen in Figure 7.4, as the percentage of confidence increases, the width of the confidence interval increases. Creation and adjustment of the confidence intervals is the basis upon which statistical analysis and hypothesis testing is based.

What we have accomplished is our first inferential statistic: to make a statement about a population parameter (μ) based on a subset of that population ($\bar{X}$). The z-test is the oldest of the statistical tests and was often called the **critical ratio** in early statistical literature. The **interval estimate** is our best guess, with a certain degree of confidence, where the actual parameter exists. We must allow for a certain amount of error (e.g., 5% or 1%) since we do not know the entire population. As shown in Figure 7.4, as our error decreases, the width of our interval estimate will increase. In

order to be 100% confident, our estimate of the interval would be from $-\infty$ to $+\infty$ (negative to positive infinity). Also as can be seen in the formula for the confidence interval estimate, with a large sample size, the standard error term will decrease and our interval width will decrease. Relating back to terms defined in Chapter 3, we can relate confidence interval in terms of precision and the confidence level is what we establish as our reliability.

As we shall see in future chapters, a basic assumption for many statistical tests (e.g., student t-test, F-test, correlation) is that populations from which the samples are selected are composed of random outcomes that approximate a normal distribution. If this is true, then we know many characteristics about our population with respect to its mean and standard deviation.

The one troublesome feature of Eq. 7.5 is the fact that it is highly unlikely that we will know a population standard deviation (σ). An example of an exception might be a quality control situation where a measurement has been repeated many times and is based on historical data. As will be shown in the next section, one could make a reasonable guess of what σ should be based on past outcomes. However, in Chapter 8 we will find an alternative test for creating a confidence interval when the population standard deviation is unknown or cannot be approximated.

Statistical Control Charts

Quality control charts represent an example of the application of confidence intervals using σ or an approximation of the population standard deviation. Traditionally, control charts have been used during manufacturing to monitor production runs and ensure the quality of the finished product. More recently these charts have been used to monitor the quality of health care systems, along with techniques such as cause-and-effect diagrams, quality-function deployment and process-flow analysis (Laffel, 1989; Wadsworth, 1985). Our discussion of control charts will focus on production issues, but the process could be easily applied to the monitoring of quality performance indicators in the provision of health services.

Statistical quality control is the process of assessing the status of a specific characteristic or characteristics, over a period of time, with respect to some target value or goal. During the production process, control charts provide a visual method for evaluating an intermediate or the final product during the ongoing process. They can be used to identify problems during production and document the history of a specific batch or run.

The use of control charts is one of the most common applications of statistics to the process of pharmaceutical quality control. The design of such charts was originally developed by Walter Shewhart of Bell Telephone Laboratories in 1931 (Shewhart, 1931). Over the years, modifications have been made, but most of the original characteristics of the **Shewhart control chart** remain today. Control charts assess and monitor the variability of a specific characteristic, which is assumed to exist under relatively homogeneous and stable conditions. There are generally two types of control charts: 1) measuring consistency of the production run around a target value (**property chart**) and 2) measuring the variability of the samples (**precision chart**).

To assess and monitor a given characteristic during a production run, we periodically sample items (e.g., tablets, vials) using random or selected sampling and

measure the specific characteristic or variable (e.g., weight, hardness). The results are plotted on a two-dimensional graph. The *x*-axis is a "time-ordered" sequence. The outcomes or changes are plotted on the *y*-axis over this time period to determine if the process is under control.

A **sampling plan** is developed to determine times, at equal intervals, during which samples are selected. In the case of a selected sampling scheme (e.g., every 15 minutes samples are selected from a production line), it is assumed that individual samples are withdrawn at random. How often should a sample be selected? The length of time between samples is dependent on the stability of the process being measured. A relatively stable process (for example, weights of finished tablets in a production run), may require only occasional monitoring, (e.g., every 30 minutes). A more volatile product or one with potential for large deviations from the target outcome may require more frequent sampling. When drawing samples for control charts, the time intervals should be consistent (every 30 minutes or 60 minutes, etc.) and the sample sizes should be equal. The size and frequency of the sample is dependent on the nature of the control process and desired precision. Sample sizes as small as four or five observations have been recommended (Bolton, 2004, p. 376).

The creation of a quality control chart is a relatively simple procedure. Time intervals are located on the *x*-axis and outcomes for the characteristic of interest (variable or property) are measured on the *y*-axis. The "property" chart uses either a single measurement (sometimes referred to as an **x-chart**) or the mean of several measurements (a **mean chart**) of a selected variable (e.g., capsule weight, tablet hardness, fill volume of a liquid into a bottle). The mean chart ($\overline{X}$ chart) would be preferable to a simple x-chart because it is less sensitive to extreme results because these would offset when all the measures are averaged. Also, an x-chart consists of a series of single measures and does not provide any information about the variance of outcomes at specific time periods.

Control charts contain a **central line** running through the chart parallel to the *x*-axis. This central line represents the "target" or "ideal" goal and is often based on historical data from scale up through initial full-scale runs. Also referred to as the **average line**, it defines the target for the variable being plotted. This is seen as the center line in Figure 7.5. In an ideal world, if a process is under control all results would fall on the central line. Unfortunately most outcomes will be observed to fall above or below the line, due to simple random error. The distance from the central line and the actual point measures variability. Thus, in addition to identifying a central line it is important to determine acceptable limits, above and below this line, within which observations should fall.

There are two types of variability that can be seen in statistical control charts: 1) common cause variability and 2) assignable cause variability. **Common cause variation** is due to random error or normal variability attributed to the sampling process. This type of error is due to natural random error or error inherent in the process. **Assignable cause variation** is systematic error or bias that occurs in excess of the common-cause variability of the process. Also called **special-cause variation,** it is the responsibility of the person controlling the process to identify the cause of this variation, correct it and maintain a process that is under control. When the control chart shows excessive variation from the ideal outcome the process is said to be "out of statistical control." Thus, the required measures for constructing a statistical quality control chart are: 1) a sampling plan (size and length of time interval); 2) a target value; and 3) an estimate of

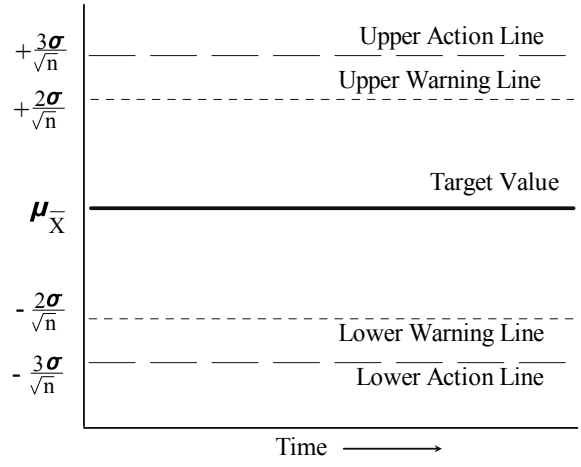


Figure 7.5 Traditional quality control chart.

the random error. As we shall see, the last measurement is based on either the expected standard deviation or range.

How much deviation from the central line is acceptable? The original Shewhart format utilized the population standard deviation (σ) and created **action lines** at three standard deviations above and below the target value. These lines were referred to as the three-sigma limits or the upper and lower control limits (UCL and LCL).

$$UCL = target + \frac{3\sigma}{\sqrt{n}} \qquad \text{Eq. 7.6}$$

$$LCL = target - \frac{3\sigma}{\sqrt{n}} \qquad \text{Eq. 7.7}$$

where n is the number of samples at each time point. These are the boundaries within which essentially all of the sample data should fall if the process is under statistical control. In order to create these upper and lower action lines we need to be able to estimate the population standard deviation. This could be based on historical data about a particular product or process, or it can be estimated from previous samples.

One method for estimating σ is to calculate an average or "pooled" sample standard deviation. This can be calculated by averaging standard deviations from previous runs:

$$S_p = \frac{S_1 + S_2 + S_3 + \ldots S_k}{k} \qquad \text{Eq. 7.8}$$

where k is the number of sample standard deviations. Averaging the sum of the squared standard deviations is sometimes referred to as the **within-sample estimate of variance** or random error:

$$\sigma^2_{WSE} = \frac{\sum S^2}{k} \qquad \text{Eq. 7.9}$$

Either the pooled sample standard deviation or the square root of the within-sample estimate of variance can provide a rough estimate of the true population standard deviation. Since the pooled sample standard deviation is an estimate sigma, it can be used to substitute for the population standard deviation

$$UCL = target + \frac{3S_p}{\sqrt{n}} \qquad \text{Eq. 7.10}$$

$$LCL = target - \frac{3S_p}{\sqrt{n}} \qquad \text{Eq. 7.11}$$

More recent control charts incorporate additional limits called **warning lines**. This method involves establishing two sets of lines: warning lines at two sigmas and action lines at three sigmas (see Figure 7.5). Because they only involve two standard deviations above and below the target line, the warning limits are always narrower and do not demand the immediate intervention seen with the action lines. The warning lines would be calculated as follows:

$$\mu_w = \mu_0 \pm \frac{2\sigma}{\sqrt{n}} \qquad \text{Eq. 7.12}$$

and the action lines are:

$$\mu_a = \mu_0 \pm \frac{3\sigma}{\sqrt{n}} \qquad \text{Eq. 7.13}$$

Some control charting systems even evaluate observations falling outside one standard deviation beyond the central line. As discussed, virtually all samples will fall between ± 3-sigma, 95% will be located within ± 2-sigma and approximately 2/3 are contained within ± 1-sigma. Therefore, deviations outside any of these three parameters can be used to monitor production. Possible indicators of a process becoming "out-of-control" would be two successive samples outside the 2-sigma limit or four successive samples outside the 1-sigma limit or any systematic trends (several consecutive samples in the same direction) up or down from the central line (Taylor, 1987, pp. 135-136).

As mentioned previously, two components that can influence a control chart are: 1) the variable or property of interest (systematic or assignable cause variability) and 2) the precision of the measurement (random or common-cause variability). The center, warning and action lines monitor a given property in a quality control chart. However, we are also concerned about the precision or variability of our sample (the random variability). Standard deviations and ranges can be used as a measure of

consistency of the samples. Variations in these measures are not seen in a simple Shewhart chart. A precision chart measures the amount of random error and consists of plotting the sample standard deviation (or the sample range) against the time-ordered sequence in parallel with the control chart for the sample means. For plotting the sample standard deviations, the pooled sample standard deviation becomes the "target" for variability and is once again substituted as an estimate of the population standard deviation for a precision chart

$$UCL_D = S_p + \frac{3S_p}{\sqrt{n}} \qquad \text{Eq. 7.14}$$

$$LCL_D = S_p - \frac{3S_p}{\sqrt{n}} \qquad \text{Eq. 7.15}$$

In addition to creating a control chart based on the standard deviation, a similar chart can be produced using the easiest of all measures of dispersion, the range. The central line for a range chart is calculated similar to the line used for the property chart. An average range is computed based on past observations.

$$\overline{R} = \frac{R_1 + R_2 + R_3 + \ldots R_k}{k} \qquad \text{Eq. 7.16}$$

Obviously the range is easier to calculate and is as efficient as the standard deviation to measure deviations from the central line if the sample size is greater than 5 (Mason, 1989, p. 66). Also, to calculate $\overline{R}$, there should be at least 8 observations and preferably at least 15 for any given time period (Taylor, 1987, p. 140). This alternative method can be used to for calculating the action lines property chart utilizing $\overline{R}$ and an A-value from Table 7.2.

$$UCL = target + A\overline{R} \qquad \text{Eq. 7.17}$$

$$LCL = target - A\overline{R} \qquad \text{Eq. 7.18}$$

Using the above formula will produce action lines similar to those created using Eq. 7.10 and 7.11.

To calculate the action lines for a precision chart for variations in the range, as a measure of dispersion, a value similar to the reliability coefficient portion of Eq. 7.5 is selected from Table 7.2. The D_L and D_U-values from the table for the lower and upper limits, respectively, are used in the following formulas:

$$UCL_D = D_U \overline{R} \qquad \text{Eq. 7.19}$$

$$UCL_D = D_L \overline{R} \qquad \text{Eq. 7.20}$$

Table 7.2 Factors for Determining Upper and Lower 3σ Limits for Mean and Range Quality Control Charts

		Factors for Range Chart	
Sample Size of Subgroup, N	A: Factor for X Chart	D_L for Lower Limit	D_U for Upper Limit
2	1.88	0	3.27
3	1.02	0	2.57
4	0.73	0	2.28
5	0.58	0	2.11
6	0.48	0	2.00
7	0.42	0.08	1.92
8	0.37	0.14	1.86
9	0.34	0.18	1.82
10	0.31	0.22	1.78
15	0.22	0.35	1.65
20	0.18	0.41	1.59

From: Bolton, S. (1997). *Pharmaceutical Statistics: Practical and Clinical Applications*, Third edition, Marcel Dekker, Inc., New York, p. 658. Reproduced with permission of the publisher.

As more is known about the total population (e.g., larger sample sizes), the values in Table 7.2 become smaller and the action lines come closer together. Also, based on the values in the table, the lines around the average range will not be symmetrical and the upper action line will always be further from the central, target range. By presenting these two plots in parallel, it is possible to monitor both the variability of the characteristic being measured, as well as the precision of the measurements at each specific time period.

As an example consider the tablet weights sampled over 12 time points presented in Table 7.3. The best estimate of center would be the average of the sample means which is 200.07 mg. The creation of the control chart by using the sample standard deviations would be as follows:

$$UCL = target + \frac{3S_p}{\sqrt{n}} = 200.07 + \frac{3(2.11)}{\sqrt{5}} = 202.90$$

$$LCL = target - \frac{3S_p}{\sqrt{n}} = 200.07 - \frac{3(2.11)}{\sqrt{5}} = 197.24$$

$$UCL_D = S_p + \frac{3S_p}{\sqrt{n}} = 2.11 + \frac{3(2.11)}{\sqrt{5}} = 4.94$$

Table 7.3. Sample Weights (mg) Observed during a Production Run

Date	Time	Samples					Mean	SD	Range
9/6	9:00	200.5	198.5	205.2	201.8	198.3	200.86	2.83	6.9
	9:30	199.4	200.4	204.8	200.6	198.6	200.76	2.40	6.2
	10:00	201.5	197.4	200.9	202.3	199.5	200.32	1.93	4.9
	10:30	199.9	196.6	200	201.5	197.9	199.18	1.93	4.9
	11:00	200.5	200.8	204.3	199.4	202.1	201.42	1.88	4.9
	11:30	200.9	198.1	203.1	199.2	203.4	200.94	2.34	5.3
	12:00	201.3	196.7	200.3	200.1	203.2	200.32	2.37	6.5
	12:30	198.1	198	200.4	200.5	199.9	199.38	1.24	2.5
	13:00	199.8	197.3	202.3	198.4	200.5	199.66	1.93	5
	13:30	198.8	196.4	203.6	199.1	197.5	199.08	2.75	7.2
	14:00	199.2	197.6	201.1	199.6	198.8	199.26	1.27	3.5
	14:30	200	196.8	202.2	201.8	197.3	199.62	2.49	5.4
						Average:	200.07	2.11	5.27

$$LCL_D = S_p - \frac{3S_p}{\sqrt{n}} = 2.11 - \frac{3(2.11)}{\sqrt{5}} = -0.72$$

Since a negative deviation is impossible the results for the lower limit for the standard deviation would be truncated at zero. The results are plotted in Figure 7.5. If instead the researcher decided to use only the range, the results would be slightly different, where the average is 5.27:

$$UCL = target + A\overline{R} = 200.07 + (0.58)(5.27) = 203.13$$

$$LCL = target - A\overline{R} = 200.07 - (0.58)(5.27) = 197.01$$

$$UCL_D = D_U \overline{R} = 2.11(5.27) = 11.12$$

$$UCL_D = D_L \overline{R} = 0(5.27) = 0$$

Notice the intervals created using the ranges are wider because less information is known about each sample, only two data points for the extreme values.

Sometime **moving averages** and/or **moving ranges** are used for control charts. In these cases, the first two or three samples are averaged and the results used as the point on the control chart. When the next sample is collected, the first value is dropped and a new average is plotted (for both the mean and the range). This process continues, averaging including a new observation and excluding the earliest previous number continued for the whole data set. This yields a series of means and ranges representing the average of multiple consecutive data points. The average range is replaced by the average moving range

$$AMR = \frac{MR_2 + MR_3 + MR_4 + ...MR_k}{k-1} \qquad \text{Eq. 7.21}$$

where each MR_i is the average of R_i+R_{i-1} and the estimate of the population standard deviation is

$$S = \frac{AMR}{1.128} \qquad \text{Eq. 7.22}$$

For the previous example the results using the moving average would be: target = 4.60; UCL = 202.74; LCL = 197.40; UCLD = 9.70; LCLD = 0 which are much closer to the results using the pooled sample standard deviation.

A second type of control chart is the **cumulative sum** or **CUSUM** charting technique. It is considered more sensitive than Shewhart control charts to modest changes in the characteristic being monitored (Mason, 1989, p. 66). The CUSUM charts are more effective in identifying gradual approaches to out-of-control conditions. The name CUSUM is from the fact that successive deviations are accumulated from a fixed reference point in the process. It provides a running, visual summation of deviations, from some preselected reference point. There is evidence of a special-cause variation when the cumulative sum of the deviations is extremely large or extremely small. Further information on CUSUM charts can be found in Mason's book (Mason, 1989, pp. 67-70).

Process Capability Indices

Process capability is a measure of the inherent variability of a process removing any undesirable special causes that might increase variability. It is the smallest variability due solely to common causes. In manufacturing it is a measurement of the degree to which the process is meeting the manufacturing requirements. It is the repeatability and consistency of that process and is relative to the customer requirements in terms of specification limits for the product.

Possible special causes of variability include different production sites, different equipment, and different operators running that equipment. One way to eliminate these special causes is to collect data using the same operator on the same machine, measuring the same batch of materials.

Studies of process capability are designed to determine what the process is "capable" of doing under controlled conditions (removing any special causes for variability). Another benefit of studying the process capability is to determine the stability of the process by comparing the output of a stable process with the process specifications or by comparing the normal variability of a stable process with the process specification limits.

Process capability compares the process outcome that is "in control" with the specification limits by measures called **capacity indices**. This comparison is a ratio of the deviation between the process specifications (called the specification width) to the deviation of the process values based on six process standard deviation units (referred to as the process width). A "capable process" is defined as one in which all the

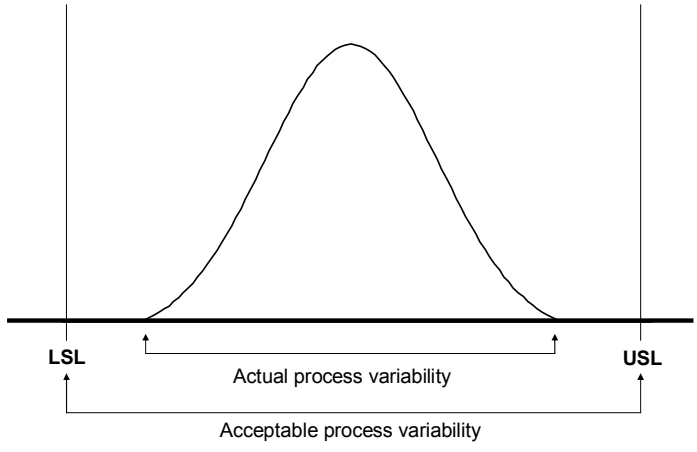


Figure 7.6 Illustration of a distribution within specification limits.

measurements fall inside the predetermined specification limits (Figure 7.6).

Capability indices are equations employed to place the distribution from a specific process in relationship to the product specifications. Capability indices are used to determine, given normal variation, if the process is capable of meeting established specifications. Thus, it is assumed that data points are sampled from a normally distributed population. Process capability is expressed as an index and there are three different indices, labeled C_p, C_{pk} and C_{pm}. These capability indices are valid only when there is a large sample size, usually a minimum of 50 data points. These should be consecutive data points, in at least 10 subgroups, each with 5 observations.

Several symbols are used in the calculations of the capability indices. T is the target value for the product. The μ is the process mean and σ is the measure of dispersion based on historical experience with the process (often T and μ are the same value). The *USL* and *LSL* are the upper and lower specification limits, respectively. The manufacture sets the specification limits. The specification range is the difference between the *USL* and *LSL*.

$$Specification\ range = USL - LSL \qquad \text{Eq. 7.23}$$

The specification range is usually from -3σ to $+3\sigma$, or a six-sigma spread. As seen in the previous chapter, approximately 99.7% of the area under a normal distribution would be within the plus or minus three sigmas. Thus, the total variability or spread in outcomes should have a total variation of approximately six sigmas.

C_p is a simple index that relates the acceptable variability of the specification limits to the natural variation of the process (expressed as 6σ). It is sometimes referred to as the **population capability** or **process potential.** The C_p calculated as follows:

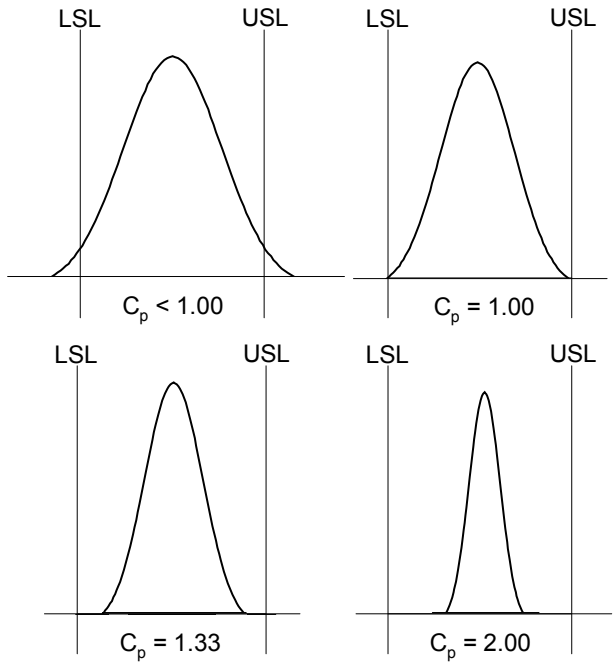


Figure 7.7 Distributions for various C_p values.

$$C_p = \frac{USL - LSL}{6\sigma} \qquad \text{Eq. 7.24}$$

Various C_p results are illustrated in Figure 7.7. If the C_p is less than one, the process variation exceeds specification, and a significant number of defects may be found. A C_p of less than one indicates that it is not a capable process; not capable of meeting specifications regardless of where the process mean is located. In these cases the process spread is greater than *USL-LSL*.

If the C_p equals one, the process is just meeting specifications and a minimum of 0.3% (100%-99.7%) defects will be detected if the process is centered at the target. This would be when a process is just barely capable; the process variability matches 6 σ. The C_p evaluates the spread of the process relative to the specification width, it does not provide information on how well the process average, μ , is centered with respect to the target value, *T*. If the process mean shifts slightly to the left or to the right, a significant amount of production output will exceed one of the two specification limits. In this case, the process must be watched closely to identify any shifts from the mean. Control charts are excellent for such monitoring.

If the C_p is greater than one, the process variation is less than the specification limits, but the defect rate might be greater if the process is not centered on the target value (*T*). Also, the C_p can be highly inaccurate and misleading if the data is not

Table 7.4 C_p Values Assuming that the Center of the Distibution is μ

USL–LSL	C_p	Rejects (parts per million)	% of Specification Used
6σ	1.000	2,700	100
8σ	1.333	64	75
10σ	1.667	0.6	60
12σ	2.000	0.002	50

sampled from a normally distributed population. Table 7.4 indicates the expected number of defects for various levels of C_p. As seen in Table 7.4 the greater the C_p the more likely the process variability will fall within the specification spread (6 sigma is less than *USL–LSL*). For example, with a C_p of 2.0 indicates a process distribution where 12 sigmas would fit between the USL and LSL. If a manufacturer can tighten its specification limits, it might be able to claim that its product is more consistent or uniform than its competitors. Some pharmaceutical manufacturers are establishing specific process capabilities targets. As a starting point they may require a C_p of 1.33 for supplier qualifications and have a desired goal of 2.0.

A second process capability index is C_{pk} and comparing it to the C_p it is possible to get an indication of the difference between μ and *T*. The C_{pk} is calculated as follows:

$$C_{pk} = min\left[\frac{USL-\mu}{3\sigma}, \frac{\mu-LSL}{3\sigma}\right] \qquad \text{Eq. 7.25}$$

and the smaller of the two values (*min*) in the parentheses is reported as the C_{pk}. If the process approaches a normal distribution and is in statistical control, the C_{pk} may be used to estimate the expected percent of defective products, similar to the C_p.

An alternative method for estimating the C_{pk} is:

$$C_{pk} = C_p(1-k) \qquad \text{Eq. 7.26}$$

where *k* is the scaled distance between the midpoint of the specification range *m* and the process mean, μ. This k-value comes from the Japanese word *katayori*, which means deviation. The specification range is calculated as follows:

$$m = \frac{USL+LSL}{2} \qquad \text{Eq. 7.27}$$

and the *k* value is derived using the equation

$$k = \frac{|m-\mu|}{\frac{USL-LSL}{2}} \qquad \text{Eq. 7.28}$$

The resultant k must be greater or equal to zero and less than or equal to one. When the m and μ are equal, $k = 0$ in Eq. 7.26 and $C_{pk} = C_p$. The difference between the C_p and C_{pk} is represented by the k-value, and indicates how much of the process capability is lost due to poor centering. For example, suppose that $k = 0.25$ and the $C_p = 2.00$, the C_{pk} would be reduced to:

$$C_{pk} = 2.00(1-0.25) = 1.50$$

If the process can be centered, this capability index would increase by 33%. This deviation from the center (T) can be calculated as follows:

$$\delta = \frac{C_p}{C_{pk}} \times 100\% \qquad \text{Eq. 7.29}$$

For this example:

$$\delta = \frac{2.00}{1.50} \times 100\% = 33.3\%$$

The third capability index is C_{pm}; sometimes referred to as the Taguchi capability (named after Genichi Taguchi). This was developed in the late 1980s and the index, similar to the C_{pk}, accounts for the proximity of the process mean to a designated target mean, T.

$$C_{pm} = \frac{USL - LSL}{6\sqrt{\sigma^2 + (\mu - T)^2}} \qquad \text{Eq. 7.30}$$

If the process mean is centered between the specification limits and the process mean equals the target mean (T), then $C_p = C_{pk} = C_{pm.}$ The C_{pk} and C_{pm} are the better indices because they account for deviations between the process center and the target center in the distribution.

If the population standard deviation (σ) is unknown, sample estimates can be used by replacing σ with the sample standard deviation (S). Slight modifications are made on the previous equations and a hat is added above each index to indicate that it is an estimate based on sample variability:

$$\hat{C}_p = \frac{USL - LSL}{6S} \qquad \text{Eq. 7.31}$$

$$\hat{C}_{pk} = min\left[\frac{USL - \overline{X}}{3S}, \frac{\overline{X} - LSL}{3S}\right] \qquad \text{Eq. 7.32}$$

$$\hat{C}_{pm} = \frac{USL - LSL}{6\sqrt{S^2 + (\overline{X} - T)^2}} \qquad \text{Eq. 7.33}$$

The *k*-deviation can also be calculated based on sample data. The sample mean ($\overline{X}$) is the best estimate of μ and the sample estimate of *k* and C_{pk} would be:

$$\hat{k} = \frac{\left|m - \overline{X}\right|}{\frac{USL - LSL}{2}} \qquad \text{Eq. 7.34}$$

and

$$\hat{C}_{pk} = \hat{C}_p(1 - \hat{k}) \qquad \text{Eq. 7.35}$$

If $0 \leq k \leq 1$; then:

$$\hat{C}_{pk} \leq \hat{C}_p \qquad \text{Eq. 7.36}$$

As an example, consider the data presented in Table 7.5 which represent 60 samples randomly selected during the production of a product. The manufacturer has set the specification limits to be within 3% of label claim (T = 100%, USL = 103%, LSL = 97%). From previous production experience with the product the expected mean (μ) and standard deviation (σ) are 100 and 1%, respectively. However, the sample results are $\overline{X}$ = 100.1 and S = 0.25. The process capability indices are:

$$\hat{C}_p = \frac{USL - LSL}{6S} = \frac{103 - 97}{6(.25)} = 4.00$$

$$\hat{C}_{pk} = min\left[\frac{USL - \overline{X}}{3S}, \frac{\overline{X} - LSL}{3S}\right] = min\left[\frac{103 - 100.1}{3(0.25)}, \frac{100.1 - 97}{3(0.25)}\right]$$

$$\hat{C}_{pk} = min\left[3.87, 4.13\right] = 3.87$$

$$\hat{C}_{pm} = \frac{USL - LSL}{6\sqrt{S^2 + (\overline{X} - T)^2}} = \frac{103 - 97}{6\sqrt{(0.25)^2 + (100.1 - 100.0)^2}} = 3.71$$

or as an alternative for C_{pk}:

$$m = \frac{USL + LSL}{2} = \frac{103 + 97}{2} = 100$$

Table 7.5. Sample Results Observed during a Production Run

Time	Sample Results			Mean	S.D.	Range
0:05	100.3	100.5	100.0	100.12	0.26	0.7
	99.8	99.9	100.2			
0:20	99.9	100.2	100.4	100.07	0.27	0.7
	100.3	99.7	99.9			
0:35	100.1	100.0	100.5	100.20	0.18	0.5
	100.3	100.2	100.1			
0:50	100.2	99.5	99.9	100.08	0.38	1.1
	100.3	100.0	100.6			
1:05	100.2	100.3	99.8	100.08	0.17	0.5
	100.0	100.1	100.1			
1:20	100.1	100.3	99.9	100.08	0.23	0.6
	100.4	99.8	100.0			
1:35	99.8	100.2	99.6	100.03	0.31	0.9
	100.5	100.0	100.1			
1:50	100.2	100.3	100.0	100.13	0.20	0.5
	100.4	99.9	100.0			
2:05	100.8	100.2	99.8	100.15	0.36	1.0
	100.0	99.9	100.2			
2:20	99.9	100.4	100.1	100.07	0.26	0.7
	100.3	100.0	99.7			
		Total for all samples:		100.10	0.25	

$$k = \frac{\left|m - \overline{X}\right|}{\frac{USL - LSL}{2}} = \frac{\left|100 - 100.1\right|}{\frac{103 - 97}{2}} = \frac{0.1}{3} = 0.033$$

$$\hat{C}_{pk} = \hat{C}_p(1 - \hat{k}) = 4.00(1 - 0.033) = 4.00(0.967) = 3.87$$

It is possible to do unilateral, or one-sided tests, for determining process capabilities. The previous examples were bilateral, or two-sided cases, and involved both the *USL* and *LSL*. For the unilateral case either the *USL* or *LSL* is used alone:

$$\hat{C}_{pu} = \frac{USL - \overline{X}}{3S} \qquad \text{Eq. 7.37}$$

$$\hat{C}_{pl} = \frac{\overline{X} - LSL}{3S} \qquad \text{Eq. 7.38}$$

and by extension the C_p is:

$$\hat{C}_p = \frac{\hat{C}_{pl} + \hat{C}_{pu}}{2} \qquad \text{Eq. 7.39}$$

and C_{pk} is the smaller value for either C_{pl} or C_{pu}:

$$\hat{C}_{pk} = min\left[\hat{C}_{pl}, \hat{C}_{pu}\right] \qquad \text{Eq. 7.40}$$

In addition, estimators can be used replacing μ and σ with $\overline{X}$ and S.

It is possible to calculate a confidence interval for the capability indices. Once again, we are assuming a normal distribution population. For the C_{pk} a confidence interval can be calculated using the following equation:

$$C_{pk} = \hat{C}_{pk} \pm z_{1-\alpha/2}\sqrt{\frac{1}{9n} + \frac{\hat{C}_{pk}^2}{2(n-1)}} \qquad \text{Eq. 7.41}$$

Like Eq. 7.4, our best estimate of the true C_{pk} is our sample estimate ($\hat{C}_{pk}$). How confident we are in our decision is controlled by the reliability coefficient ($z_{1-\alpha/2}$) and error term, which in this case is the portion of the equation included in the square root term. This equation is similar to Eq. 7.5 and if we wish to be 95% confident in our decision the reliability coefficient would be 1.96. The resulting confidence interval is evaluated base on its proximity to 1.0, because a capability index of 1.0 just meets specifications (the ratio of the process specifications to the deviation of the process values is 1.0). The concept of interpreting ratios will be discussed in greater detail in Chapter 18. However, at this point assume that if our confidence interval has values that are all greater than 1.0 that we are 95% confident that we have a capable process. Using our previous example, where $\hat{C}_{pk}$ = 3.87 and n = 60, the confidence interval would be:

$$C_{pk} = 3.87 \pm 1.96\sqrt{\frac{1}{9(60)} + \frac{(3.87)^2}{2(59)}} = 3.87 \pm 0.70$$

$$3.17 < C_{pk} < 4.57$$

Our interpretation is that we are 95% confident that the true C_{pk} is somewhere between 3.17 and 4.57. A value of 1.0 or less cannot possibly fall within this interval; therefore we have good process capability. Confidence intervals can be calculated for

other capability indices, but unfortunately these intervals involve distributions that will not be covered until later chapters in this book (the chi-square distribution for C_p and the one-tailed t-distribution for C_{pu} and C_{pl}) and are beyond the scope of this book. A reference for calculating these intervals is Bissell (1990).

If sample data comes from a process that does not appear to be normally distributed it is recommended that the data be transformed to create normality or use a nonparametric alternative index (C_{npk}), which is based on the median:

$$\hat{C}_{npk} = min\left[\frac{USL - median}{p(.995) - median}, \frac{median - LSL}{median - p(.005)}\right] \qquad \text{Eq. 7.42}$$

where *p(0.995)* and *p(0.005)* are the 99.5th and 0.5th percentiles of the sample data. More Information about these tests can be found in Johnson and Kotz (1993) or Bothe (1997).

Tolerance Limits

In the discussion of confidence intervals we employed the process of estimating a range of possible values for the population mean (μ) based on sample data ($\overline{x}$). The result was an interval within which we predicted the true population mean was located. However, sometimes the investigator might be more interested in the approximate range of values for a particular population (e.g., tablets produced during a specific run). In this case, **tolerance limits** indicate the limits (above, or below) within which we would expect to find a given proportion of items from the population. It is possible to create both one-sided and two-sided limits. In the case of the two-sided limits tolerance test, with statistical manipulation it is possible to calculate two values (the lower tolerance limit or *LTL* and the upper tolerance limit or *UTL*) between which we have a certain degree of confidence that a given proportion (p) of the population will exist. With the one-sided test, we can identify a single value (X_L), above which at least a proportion (p) will occur with a certain level of confidence.

Suppose we are producing a specific batch of tablets and we know that there is a certain amount of variation in the process. Therefore, over time, the weights of the tablets will vary slightly. Obviously, it is possible to take samples during the production run and calculate the mean ($\overline{X}$) and standard deviation (S). But we would like to know lower and upper limits on the tablet weights produced during this specific batch. Therefore we need to use a test that can estimate prescribed extremes in our data, rather than estimate the true center for the population.

In most cases it is impossible to measure the entire population and know the "real world" limits for all tablets produced during a specific run. However, we can determine limits within which we would expect to find 90%, 95%, or 99% of all the tablets produced. If we wanted to know the limits for 99% of all tablets we could create a "tolerance limit for 99% of the population." However as seen previously, we can never be 100% confident in our projection based on sample data, but we can predict with 95% confidence in our decision. Therefore, it should be possible to perform a statistical test to identify a "95% tolerance limits for 99% of the

population." This reliability coefficient or confidence coefficient is sometime noted by the Greek letter gamma (γ).

If it is assumed that the weights of all tablets, when plotted, would produce a bell-shaped curve (data are normally distributed) then we can calculate the tolerance limits using the following formulas:

$$LTL = \overline{X} - KS \qquad \text{Eq. 7.43}$$

$$UTL = \overline{X} + KS \qquad \text{Eq. 7.44}$$

where *K* is a new reliability coefficient. *K*-values for the two-tailed test can be found in Table B3 (Appendix B) and represent the two-tailed test for creating both the upper and lower tolerance limits.

Table B3 is divided into three major columns, each representing our traditional confidence level ($1 - \alpha/2$). Numbers in the center third of Table B3 would be used if we wish to be 95% confident (γ) in our decision. Each major section of the table is further divided into the subsections, or columns, that represent the proportion of the population we wish to define. For example, between our tolerance limits we would expect 95%, 99%, or 99.9% of all the population outcomes to be located.

Assume we randomly sample 30 tablets during the course of a production run (Table 7.6) and find the sample mean ($\overline{X}$) and standard deviation (*S*) to be 99.96% label claim and 0.286%, respectively. Within what limits would we expect 99% of all the tablets to fall with 95% confidence? Using Table B3 for this example, with 95% certainty, we want to identify the limits within which we would expect 99% of our population. The 95% certainty is found in the center third of the table and 99% of the population is defined by the *K*-value in the sixth column from the left. If our sample size involves 30 tablets, then the *K*-value of 3.350 is found in the sixth column on the row where n = 30. The calculation for the tolerance limits would be as follows:

$$LTL = 99.96 - (3.350)(0.286) = 99.00\%$$

$$UTL = 99.96 + (3.350)(0.286) = 100.92\%$$

Thus, with 95% confidence, we would expect 99% of all tablets to contain between 99.0% and 100.9% of the label claim.

The same procedure is used for the one-tailed test, except new K-values are taken from a one-tailed table (Appendix B, Table B4) and a new equation is used to determine a proportion of the population above a given value:

$$X_L = \overline{X} - KS \qquad \text{Eq. 7.45}$$

or below a given point:

$$X_U = \overline{X} + KS \qquad \text{Eq. 7.46}$$

Table 7.6 Tablets Randomly Sampled from a Production Run (% label claim)

100.0	100.3	99.1	100.1	99.9	99.8
99.5	99.9	100.0	99.9	100.1	99.9
100.4	99.8	100.2	100.3	100.0	100.1
100.2	100.2	100.1	100.0	99.6	100.0
100.0	99.8	100.3	100.2	99.4	99.8

Using the same data presented in Table 7.6, 99% of the population would be above (with $K = 3.064$ for $p = 0.99$ and $\gamma = 0.95$):

$$X_L = 99.96 - (3.064)(0.286) = 99.08\%$$

with 95% confidence.

The previously calculated tolerance limits assume that the sample is taken from a normal distribution population. If the distribution is not normal, then the true proportion p of the population between the tolerance limits will vary from the intended p depending on the amount of departure from normality. The greater the departure from normality the greater the difference and the tolerance limits obtained tend to be substantially wider than those assuming normality. Natrella (1963) provides guidance for nonnormal conditions and statistical tables for such situations.

Using Excel® and Minitab® for Applications Discussed in this Chapter

Excel 2010 has several function (fx) options that can be used with a normal distribution and for confidence intervals for the z-test. Instead of referring to Table B2 to determine critical area under the curve for a normal distribution, various function options are available. For a standardized normal distribution, the area under the curve below any point in the distribution can be determined using **NORM.S.INV** (**NORMSINV** in versions before 2010). Excel will request probability for the area below a given point. The result will be the z-value (e.g., if provided 0.025, Excel will give –1.96). Conversely, if the interest is in the proportion of the curve falling below a certain point in a standardized normal distribution this can be determined using **NORM.S.DIST** (**NORMSDIST** in versions before 2010). Excel will request z-value and a "true" to a logic statement regarding the cumulative distribution below that point. The result will be the proportion under the curve below that point in the curve (e.g. if provided +1.96, Excel will give 0.975).

Instead of standardized normal scores, if evaluating actual data and one wishes to determine similar results where values are represented by raw scores there are two more options. Assuming a normal distribution and a known or estimated population standard deviation, the area under the curve below any point in the distribution can be determined using **NORM.INV** (**NORMINV** in versions before 2010). Excel will request the population mean and standard deviation, and the logic request of "true" for cumulative probability. The result will be a point in the distribution measures in the appropriate units. For any point in the distribution (assuming normality and given the population standard deviation) the area under the curve below that point can be

determined using **NORM.DIST** (**NORMDIST** in versions before 2010). Excel will request the value of interest, the mean and standard deviation for the population, and the logic statement regarding a cumulative distribution. The result will be the proportion below that point in the curve. Since we know that the area below and above any given point must sum to one, it is possible to determine the proportion of the area above any given point by subtracting the probability of being below that point from one (1 - NORM.DIST).

There is a function option to help create the confidence interval for a one-sample z-test. The function is **CONFIDENCE.NORM** (in Excel 2010) which will create that portion of the equation (Eq. 7.5) that includes the reliability coefficient and error term. Excel will prompt for the "alpha" (amount of Type I error), the population standard deviation and the sample size. The resultant value needs to be added and subtracted from the sample mean to create the confidence interval. Older versions of Excel labeled this function as **CONFIDENCE**.

Minitab offers an application that directly calculates the confidence interval for the one-sample z-test if the population standard deviation is known.

Stat ➤ Basic Statistics ➤ 1-sample Z…

Figure 7.8 illustrates the decisions required for a one-sample z-test for the data from Sample B in Table 7.1 with the known $\sigma = 16.8$. The variable(s) to be evaluated can be selected by double clicking on those available in the box to the left. "Graphic" options include a histogram, an individual value plot or a box plot of the data. "Options" allows one to change in the confidence interval from the default value on 95% or to create a one-tailed interval. The results for a 95% confidence interval are presented in Figure 7.9, which are the same (with slight discrepancy due to rounding) as the hand calculation earlier in this chapter.

Minitab can be used to create the control charts described in the chapter, determine the process capability indices and establish confidence and tolerance intervals. Note that there are variations of the formulas presented in this chapter and Minitab may give slightly different results, based on the formulas used and rounding during hand calculations.

A variety of control charts can be produced using Minitab including mean charts (*Xbar*), range charts (*R*...), and charts based on the pooled sample standard deviation (*S*...). All the charts are located at

Stat ➤ Control Charts ➤ Variables Charts for Subgroups

Single charts can be selected or combinations such as "Xbar-R…" for paralled mean and range charts or "Xbar-S…" for mean and standard deviations charts (an example is Figure 7.10). For a simple x-chart or moving range chart the option would be

Stat ➤ Control Charts ➤ Variables Charts for Individuals

Options include *Individual* for a simple x-chart and *Moving Range* for a moving range chart.

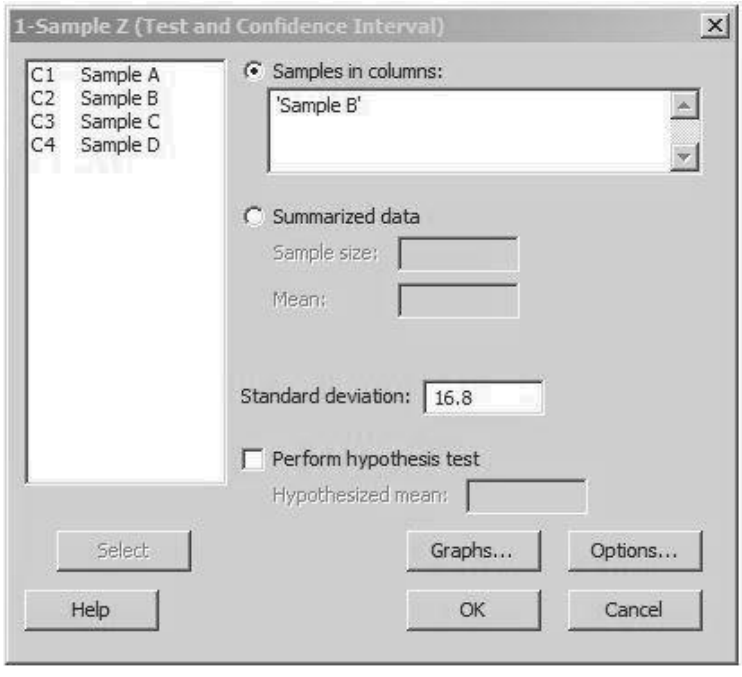


Figure 7.8 Options for one-sample z-test with Minitab.

One-Sample Z: Sample B

```
The assumed standard deviation = 16.8

Variable  N    Mean  StDev  SE Mean       95% CI
Sample B  5  752.80  21.49     7.51  (738.07, 767.53)
```

Figure 7.9 Outcome report for a one-sample z-test with Minitab.

For evaluating statistical process capability Minitab offers a variety of options and the most comprehensive is the "Capacity Sixpack". All of the previous discussion assumed that the sample came from a normally distributed population. Minitab offers the options for data from nonnormal distributions.

Stat ➤ Quality Tools ➤ Capacity Sixpack ➤ Normal

All data should be placed in one column in sequential order by the times the data were collected (*Single Column*:). The number of observations per time period can be noted by time or a consistent number for each time period (*use a constant or an ID column*). The USL and LSL must be included and data could consist of only sample information or estimates of the population added as optional information. The output

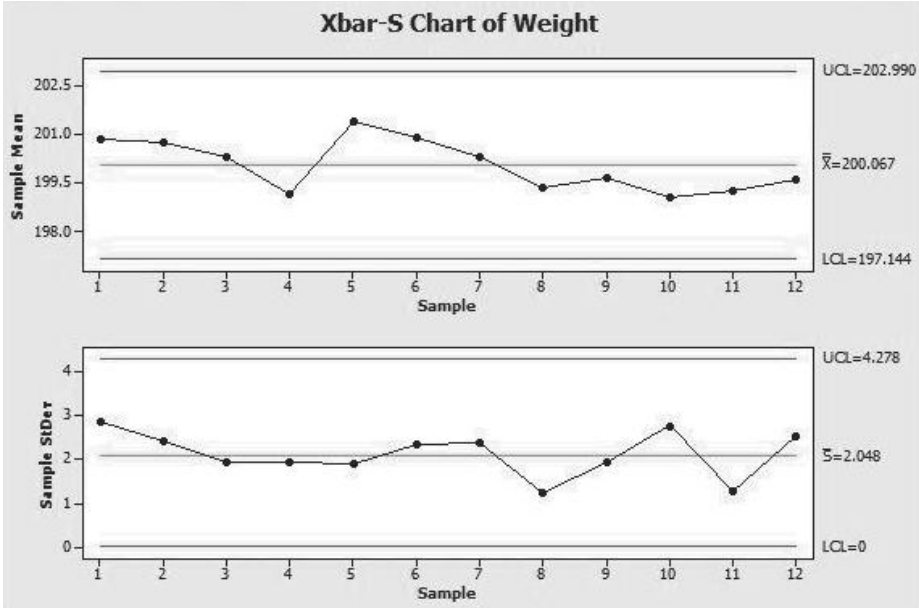


Figure 7.10 Quality control charts for the means and ranges for the data presented in Table 7.3.

form Capacity Sixpack provides much more information than discussed in this chapter, as well as visual graphics for the data. The evaluations of data presented and previously calculated for Table 7.5 are presented in Figure 7.11. In addition to the graphic presentations, note that the C_p and C_{pk} are reported in the lower right corner under "Capability Plot". If concerned that the sample distribution (best estimate of the population distribution) may not be normally distributed, a quick check to see if the Anderson Darling normality test (Chapter 6) has a *p*-value is greater than 0.05 is possible (Chapter 6).

Stat ➤ Basic Statistics ➤ Graphical Summary

The confidence interval discussed in this chapter requires knowledge of the population standard deviation (σ). The confidence intervals created by Minitab use the sample standard deviation (S) which may result in completely different outcomes based on the sample size. A discussion of these types of confidence intervals will be deferred to Chapter 9. The tolerance limits are available at

Stat ➤ Quality Tools ➤ Tolerance Interval

Enter the column containing the information and select "Option" where you can select: 1) the level of confidence in the decision; 2) the percent of products within the

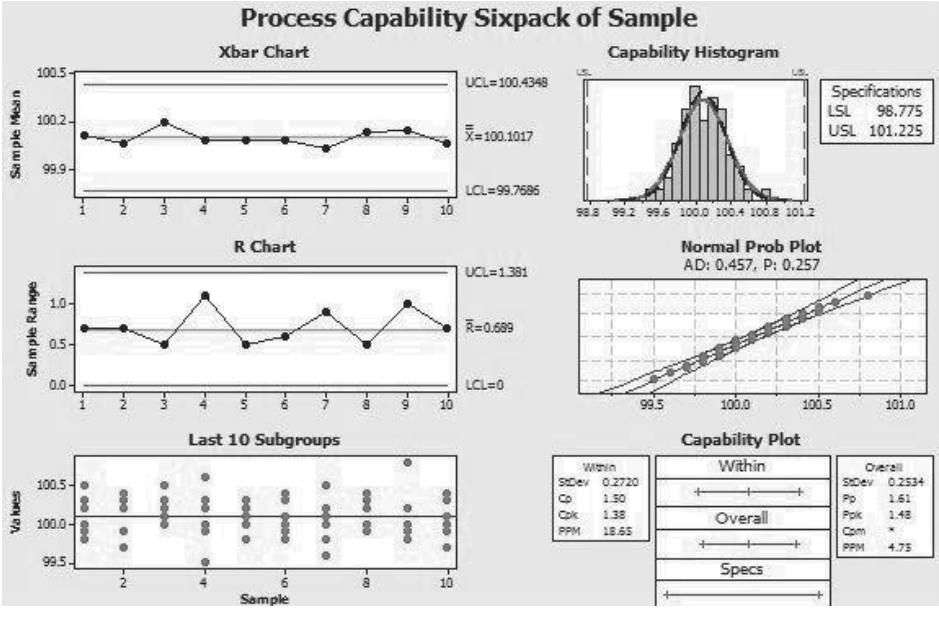


Figure 7.11 Example of output using Minitab "Capacity Sixpack."

interval; and 3) whether the tolerance interval is two-tailed or setting only a single upper or lower limit. Results from the data presented in Table 7.6 are shown in Figure 7.12.

References

Bissell, A. F. (1990). "How Reliable is Your Capability Index?" *Applied Statistics* 39:331-340.

Bolton, S. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, Inc. New York, pp. 376.

Bothe, D.R. (1997). *Measuring Process Capability: Techniques and Calculations for Quality and Manufacturing Engineers*, McGraw Hill, New York.

Kachigan, S.K. (1991). *Multivariate Statistical Analysis*, Second edition, Radius Press, New York, pp. 89, 90.

Kotz, S. and Johnson, N. L. (1993). *Process Capability Indices*, Chapman & Hall, New York.

Laffel, G. and Blumenthal, D. (1989). "The case for using industral quality management science in health care organizations," *Journal of the American Medical Association* 262:2869-2873.

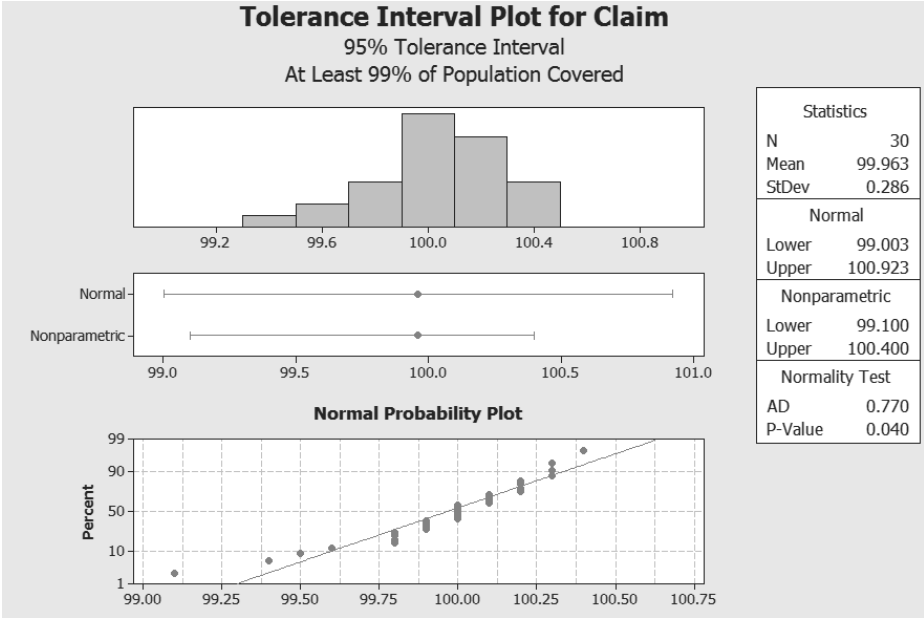


Figure 7.12 Example of output using Minitab "Tolerance Interval."

Mason, R.L., Gunst, R.F., and Hess, J.L. (1989). *Statistical Design and Analysis of Experiments with Applications to Engineering and Science*, John Wiley and Sons, New York.

Natrella, M.G. "The use of transformations," *Experimental Statistics*, National Bureau of Standards Handbook 9, U.S. Department of Commerce, Washington, DC, 1963, pp. 2-15.

Shewhart, W.A. (1931). *Economic Control of Quality of Manufactured Product*. Van Nostrand Reinhold, Princeton, NJ.

Taylor, J.K. (1987). *Quality Assurance of Chemical Measurements*, Lewis Publications, Chelsea, MI.

Wadsworth, H.M., Stephens, K.S., and Godfrey, A.B. (1986). *Modern Methods for Quality Control and Improvement*, John Wiley and Sons, New York.

Suggested Supplemental Readings

Bolton, S. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, Inc. New York, pp. 373-415.

Cheremisinoff, N.P. (1987). *Practical Statistics for Engineers and Scientists*, Technomic Publishing, Lancaster, PA, pp. 41-50.

Mason, R.L., Gunst, R.F. and Hess, J.L. (1989). *Statistical Design and Analysis of Experiments with Applications to Engineering and Science*, John Wiley and Sons, New York, pp. 62-70.

Ryan, B., Joiner, B. and Cryer, J. (2005). Minitab Handbook, Updated for Release 14, Brooks/Cole, Belmont, CA, pp. 402-418.

Taylor, J.K. (1987). *Quality Assurance of Chemical Measurements*, Lewis Publications, Chelsea, MI, pp. 129-146.

Example Problems (Answers are provided in Appendix D)

1. Assume that three assays are selected at random from the following results:

Tablet Number	Assay (mg)	Tablet Number	Assay (mg)	Tablet Number	Assay (mg)
1	75	11	73	21	80
2	74	12	77	22	75
3	72	13	75	23	76
4	78	14	74	24	73
5	78	15	72	25	79
6	74	16	74	26	76
7	75	17	77	27	73
8	77	18	76	28	75
9	76	19	74	29	76
10	78	20	77	30	75

   The resultant sample consists of tablets 05, 16, and 27.

   a. Based on this one sample and assuming that the population standard deviation (σ) is known to be 2.01, calculate 95% confidence intervals for the population mean.

   b. Again, based on this one sample, calculate the 90% and 99% confidence intervals for the population mean. How do these results compare to the 95% confidence interval for the same sample in the previous example?

   c. Assuming the true population mean (μ) is 75.47 for all 30 data points, did our one sample create confidence intervals at the 90%, 95%, and 99% levels, which included the population mean?

2. Assuming the true population mean (μ) is 75.47 and the population standard deviation (σ) is 2.01 for the question 1, calculate the following:

   a. How many different samples of n = 3 can be selected from the above population of 30 data points?

b. What would be the grand mean for all the possible samples of n = 3?

c. What would be the standard deviation for all the possible samples of n = 3?

3. During scale-up and initial production of an intravenous product in a 5-cc vial, it was found that the standard deviation for volume fill was 0.2 cc. Create a Shewhart control chart to monitor the fill rates of the production vials. Monitor the precision assuming the range is 0.6 cc ($6 \times \sigma$) and the each sample size is 10 vials.

4. During a production run of an injectable agent, 20 ampules are randomly sampled. Listed below are the volumes contained in each ampule. What are the tolerance limits, by volume, within which we would expect to find 99% of the total ampules in the run and have 99% confidence in our decision?

1.99	2.00	2.02	1.98
2.01	2.01	2.01	2.02
2.00	1.98	1.99	2.00
1.98	1.99	2.00	2.01
2.03	2.00	2.00	1.99

5. Assume that a manufacturer has set the upper and lower specification limits to be within 20% of the target for a given process (USL = 1.20 and LSL = 0.80). A random sample of 100 samples during a production run presents with $\overline{X}$ = 0.93 and S = 0.06. Is this a capable process? Assuming a normally distributed population, use the three difference indices described in the chapter.

6. During the production of a specific solid dosage form it is expected that the standard deviation (σ) for the specific strength will be approximately 3.5 mg, based on experience with the product. Twenty tablets are sampled at random from Batch #1234 and found to have a mean assay of 48.3 mg. With 95% confidence, does this sample come from a batch with the correct strength (50 mg) or is this batch subpotent?

8

Hypothesis Testing

Hypothesis testing is the process of inferring from a sample whether to reject a certain statement about a population or populations. The sample is assumed to be a small representative proportion of the total population. Hypotheses are established and two errors can occur, rejection of a true hypothesis or failing to reject a false hypothesis.

As mentioned in the beginning of Chapter 1, inferential statistical tests are intended to help answer questions confronting the researcher. Statistical analysis is based on hypotheses that are formulated and then tested. Often in published articles, these hypotheses or questions are described as the "objectives" or "purposes" of the study.

Hypothesis Testing

Sometimes referred to as **significance testing**, hypothesis testing is the process of inferring from a sample whether to reject a certain statement about the population from which the sample was taken.

Hypothesis:	Fact A
Alternative:	Fact A is false

Researchers must carefully define the population about which they plan to make inferences and then randomly select samples or subjects that should be representative of this population. For example, if 100 capsules were drawn at random from one particular batch of a medication and some analytical procedure was performed on the sample, this measurement could be considered indicative of the population. In this case, the population is only those capsules in that specific batch and cannot be generalized to other batches of the same medication. Similarly, pharmacokinetic results from a Phase I clinical trial performed only on healthy male volunteers between 18 and 45 years old, are not necessarily reflective of the responses expected in females, children, geriatric patients, or even individuals with the specific illness for which the drug is intended to treat.

In addition, with any inferential statistical test it is assumed that the individual measurements are independent of one another and any one measurement will not

influence the outcome of any other member of the sample. Also, the stated hypotheses should be free from apparent prejudgment or bias. Lastly, the hypotheses should be well-defined and clearly stated. Thus, the results of the statistical test will determine which hypothesis is correct.

The hypothesis may be rejected, meaning the evidence from the sample casts enough doubt on the hypothesis for us to say with some degree of certainty that the hypothesis is false. If the null hypothesis is rejected we accept the **alternative hypothesis**, which is the statement the researcher is usually trying to prove. On the other hand, the hypothesis may not be rejected if we are unable to statistically contradict it. Using an inferential statistic there are two possible outcomes:

H_0: Null hypothesis (**hypothesis under test**)
H_1: Alternative hypothesis (**research hypothesis**)

By convention, the **null hypothesis** is stated as no real differences in the outcomes or a relationship of zero (a null relationship). For example, if we are comparing three levels of a discrete independent variable (μ_1, μ_2, μ_3), the null hypothesis would be stated $\mu_1 = \mu_2 = \mu_3$. The evaluation then attempts to nullify the hypothesis of no significant difference in favor of an alternative research hypothesis. The type of null hypothesis will depend upon the types of variables and the outcomes the researcher is interested in measuring. Examples of other hypotheses that will be discussed in later chapters are presented in Table 8.1.

The two hypotheses must be mutually exclusive and exhaustive. They cannot both occur and they include all possible outcomes.

H_0: Hypothesis A
H_1: Hypothesis A is false

The sample values, if they are randomly sampled and measured independently, are the best estimate of the population values; therefore, in the case of two levels of a discrete independent variable:

$$\overline{X}_1 \approx \mu_1 \qquad \text{and} \qquad \overline{X}_2 \approx \mu_2$$

With the null hypothesis as a hypothesis of no difference, we are stating that the two populations under the hypothesis are the same:

$$H_0: \ \mu_1 = \mu_2$$

We are really testing our sample data $\overline{X}_1 = \overline{X}_2$ and inferring that these data are representative of the population $\mu_1 = \mu_2$, allowing for a certain amount of error in our decision. The alternative hypothesis is either accepted or rejected based upon the decision about the hypothesis under test. Thus, an **inference** can be defined as any conclusion that is drawn from a statistical evaluation.

Statistics from our sample provide us with a basis for estimating the probability that some observed difference between samples should be expected due to sampling

Table 8.1 Examples of Null Hypotheses

Chapters	Statistical Tests	Null Hypothesis
9	Two-sample t-test	$\mu_1 = \mu_2$ or $\mu_1 - \mu_2 = 0$
9	Paired t-test	$\mu_d = 0$
10	One-way analysis of variance	$\mu_1 = \mu_2 = \mu_3 = \ldots \mu_k$
13	Correlation	$r_{xy} = 0$
14	Linear regression	No linear regression
16-18	Tests of association	No association
21	Nonparametric tests	Same population

error. Two approaches could be used: 1) create a confidence interval or 2) establish a ratio and compare the resultant test statistic to a predetermined critical value. The former has already been employed in the previous chapter, with the establishment of a confidence interval for a population parameter based on sample results.

$$\begin{matrix} \textit{Population} \\ \textit{Mean} \end{matrix} = \begin{matrix} \textit{Estimate} \\ \textit{Sample Mean} \end{matrix} \pm \begin{matrix} \textit{Reliability} \\ \textit{Coefficient} \end{matrix} \times \begin{matrix} \textit{Standard} \\ \textit{Error} \end{matrix}$$

In the second method we would calculate a "test statistic" (a value based on the manipulation of sample data). This value is compared to a preset "critical" value (usually found in a special table) based on a specific acceptable error rate (e.g., 5%). In most cases this involves a ratio, simplified to the following:

$$\textit{Test Statistic} = \frac{\textit{Measure of Comparison}}{\textit{Standard Error}}$$

If the test statistic is extremely rare it will be to the extreme of our critical value and we will reject the hypothesis under test in favor of the research hypothesis, which is the only possible alternative. For example, assume that we are interested in the hypothesis H_0: $\mu_1 = \mu_2$. If we calculate a test statistic to evaluate this hypothesis we would expect our calculated statistic to equal zero if the two populations are identical. As this test statistic becomes larger, or to an extreme of zero (either in the positive or negative direction), it becomes more likely that the two populations are not equal. In other words, as the absolute value of the calculated test statistic becomes large, there is a smaller probability that H_0 is true and that this difference is not due to chance error alone. The critical values for most statistical tests indicate an extreme at which we reject H_0 and conclude that H_1 is the true situation (in this case that $\mu_1 \neq \mu_2$).

The statistical test results have only two possible outcomes, either we cannot reject H_0 or we reject H_0 in favor of H_1. At the same time, if all the facts were known (the real world) or we had data for the entire population(s), the hypothesis (H_0) is either true or false for the population(s) that the sample represents. This is represented

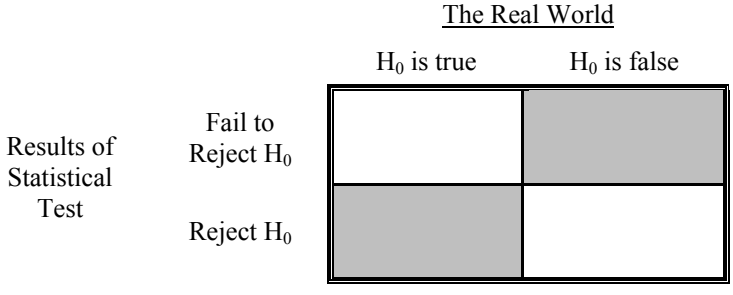


Figure 8.1 Types of errors that can occur with hypothesis testing.

in Figure 8.1 where we want our results to fall into either of the two clear areas. If the results fall into either of the shaded areas, these are considered mistakes or errors.

An analogy to hypothesis testing can be seen in American jurisprudence (Kachigan, 1991). Illustrated below are the possible results from a jury trial.

H_0: Person is innocent of crime
H_1: Person is guilty of crime

During the trial, the jury will be presented with data (information, exhibits, testimonies, evidence) that will help, or hinder, their decision-making process (Figure 8.2). The original hypothesis is that the person is innocent until proven guilty. Evidence will conflict and the jury will never know the true situation, but will be required to render a decision. They will find the defendant either guilty or not guilty, when in fact if all the data were known, the person is either guilty or innocent of the crime. Two errors are possible: 1) sending an innocent person to prison (error I) or 2) freeing a guilty person (error II). For most, the former error would be the more grievous of the two mistakes.

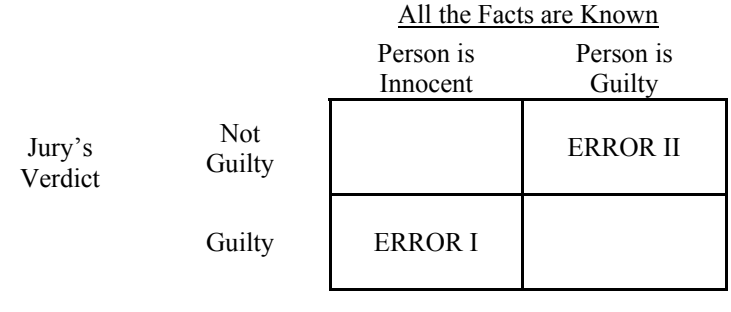


Figure 8.2 Jurisprudence example.

Note that in this analogy, if the jury fails to find the person guilty their decision is not that the person is "innocent." Instead they render a verdict of "not guilty" (they failed to have enough evidence to prove guilt). In a similar vein, the decision is not to accept a null hypothesis, but to fail to reject it. If we cannot reject the null hypothesis, it does not prove that the statement is actually true. It only indicates that there is insufficient evidence to justify rejection. One cannot prove a null hypothesis and can only fail to reject it.

It is hoped that outcomes from our court system will end in the clear areas of the previous illustration and the innocent are freed and the guilty sent to jail. Similarly, it is hoped that the results of our statistical analysis will not fall into the shaded error regions. Like our system of jurisprudence, a statistical test can only disprove the null hypothesis; it can never prove the hypothesis is true.

Types of Errors

Similar to our jurisprudence example, there are two possible errors associated with hypothesis testing. Type I error is the probability of rejecting a true null hypothesis (H_0) and Type II error is the probability of accepting a false H_0. Type I error is also called the **level of significance** and uses the symbol α or p. Like sending an innocent person to jail, this is the most important error to minimize or control. Fortunately, the researcher has more control over the amount of acceptable Type I error. Alternatively, our level of confidence in our decision, or **confidence level**, is $1 - \alpha$ (the probability of all outcomes less Type I error).

Type II error is symbolized using the Greek letter beta (β). The probability of rejecting a false H_0 is called **power** $(1 - \beta)$. In hypothesis testing we always want to minimize the α and maximize $1 - \beta$. Continuing with our previous example of two populations being equal or not equal, the hypotheses are

$$H_0: \mu_1 = \mu_2$$
$$H_1: \mu_1 \neq \mu_2$$

with the four potential outcomes presented in Figure 8.3:

$1 - \alpha$: Do not reject H_0 when in fact $\mu_1 = \mu_2$ is true
α: Reject H_0 when in fact $\mu_1 = \mu_2$ is true
$1 - \beta$: Reject H_0 when in fact $\mu_1 = \mu_2$ is false
β: Do not reject H_0 when in fact $\mu_1 = \mu_2$ is false

In Chapter 3 we discussed the different types of errors in research (random and systematic). Statistics allow us to estimate the extent of our random errors or establish acceptable levels of random errors. Systematic error is controlled through the experimental design used in the study (including random sampling and independence). In many cases systematic errors are predictable and often unidirectional. Random errors are unpredictable and relate to sample deviations that were discussed in the previous chapter.

Results of Statistical Test	The Real World: H_0 is true	The Real World: H_0 is false
Fail to Reject H_0	$1 - \alpha$	β
Reject H_0	α, p	$1 - \beta$

Figure 8.3 Illustration of possible results from hypothesis testing.

Type I Error

The Type I error rate (α) should be established before making statistical computations. By convention, a probability of less than 5% ($\alpha < 0.05$ or a 1/20 chance) is usually considered an unlikely event. However, we may wish to establish more stringent criteria (i.e., 0.01, 0.001) or a less demanding level (e.g., 0.10, 0.20) depending on the type of experiment and impact of erroneous decisions. For the purposes of this book, the error rates will usually be established at either 0.05 or 0.01. The term "statistically significant" is used to indicate that the sample data is incompatible with the null hypothesis for the proposed population and that it is rejected in favor of the alternate hypothesis.

If Type I error (α) must be chosen before the data is gathered, it prevents the researcher from choosing a significance level to fit the test statistic resulting from statistical testing of the data. A **decision rule** is established, which is a statement in hypothesis testing that determines whether the hypothesis under test should be rejected; for example, "with $\alpha = 0.05$, reject H_0 if … ." After the data is analyzed (by hand or via computer) the p-value is reported to indicate the amount of possible error in the decision if the null hypothesis is rejected. Both symbols represent Type I errors: α is an *a priori* determination and the *p* value is a *post hoc* measure of error.

In the previous illustration of pharmacokinetic data (Table 4.3), we found that there were over 234 million possible samples ($n = 5$), which produced a normally distributed array of possible outcomes. Using any one of these samples it is possible to estimate the population mean (Eq. 7.5):

$$\mu = \overline{X} \pm Z_{(1-\alpha/2)} \times \frac{\sigma}{\sqrt{n}}$$

Using this equation we can predict a range of possible values within which the true population would fall. If we set the reliability coefficient to $\alpha = 0.05$, then 95% of the possible samples would create intervals that correctly include the population mean (μ) based on the sample mean ($\overline{X}$). Conversely, only 5% of the potential samples produce estimated ranges that do not include the true population mean.

As will be shown in the next chapter, the reverse of this procedure is to use a

statistical formula, calculate a "test statistic" and then compare it to a critical number from a specific table in Appendix B. If the "statistic" is to the extreme of the table value, H_0 is rejected. Again, if we allow for a 5% Type I error rate, 95% of the time our results should be correct. However, through sampling distribution and random error, we could still be wrong 5% (α) of the time due to chance error in sampling.

The **acceptance region** is that area in a statistical distribution where the outcomes will not lead to a rejection of the hypothesis under test. In contrast, the **rejection region**, or **critical region**, represents outcomes in a statistical distribution, which lead to the rejection of the hypothesis under test and acceptance of the alternative hypothesis. In other words, outcomes in the acceptance region could occur as a result of random or chance error. However, the likelihood of an occurrence falling in the critical region is so rare that this result cannot be attributed to chance alone.

The critical value is that value in a statistical test that divides the range of all possible values into an acceptance and a rejection region for the purposes of hypothesis testing. For example (to be further discussed in Chapter 10):

$$\text{With } \alpha = .05, \text{ reject } H_0 \text{ if } F > F_{3,120}(0.95) = 2.68$$

In this particular case, if "F" (which is calculated through a mathematical procedure) is greater than "$F_{3,120}(0.95)$" (which is found in a statistical table), then the null hypothesis is rejected in favor of the alternative.

To illustrate the above discussion, assume we are testing the fact that two samples come from different populations ($\mu_A \neq \mu_B$). Our null hypothesis would be that the two populations are equal and, if mutually exclusive and exhaustive, the only alternate hypothesis would be that they are not the same.

$$H_0: \ \mu_A = \mu_B$$
$$H_1: \ \mu_A \neq \mu_B$$

The best, and only, estimate of the populations are the two sample means ($\overline{X}_A$, $\overline{X}_B$). Based on the discussion in the previous chapter on sampling distributions, we know that sample means can vary and this variability is the standard error of the mean. Obviously, if the two sample means are the same we cannot reject the null hypothesis. But what if one is 10% larger than the other? Or 20%? Or even 100%? Where do we "draw the line" and establish a point at which we must reject the null hypothesis of equality? At what point can the difference no longer be attributed to random error or chance alone? As illustrated in Figure 8.4, this point is our critical value. If we exceed this point there is a significant difference. If the sample difference is zero or less than the critical value, then this difference could be attributed to chance error due to the potential distribution associated with samples.

Statistics provides us with tools for making statements about our certainty that there are real differences, as opposed to only chance differences between populations based on sample observations. The decision rule, with assistance from tables in Appendix B, establishes the **critical value**. The numerical manipulations presented in the following chapters will produce the **test statistic**. If we fail to reject the null

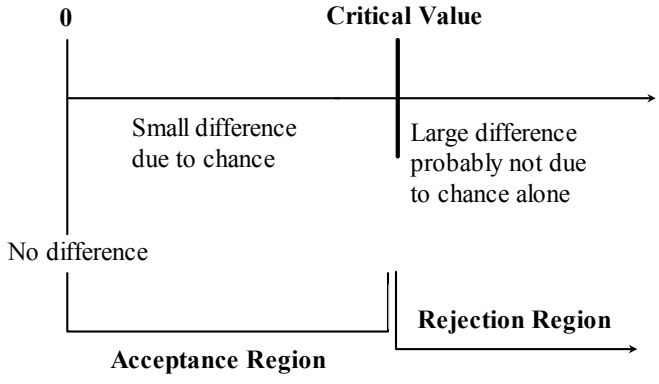


Figure 8.4 The critical value.

hypothesis, then there is insufficient evidence available to conclude that H_0 is false.

Our hypothesis can be bidirectional or unidirectional. For example, assume we are not making a prediction that one outcome is better or worse than the other. Using the previous example:

$$H_0: \mu_A = \mu_B$$
$$H_1: \mu_A \neq \mu_B$$

In this case the alternate hypothesis only measures that there is a difference and μ_A could be significantly larger or smaller than μ_B. If $\alpha = 0.05$, then we need to divide it equally between the two extremes of our sampling distribution of outcomes and create two rejection regions (Figure 8.5). We then demarcate finite regions of their distribution. The range of these demarcations define the limits beyond which the null hypothesis will be rejected.

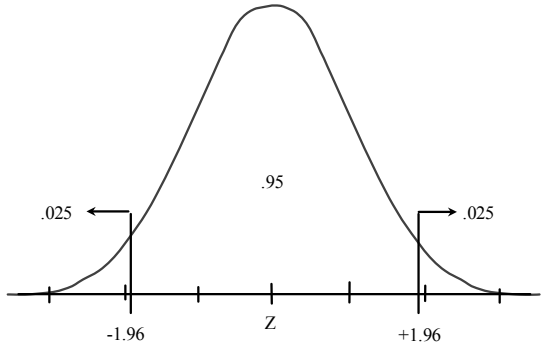


Figure 8.5 Two-tailed 95% confidence interval.

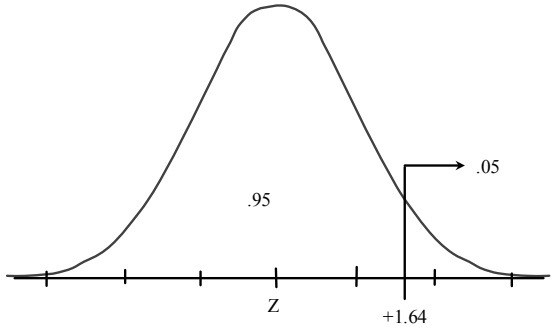


Figure 8.6 One-tailed 95% confidence interval.

An alternative approach would be to create a **directional hypothesis** where we predict that one population is larger or smaller than the other:

$$H_0: \quad \mu_A \leq \mu_B$$
$$H_1: \quad \mu_A > \mu_B$$

In this case, if we reject H_0 we would conclude that population A is significantly larger than population B (Figure 8.6). Also referred to as **truncated**, **curtailed**, or **one-sided hypotheses**, we must be absolutely certain, usually on logical grounds, that the third omitted outcome ($\mu_A < \mu_B$) has a zero probability of occurring. The one-tailed test should never be used unless there is a specific reason for being directional.

Type II Error and Power

Type II error and power are closely associated with sample size and the amount of difference the researcher wishes to detect. We are primarily interested in power, which is the complement of Type II error (β). Symbolized as $1 - \beta$, **power** is the ability of a statistical test to identify a significant difference if such a difference truly exists. It is dependent on several factors including the size of the groups as well as the size of the difference in outcomes. In hypothesis testing, it is important to have a sizable sample to allow statistical tests to show significant differences where they exist.

Power is more difficult to understand than Type I error, where we simply select from a statistical table the amount of error we will tolerate in rejecting a true null hypotheses. We are concerned with the ability to reject a false H_0. In the simplest example (H_0: $\mu_1 = \mu_2$), we need the ability to reject this hypothesis if it is false and accept the alternative hypothesis (H_1: $\mu_1 \neq \mu_2$).

Let us assume for the moment that we know, or can approximate the population variance as a measure of dispersion. We could estimate our Type II error using the following equation:

$$z_\beta = \frac{\delta}{\sqrt{\frac{2\sigma^2}{n}}} - z_{\alpha/2} \qquad \text{Eq. 8.1}$$

In this equation, σ^2 represents the variance of the population (assuming the two samples are the same $\{\mu_1=\mu_2\}$, then the dispersion will be the same for σ_1 and σ_2); δ is the detectable difference we want to be able to identify if $\mu_1 \neq \mu_2$; n is the sample size for each level of the independent variable (assuming equal n); and $z_{\alpha/2}$ is the amount of Type I error preselected for our analysis. $z_{\alpha/2}$ is expressed as a z-value from the normal standardized distribution (Table B2 in Appendix B). Obviously, z_β represents the amount of Type II error, again expressed as a value in the normalized standard distribution and reporting the probability (β) of being greater than z_β. The complement of β would be the power associated with our statistical test $(1 - \beta)$.

As seen in Eq. 8.1, Type II error is a one-tailed distribution (z_β); whereas the Type I error rate may be set either unidirectional (z_α) or bidirectional ($z_{\alpha/2}$). This can be explained through using the simplest hypothesis (H_0: $\mu_1 - \mu_2 = 0$), where we want to be able to reject this hypothesis if there is a true difference and accept the alternative hypothesis (H_0: $\mu_1 - \mu_2 \neq 0$). The question that needs to be asked is how large should the difference be in order to accept this alternative hypothesis? Figure 8.7 illustrates the relationship between Type I and II errors. In this figure, Type I error is divided equally between the two tails of our null hypothesis and the Type II errors to the left side of the distribution for the alternative hypothesis (if it is true). Notice the common point where both types of errors end, which becomes our decision point to accept or reject the null hypothesis.

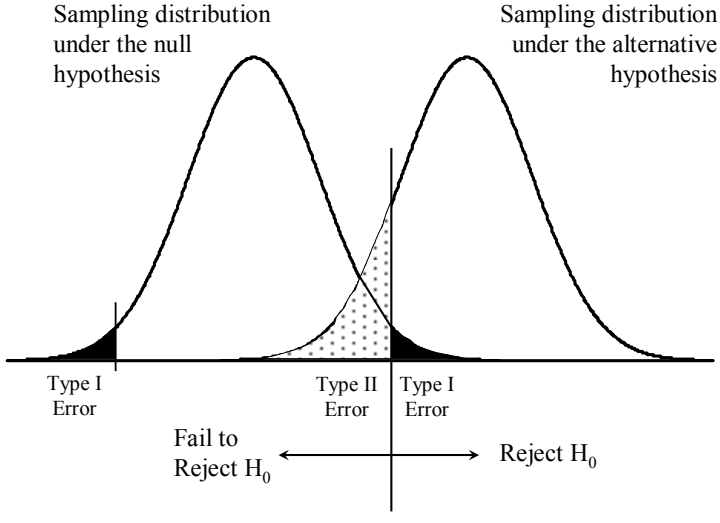


Figure 8.7 Comparisons of sampling distributions under H_0 and H_1.

To illustrate this point, assume we are comparing samples from two tablet production runs (batches) and are concerned that there might be a difference in the average weights of the tablets. Based on historical data for the production of this dosage form, we expect a standard deviation of approximately 8 mg ($\sigma^2 = 64$). If the two runs are not the same with respect to tablet weight ($\mu_1 \neq \mu_2$), we want to be able to identify true population differences as small as 10 mg (δ). At the same time, we would like to be 95% confident in our decision ($z_{\alpha/2} = 1.96$). We sample 6 tablets from each batch. The Type II error calculation is as follows:

$$z_\beta = \frac{\delta}{\sqrt{\frac{2\sigma^2}{n}}} - z_{\alpha/2}$$

$$z_\beta = \frac{10}{\sqrt{\frac{2(8)^2}{6}}} - 1.96$$

$$z_\beta = 2.17 - 1.96 = 0.21$$

The value z_β represents the point on a normal distribution, below which the β proportion of the curve falls. In other words the probability of being below this point is the Type II error. Looking at the normal standardized distribution table we see that the proportion of the curve between 0 and $z = 0.21$ is 0.0832. The area below the curve (Table B2, Appendix B), representing the Type II error, is 0.4168 (0.5000 – 0.0832). Thus, for this particular problem we have power less than 60% (1 – 0.4168) to detect a 10-mg difference, if such a difference exists.

As will be discussed later, if we can increase our sample size we will increase our power. Let us assume that we double our sample, collecting 12 tablets from each batch, then z_β would be:

$$z_\beta = \frac{10}{\sqrt{\frac{2(8)^2}{12}}} - 1.96 = 3.06 - 1.96 = 1.10$$

Once again referring to the normal standardized distribution table, we see that the proportion of the curve between 0 and $z = 1.10$ is 0.3643. In this case the area below the curve, representing the Type II error, is 0.1357 (0.5000 – 0.3643). In this second case, by doubling the sample size we produce power greater than 86% (1 – 0.1357) to detect a 10-mg difference, if such a difference exists.

We can modify Eq. 8.1 slightly to identify the sample size required to produce a given power.

$$n \geq \frac{2\sigma^2}{\delta^2}(z_{\alpha/2} + z_{\beta})^2 \qquad \text{Eq. 8.2}$$

Using the same example, assume that we still wish to be able to detect a 10-mg difference between the two batches with 95% confidence ($z_{\alpha/2} = 1.96$). In this case, we also wish to have at least 80% power (the ability to reject H_0 when H_0 is false). Therefore β (1 − power) is the point on our normal standardized distribution below which 20% (or 0.20 proportion of the area of the curve) falls. At the same time 0.30 will fall between that point and 0 (0.50 − 0.20). Once again looking at Table B2 in Appendix B we find that proportion (0.2995) to be located at a *z*-value of 0.84. Note in Figure 8.7 that the critical values are based on $\alpha/2$ (two-tailed) and 1 − β (one-tailed).

$$n \geq \frac{2\sigma^2}{\delta^2}(z_{\alpha/2} + z_{\beta})^2 = \frac{2(64)}{(10)^2}(1.96 + 0.84)^2$$

$$n \geq (1.28)(2.80)^2 = 10.04$$

Therefore, to ensure a power of at least 80% we should have 11 samples (rounding up the 10.04 to the next whole number).

Four characteristics are considered regarding power: 1) sample size; 2) the dispersion of the data; 3) amount of Type I error; and 4) the amount of difference to be detected.

$$Type\ II\ Error = \frac{Detectable\ Difference}{\sqrt{\frac{Dispersion}{n}}} + Type\ I\ Error \qquad \text{Eq. 8.3}$$

Using Eq. 8.1, it is possible to modify one of the four factors affecting power to detect differences: 1) as the detectable difference increases the power will increase; 2) as sample size increases the denominator decreases and the power once again increases; 3) as the dispersion increases the denominator increases and the power decreases; and 4) as the amount of Type I error decreases it will result in a decreased power. These are graphically illustrated in the following series of figures (Figures 8.8 through 8.11).

The only way to reduce both types of error is to increase the sample size. Thus, for a given level of significance (α), larger sample sizes will result in greater power. Using data from the previous example, Figure 8.8 illustrates the importance of sample size. With α, δ and the dispersion remaining the same, as we increase the sample size, the Type II error decreases and the power increases. Therefore, small sample sizes generally lack statistical power and are more likely to fail to identify important differences because the test results will be statistically insignificant.

Obviously, if they exist, it is easier to detect large differences than very small ones. The importance of detectable differences is seen in Figure 8.9 where the sample

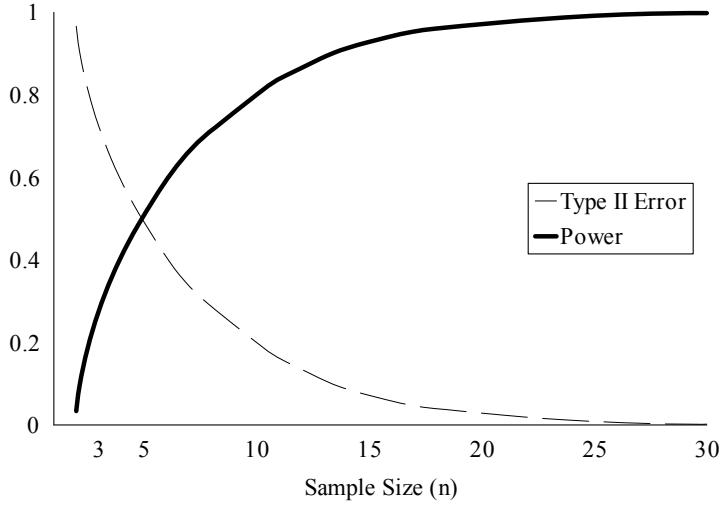


Figure 8.8 Effect of changes in sample size on statistical power (constants $\delta = 10$, $\sigma^2 = 68$, $\alpha = 0.05$).

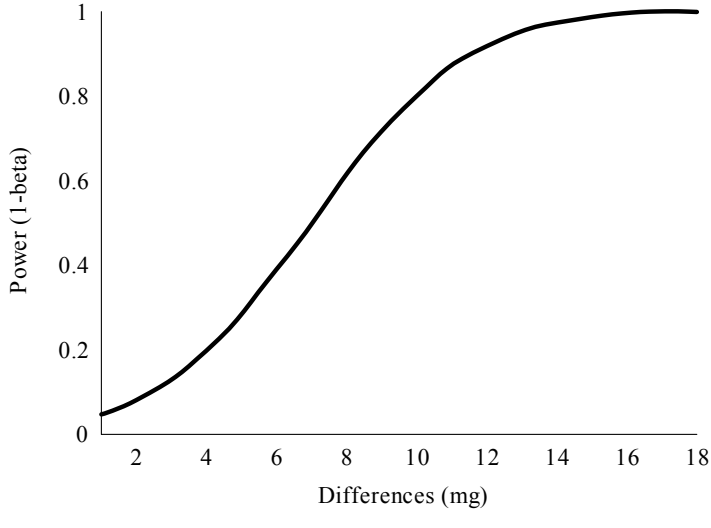


Figure 8.9 Effect of changes in detectable differences on statistical power (constants n =10, $\sigma 2 = 64$, $\alpha = 0.05$).

size is constant (n = 10), the estimated variance is 64 and α remains constant at 0.05. The only change is the amount of difference we wish to detect. As difference increases, power also increases. If we are interested in detecting a difference between two populations, obviously the larger the difference, the easier it is to detect. Again,

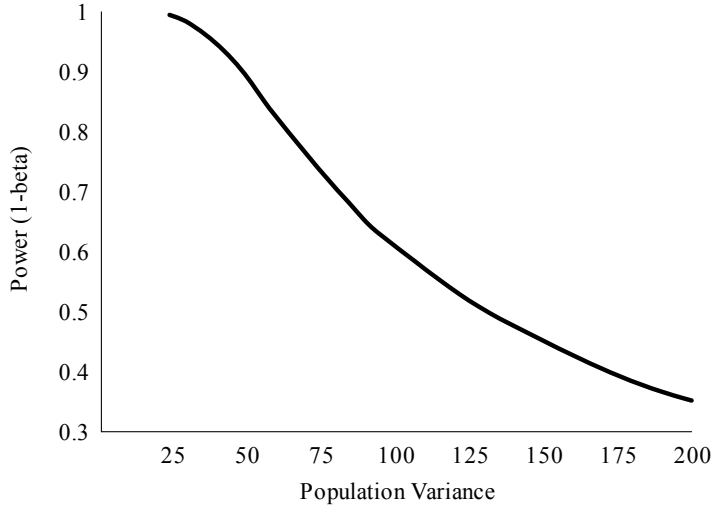


Figure 8.10 Effect of changes in variance on statistical power (constants n = 10, $\delta = 10$, $\alpha = 0.05$).

the question we must ask ourselves is how small a difference do we want to be able to detect or how small should a difference be to be worth detecting?

As seen in Eq. 8.1, the amount of dispersion or uncertainty can also influence power. Figure 8.10 displays the decrease in power that is associated with greater variance in the sample data. Conversely, as the variance within the population decreases, the power of the test to detect a fixed difference (δ) will increase.

Generally Type II error is neither known nor specified in an experimental design. Both types of error (I and II) are related inversely to each other. If we lower α without changing the sample size, we will increase the probability of having a Type II error and consequently decrease the power. Figure 8.11 illustrates changes in power for two different levels of Type I error with increasing sample sizes. As we increase our confidence that there is a difference (making α smaller), we also increase the chance of missing a true difference, increasing β or decreasing power.

In addition to the above four factors, the number of treatment levels must also be factored in when considering designs that are more complicated than comparing two levels of a discrete independent variable.

The size of the sample, or number of observations, is extremely important to statistical research design. We can increase the power of a statistical test without sacrificing our confidence level ($1 - \alpha$) solely by increasing our sample size. Unfortunately, sometimes the sample sizes required to satisfy the desired power are extremely large with respect to time and cost considerations. The ways to reduce the required sample size are to increase the precision of the test (or instrument) or to increase the minimal acceptable level of detectable differences.

The problem with the previous example is that the formula is limited to only two levels of discrete independent variables. Also, we must know the population variance.

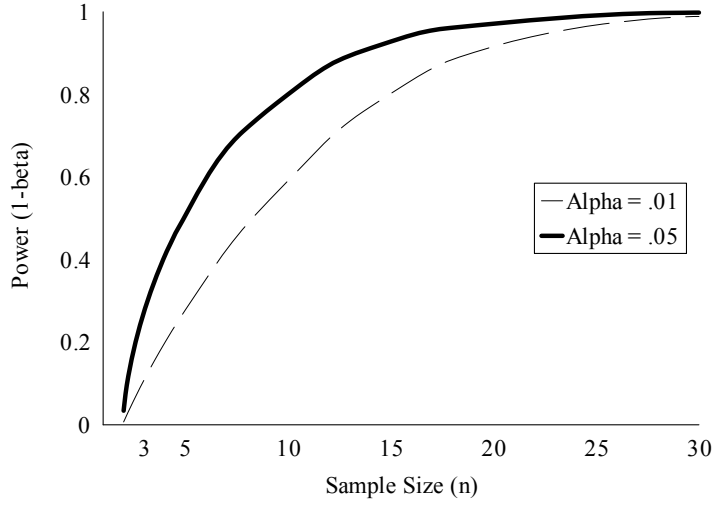


Figure 8.11 Effect of changes in sample sizes on statistical power for two levels of Type I error (constants $\delta = 10$, $\sigma^2 = 68$).

Therefore, Eq. 8.1 represents only one unique method of determining Type II error (specifically for the alternative hypothesis that two population means are not equal). Numerous formulas exist that can be used to calculate the appropriate sample size under different criteria. These include power curves presented by Kirk (1968), based on α, $1 - \beta$, and the number of levels of the independent variable; and Young's nomograms (1983) for sample size determination. An excellent reference for many of these methods is presented by Jerrold Zar. Listed in Table 8.2 are pages from Zar's book for power and sample size determination for many of the statistical tests presented in the remainder of this book. A discussion of power and sample size for binomial tests was given by Bolton (2004).

Power is often calculated after the experiment has been completed. In these *post hoc* cases the sample standard deviation can be substituted for σ. In general, Type II error is neither known nor specified in an experimental design. For a given sample size, α and β are inversely proportional. If we lower α without changing the sample size, we will increase the probability of a Type II error and consequently decrease the power ($1 - \beta$). The more "powerful" the test, the better the chances are that the null hypothesis will be rejected, when the null hypothesis is in fact false. The greater the power, the more sensitive the statistical test.

Even though it is important to have a large enough sample size to be able to detect important differences, having too large a sample size may result in a significant finding even though for all practical purposes the difference is unimportant, thus producing results that are statistically significant, but have clinically insignificant differences. For example, in a recent article by Al-Khatib and others (2011), a demographic significant difference ($p < 0.001$) was reported between the ages for the two groups compared. Data from a non-evidence-based group had an average age of 67 years, whereas the average age for the evidence-based group was 66 years. Was

Table 8.2 Formulas for Determination of Statistical Power and Sample Size Selection

Chapter	Statistical Test	Page(s) in Zar
9	One-sample t-test	114-118
9	Two-sample t-test	147-151
9	Paired t-test	182
10	One-way analysis of variance	207-214
12	Two-way analysis of variance	275-277
13	Correlation	386-390
14	Linear regression	355
15	One-sample Z-test of proportions	539-542
15	Two-sample Z-test of proportions	552-555

From: Zar, J.H. (2010). *Biostatistical Analysis*, Fifth edition, Prentice Hall, Upper Saddle River, NJ.

the one year difference significant? Yes, if one considers the sample size (25,145 for the first groups and n = 86,562 for the second). Clearly the large sample size resulted in the significant difference, even though a one year age difference was probably unimportant.

One of the advantages of statistical analysis and hypothesis testing is that its principles are general and applicable to data from any field of study (i.e., biological, physical, or behavioral). All of the tests presented in this book can be applied to data regardless of the source of the information or the branch of science or academia from which it was derived. As mentioned in Chapter 1 and seen in Appendix A, the most important first step in selecting the most appropriate statistic is to identify the independent and dependent variables and define them as discrete or continuous.

In evaluating different tests for analyzing the same data set, we would like to use the most efficient test possible. **Efficiency** is a relative term, but provides a method for comparing the same sample size required with different tests that will provide the same amount of Type I and Type II errors. Obviously the test requiring the smallest sample size is the most efficient. Assume that Test A and Test B represent two statistical methods for testing the same H_1 against the same null H_0, with the same critical levels for α and β. The **relative efficiency** of Test A to Test B is the ratio of the sample size (n_1/n_2). The problem is finding power determination to estimate the sample size required for the desired levels of α and β. Finally, we can assess a potential bias nature of a test by evaluating α and β. An **unbiased test** is one in which the probability of rejecting H_0 when H_0 is false is always greater than or equal to the probability of rejecting H_0 when H_0 is true (i.e., $1 - \beta \geq \alpha$). It is possible to have a test result in $p = 0.60$ (obviously not significant) and a failure to reject the null hypothesis. But at the same time we calculate the power (after the fact) to be 0.75. In this example we would have a biased test result.

Experimental Errors and Propagation of Errors

In evaluating the results of data collected in a study, the values for the data will be dependent upon the accuracy of the experimental measurement. This accuracy will be reflected in the subsequent conclusions and recommendations based on the study. The **experimental error** is the amount of uncertainty that is associated with any data set. The **true error** is the difference between the observed measurement and the true value of that quantity. For example this difference could be between a sample mean and the actual mean of the population from which the sample was taken. In the real world that true value is rarely known. This true error is composed of both systematic error and random error. As discussed previously, inaccuracy is a reflection of systematic error and can be reduced or eliminated using care in designing a study and measuring the results. Random error is represented by Type I and Type II errors and is the uncertainty inherent in the variable being measured. One of the most effective ways to reduce random errors is through repeated measures or by replicating the experiment.

The process of error analysis is studying and evaluating experimental errors (both systematic and random). The primary goals are to: 1) estimate the magnitude of experimental errors and 2) reduce the amount of errors. The challenge is to minimize errors so that proper conclusion can be drawn from the experiment. Since "good" science is based on measurements and the interpretation of those measurements, it is important to keep uncertainties at a minimum. The topic of systematic error has already been discussed in Chapter 3. Control of random error has been the focus of this chapter.

Random error exists in all measurement; if none exists, one needs a measurement instrument with greater precision. As seen in Chapter 5 the random error in a sample can be expressed by either the standard deviation (*S*) or the *RSD* (relative standard deviation). If we can directly measure our variable of interest, the *S* and *RSD* provide an assessment of the precision of the measurement. What if we cannot measure something directly, but need to calculate it based on several different variables? For example, consider the area of a flat rectangular surface. In this case we could measure the length and width of the rectangle and compute the area as $A = L \cdot W$. However, several different measures of these same distances could have variable results (by different individuals, at different times, under different conditions, using different instruments, etc.). Both the length and width measurements could have an amount of associated uncertainty (measured as the standard deviation):

$$L = \overline{X}_L \pm S_L \qquad W = \overline{X}_W \pm S_W$$

For calculation of the area our best estimate would involve the averages for the length and width.

$$A = \overline{X}_L \cdot \overline{X}_W$$

What about the measure of dispersion for this area? Could we simply sum the two standard deviations (for length and width), take the larger of the two, or create some

average standard deviation? To handle this type of situation we need a method for dealing with the proliferation of error associated with the two dispersions that are related to each other, in this example the calculation of the area.

Often in experiments the final results may not be measurable, but are the results of some adding, subtracting, multiplying, or dividing of the results of the other original measurements. It becomes necessary to estimate the errors based on these types of mathematical manipulations. This combining of uncertainties from separate measures is referred to as **propagation of errors**. It is the resultant measure of dispersion where the results are dependent on a number of different independent variables, each of which is measured. Each independent variable will be associated with the total measure of uncertainty (error). Similar to the previous example of surface areas, error components are estimated from repeating the measurement several times (or taking numerous samples) to calculate a measure of dispersion for the results (sample standard deviations or the relative standard deviations). The question is how to handle the variability of these independent variables.

Assume there is a serial progression and the first step involves a certain amount of error. The error would be compounded with the error associated with the second step in the procedure. This is further compounded with the third step, and so forth until the last step in a procedure.

There are two methods for dealing with the propagation of error and the choice depends on the mathematical process that takes place. For addition or subtraction (i.e., the previous serial example) the error term is based on the uncertainty measured by the variances of the independent measurements:

$$S_{Total} = \sqrt{S_1^2 + S_2^2 + S_3^2 + ...S_K^2} \qquad \text{Eq. 8.4}$$

For multiplication or division (e.g., surface area example) the error term is based on the relative uncertainty (RSD) of the independent measurements:

$$RSD_{Total} = \sqrt{RSD_1^2 + RSD_2^2 + RSD_3^2 + ...RSD_K^2} \qquad \text{Eq. 8.5}$$

This relative term is then converted to the standard deviation:

$$S_{Total} = \frac{RSD_{Total}(Final\ Mean)}{100} \qquad \text{Eq. 8.6}$$

To illustrate these methods consider the following example. To calculate the molarity for mercuric nitrate it is necessary to calculate both a mass and volume measurement:

$$Molarity = \frac{Mass\ NaCl\ (mg)}{(58.44)(2)(ml_{titrant} - ml_{blank})}$$

Table 8.3 Results of Experiment for Mercuric Nitrate

Mass (NaCl)	ml titrant	ml blank	ml used	Molarity
16.24	6.5045	0.1904	6.3141	0.02201
16.22	6.5143	0.1904	6.3239	0.02194
16.27	6.5287	0.1904	6.3383	0.02196
16.17	6.5017	0.1904	6.3113	0.02192
16.23	6.5157	0.1904	6.3253	0.02195
16.24	6.5293	0.1904	6.3389	0.02192
16.228	= Mean =		6.3253	0.02195
0.033	= SD =		0.0116	0.00003

Taking six samples, the mass (weight) will have a variance term and the volume will also have some variability. At the same time the blank used to measure the volume will vary. Listed in Table 8.3 are the results of the experiment. Without compensating for propagation of error the results for the six samples would be a mean of 0.02195 ± a standard deviation of 0.00003. However, the molarity is based on a division of the mass by the volume, but first there is the issue of variability in the volume term. The ml blank was based on the following triplicate measure:

ml titrant	ml blank	ml used
0.2003	0	0.2003
0.1754	0	0.1754
0.1956	0	0.1956
	mean =	0.1904
	SD =	0.0132

Therefore the propagation of error for the ($ml_{titrant} - ml_{blank}$) is calculated as follows:

$$S_{Total} = \sqrt{S_1^2 + S_2^2 + S_3^2 + \ldots S_K^2} = \sqrt{(0.0116)^2 + (0.0132)^2} = 0.0176$$

The calculation of the propagation of error for the molarity is further based on division and thus the relative deviations of the mass ($0.033/16.228 \cdot 100 = 0.2034\%$) and already propagated volume ($RSD = 0.0176/6.3253 \cdot 100 = 0.2782\%$):

$$RSD_{Total} = \sqrt{RSD_1^2 + RSD_2^2 + RSD_3^2 + \ldots RSD_K^2}$$

$$RSD_{Total} = \sqrt{(0.2034)^2 + (0.2782)^2} = 0.3446$$

$$S_{Total} = \frac{RSD_{Total}(Final\ Mean)}{100} = \frac{(0.3446)(0.02195)}{100} = 0.00008$$

As a result a more accurate measure of uncertainty associated with molarity, correction for the propagation of error, would be 0.02195 ± 0.00008.

For additional information on propagation of error refer to Taylor (1997).

References

Al-Khatib, SM, et al. (2011) "Non-Evidence-Based ICD Implantations in the United States," *JAMA* 305:43-49.

Bolton, S. and Bon, C. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, Inc., New York, pp. 159-161.

Kachigan, S.K. (1991). *Multivariate Statistical Analysis*, Radius Press, New York, pp. 112, 113.

Kirk, R.E. (1968). *Experimental Design: Procedures for the Behavioral Science*, Brooks/Cole Publishing Co., Belmont, CA, pp. 9-11, 540-546.

Taylor, J.R. (1997) *An Introduction to Error Analysis: The Study of Uncertainties in Physical Measurements*, University Science Books, Sausalito, CA, pp. 45-92.

Young, M.J., et al. (1983). "Sample size nomograms for interpreting negative clinical studies," *Annals of Internal Medicine* 99:248-251.

Suggested Supplemental Readings

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Eighth edition, John Wiley and Sons, New York, pp. 211-278.

Kachigan, S.K. (1991). *Multivariate Statistical Analysis*, Radius Press, New York, pp. 104-116.

Snedecor, G.W. and Cochran W.G. (1989). *Statistical Methods*, Iowa State University Press, Ames, IA, pp. 64-82.

Taylor, J.R. (1982). *An Introduction to Error Analysis: The Study of Uncertainty in Physical Measurements*, University Science Books, Mill Valley, CA, 1982.

Example Problems (Answers are provided in Appendix D)

1. Write the alternate hypothesis for each of the following null hypotheses:
   a. $\mu_A = \mu_B$
   b. $\mu_H \geq \mu_L$
   c. $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6$
   d. $\mu_A \leq \mu_B$
   e. $\mu = 125$
   f. Populations C, D, E, F, and G are the same
   g. Both samples come from the same population

2. If power is calculated to be 85%, with a Type I error rate of 5%, what are the percentages associated with the four possible outcomes associated with hypothesis testing? What if the power was only 72%?

3. In order to calculate the average density of objects it is necessary to calculate the weight and volume of each object and calculate density using the formula: *density = mass/volume*. Initial data based on 10 objects provide the following information (means and standard deviations):

   Weight = 10.6 ± 0.6 gm
   Volume = 4.9 ± 0.3 ml

   Report the mean and standard deviation for the density of these objects.

4. Analyzing a drug substance compared to its reference standard produces the following results.

103.5%	99.2%	Mean = 99.97
101.2%	100.9%	SD = 2.42
96.6%	98.4%	

   If the reference standard has a 2% variability, what is the total uncertainty?

9

The t-Tests

The initial eight chapters of this book focused on the "threads" associated with the statistical tests that will be discussed in the following chapters. The order of presentation of these statistical tests is based on the types of variables (continuous or discrete) that researchers may encounter in their design of experiments. As noted in Chapter 1, independent variables are defined as those which the researcher can control (i.e., assignment to a control or experimental group); whereas, dependent variables fall outside the control of the researcher and are measured as outcomes or responses (i.e., pharmacokinetic responses). It should be noted that other authors may use the terms **factors** or **predictor variables** to describe what we have defined as independent variables or **response variables** to describe dependent variables. We will continue to use the terms used in the preceding chapters.

Chapters 9 through 12 (t-tests, one-way analysis of variance, *post hoc* procedures and factorial designs) are concerned with independent variables that are discrete and outcomes measured on some continuum (dependent variable). Chapters 13, 14, and 20 discuss tests where both the dependent and independent variables are presented on continuous scales (i.e., correlation, regression, survival analyses). Chapters 15 through 19 (z-test of proportions, chi square tests, and measures of association) continue the presentation of tests concerned with discrete independent variables, but in these chapters the dependent variable is measured as a discrete outcome (i.e., pass or fail, live or die). Chapter 21 provides nonparametric or distribution-free statistics for evaluating data that does not meet the criteria required for many of the tests presented in Chapters 9 through 14.

Parametric Procedures

The parametric procedures include the t-tests, analysis of variance (ANOVAs or F-tests), correlation and linear regression; Chapters 9, 10, 13, and 14 respectively. In addition to the requirements that the samples must be randomly selected from their population and independently measured, two additional parameters must be met. First, it must be assumed that the sample is drawn from a population whose distribution approximates that of a normal distribution. Second, when two or more distributions are being compared, there must be **homogeneity of variance** or **homoscedasticity** (sample variances must be approximately equal). A rule of thumb

is that if the largest variance divided by the smallest variance is less than two, then homogeneity may be assumed. More specific tests for homoscedasticity will be discussed in the next chapter. With both the t-tests and F-tests there is an independent discrete variable containing one or more levels and a dependent variable that is measured on a continuous scale. Three types of parametric tests are presented in this chapter: 1) one-sample t-test; 2) two-sample t-test; and 3) paired t-test. In each case, the independent variable is discrete and the dependent variable represents continuously distributed data.

The t-Distribution

In Chapters 6 and 7, discussion focused on the standardized normal distribution, the standard error of the mean and the use of the z-test to create a confidence interval. This interval is the researcher's "best guess" of a range of scores within which the true population mean will fall (Eq. 7.5):

$$\mu = \overline{X} \pm (1.96)\frac{\sigma}{\sqrt{n}}$$

The disadvantage with this formula is the requirement that the population standard deviation (σ) must be known. In most research, the population standard deviation is unknown or at best a rough estimate can be made based on previous research (i.e., initial clinical trials or previous production runs). As seen in Figure 7.1, the larger the sample size the more constant the value of the standard error of the mean; therefore, the z-test is accurate only for large samples. From this it would seem logical that the researcher should produce a more conservative statistic as sample sizes become smaller and less information is known about the true population variance. This was noted and rationalized by William S. Gossett in an excellent 1908 article (Student, 1908). He published this work under the pseudonym "Student" because he worked for Guinness Brewing Company. At that time writing and publishing scientific papers was against company policy (Salsburg, 2002). The distribution became known as the Student t-distribution and subsequent tests are called **Student t-tests** or simply **t-tests**.

The t-tests, and their associated frequency distributions, are used 1) to compare one sample to a known population or value or 2) to compare two samples to each other and make inferences to their populations. These are the most commonly used tests to compare two samples because in most cases the population variances are unknown. To correct for this, the t-tables are used, which adjust the *z*-values from a normal distribution to account for sample sizes. Note in the abbreviated t-table below (Table 9.1), that any *t*-value at infinity degrees of freedom is equal to the corresponding *z*-value for a given Type I error (α). In other words, the t-table is nothing more than a normal standardized distribution (z-table), which corrects for the number of observations per sample.

Like the normal distribution, the shape of the Student t-distribution is symmetrical and the mean value is zero. The exact shape of the curve depends on the degrees of freedom, which was previously defined at n – 1. As the sample sizes get smaller, the amplitude of the curve becomes shorter and the range becomes wider

Table 9.1 Selected Values for the t-distribution for $(1 - \alpha/2)$

d.f.	$t_{.95}$	$t_{.975}$	$t_{.995}$
5	2.015	2.570	4.032
10	1.812	2.228	3.169
20	1.724	2.086	2.845
30	1.697	2.042	2.750
60	1.670	2.000	2.660
120	1.657	1.979	2.617
∞	1.645	1.960	2.576

(Figure 9.1). A more complete table of *t*-values is presented in Table B5 in Appendix B. Note that Table 9.1 is designed for two-tailed bidirectional tests. The Type I error rate is divided in half ($\alpha/2$). In the case of 95% confidence, there is a 2.5% chance of being wrong to the high side of the distribution and a 2.5% chance of error to the lower tail of the distribution. Therefore, allowing for a 5% error divided in half and subtracted from all possible outcomes $(1 - \alpha/2)$ the symbol of $t_{.975}$ presented in the second column of Table 9.1 represents the column for 95% confidence.

$$\alpha = 0.05$$
$$\alpha/2 = 0.025$$
$$1 - \alpha/2 = 0.975$$

Reviewing the table, the first column is degrees of freedom $(n - 1)$, the third column represents critical values for 90% confidence levels, the fourth for 95%, and the last for 99.99% confidence intervals. As the number of observations decreases, the Student *t*-value increases and the spread of the distribution increases to give a more conservative estimate, because less information is known about the population variance.

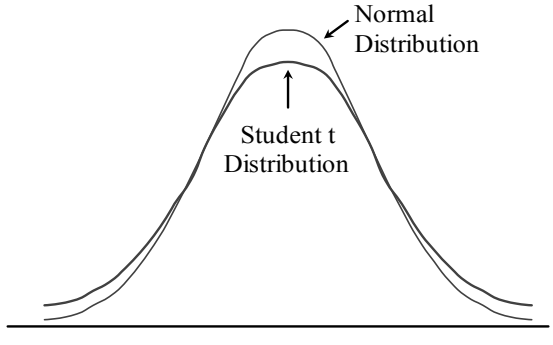


Figure 9.1 Comparison of curves for a t-distribution and a z-distribution.

One-Tailed versus Two-Tailed Tests

There are two ways in which the Type I error (α) can be distributed. In a **two-tailed test** the rejection region is equally divided between the two ends of the sampling distribution ($\alpha/2$) as described above. For example, assume we are comparing a new drug to a traditional therapeutic modality. With a two-tailed test we are not predicting that one drug is superior to the other. The alternative hypothesis is that they are different.

$$H_0: \quad \mu_{\text{new drug}} = \mu_{\text{old drug}}$$
$$H_1: \quad \mu_{\text{new drug}} \neq \mu_{\text{old drug}}$$

Assuming we would like to be 95% confident in our decision, the sampling error could result in a sample that is too high (2.5%) or too low (2.5%) based on chance sampling error. This would represent a total error rate of 5%. The rejection region for a two-tailed test where $p < 0.05$ and $df = \infty$ is illustrated as Figure 9.2.

In contrast, a **one-tailed test** is a test of hypothesis in which the rejection region is placed entirely at one end of the sampling distribution. In our current example, assume we want to prove that the new drug is superior to traditional therapy:

$$H_0: \quad \mu_{\text{new drug}} \leq \mu_{\text{old drug}}$$
$$H_1: \quad \mu_{\text{new drug}} > \mu_{\text{old drug}}$$

If a one-tailed test is used, all the α is loaded on one side of the equation and the decision rule with $\alpha = 0.05$, would be to reject H_0 if $t > t_{df}(1 - \alpha)$. Once again we would like to be 95% confident in our decision. The rejection region for a one-tailed test where $p < 0.05$ and $df = \infty$ is seen in Figure 9.3. In our example, what if our drug was truly inferior to the older drug? Using a one-tailed test we would not be able to prove this result. For that reason, as noted in the previous chapter, the one-tailed test should never be used unless there is a specific justification for being directional. Table B6 in Appendix B provides critical *t*-values for both one-tailed and two-tailed tests.

Failure to reject the null hypothesis does not mean that this hypothesis is accepted as truth. Much like the jurisprudence example in the previous chapter, the defendant is acquitted as "not guilty," as contrasted to "innocent." Thus, we fail to reject the null hypothesis, we do not prove that the null hypothesis is true. Insufficient evidence is available to conclude that H_0 is false.

One-Sample t-Tests

The one-sample case can be used to either estimate the population mean or compare the sample mean to an expected population mean. In the first method, a sample is taken on some measurable continuous data and the researcher wishes to "guess" at the true population mean. In a previous chapter, 30 bottles of a cough syrup are randomly sampled from a production line (Table 5.1). From this

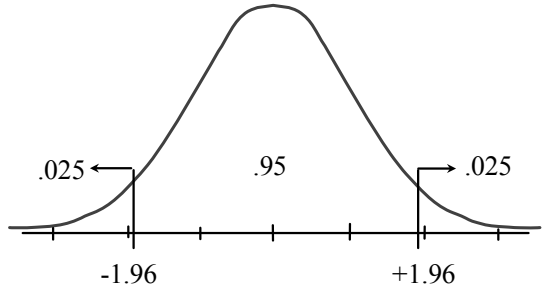


Figure 9.2 Graphic representation of a two-tailed test.

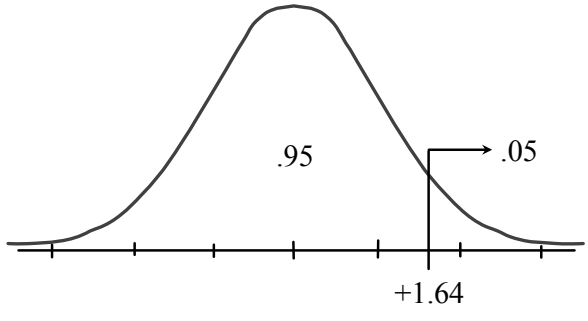


Figure 9.3 Graphic representation of a one-tailed test.

information, it was found that the sample mean equaled 120.05 ml with a standard deviation of 0.84 ml. This data could be used to predict the mean for the entire population of cough syrup in this production lot. With 95% certainty, the confidence interval would be:

$$\mu = \overline{X} \pm t_{n-1}(1-\alpha/2) \cdot \frac{S}{\sqrt{n}} \qquad \text{Eq. 9.1}$$

Notice that the population standard deviation in the error term portion of the z-test (Eq. 7.5) has been replaced with the sample standard deviation. The expression $t_{n-1}(1 - \alpha/2)$ is the *t*-value in Tables B5 or B6 in Appendix B for 29 observations or $n - 1$ degrees of freedom.[1]

---

[1] Note that exactly 29 degrees of freedom are not listed in Tables B5 or B6, but the value can be interpolated from a comparison of values for 25 and 30 df. The difference between 25 and 30 df is equivalent to 0.017 (2.059 − 2.042).

1/5 = x/0.017 x = 0.003

Therefore, $t_{29}(.975) = 2.042 + 0.003 = 2.045$

Alternatively, as described later in this chapter, Excel can be used to identify the exact value for any number of degrees of freedom.

$$\mu = 120.05 \pm (2.045) \cdot \frac{0.84}{\sqrt{30}} = 120.05 \pm 0.31$$

$$119.74 < \mu < 120.36$$

The value 2.045 is an interpolation of the *t*-value between 30 and 25 degrees of freedom in Table B5. Therefore, based on our sample of 30 bottles, it is estimated with 95% confidence that the average volume per bottle for the true population (all bottles in the production lot) is between 119.74 and 120.34 ml.

With 99% confidence the values would be calculated as follows:

$$\mu = 120.05 \pm (2.757) \frac{0.84}{\sqrt{30}} = 120.05 \pm 0.42$$

where 2.757 represents an interpolated value from Tables B5 or B6 for 29 degrees of freedom at $\alpha = 0.01$.

$$119.63 < \mu < 120.47$$

Note that in order to express greater confidence in our decision regarding the population mean, the range of our estimate increases. If it were acceptable to be less confident (90% or 80% certain that the population mean was within the estimated range) the width of the interval would decrease.

One method for decreasing the size of the confidence interval is to increase the sample size. As seen in Equation 9.1, an increase in sample size will not only result in a smaller value for $t_{n-1}(1 - \alpha/2)$, but the denominator (square root of *n*) will increase causing a decrease in the standard error portion of the equation. To illustrate this, assume the sample standard deviation remains constant for Sample B in the example of C_{max} presented in Chapter 7 (Table 7.1), where the $\overline{X} = 752.8$, S = 21.5, and n = 5. With $t_4(.975) = 2.78$, our best guess of the population mean would be:

$$\mu = \overline{X} \pm t_{n-1}(1-\alpha/2) \frac{S}{\sqrt{n}}$$

$$\mu = 752.8 \pm (2.78) \frac{21.5}{\sqrt{5}} = 752.8 \pm 26.73$$

$$726.07 < \mu < 779.53$$

If the sample size were increased to 25, where $t_{24}(0.975) \approx 2.06$, the new confidence interval would be:

$$\mu = 752.8 \pm (2.06)\ \frac{21.5}{\sqrt{25}} = 752.8 \pm 8.86$$

$$743.94 < \mu < 761.66$$

If we had the ability, funds and time to have another five-fold increase to 125 samples, where $t_{124}(.975) \approx 1.98$, the confidence interval would shrink to the following size:

$$\mu = 752.8 \pm (1.98)\ \frac{21.5}{\sqrt{125}} = 752.8 \pm 3.81$$

$$748.99 < \mu < 756.61$$

This "shrinking" in the size of the confidence interval can be graphically seen in Figure 9.4. Obviously, the more we know about the population, as reflected by a large sample size, the more precisely we can estimate the population mean.

Two-Sample t-Tests

A two-sample t-test compares two levels of a discrete independent variable to determine, based on the sample statistics, if their respective populations are the same or different. Two approaches can be taken in performing a two-sample t-test: 1) establish a confidence interval for the population differences or 2) compare test results to a critical value. Either method will produce the same results and the same decision will be made with respect to the null hypothesis. The same example for the two-sample t-test will be used to illustrate these two methods of hypothesis testing. The hypotheses can be written as two identical statements.

	Confidence Interval	Critical Value
The population means are the same:	H_0: $\mu_1 - \mu_2 = 0$	H_0: $\mu_1 = \mu_2$
The population means are different:	H_1: $\mu_1 - \mu_2 \neq 0$	H_1: $\mu_1 \neq \mu_2$

Note that the hypotheses are saying the same thing. For the null hypothesis μ_1 and μ_2 are the same and the alternative (mutually exclusive and exhaustive) statement is that they are different.

The first method for calculating a two-sample t-test is an extension of the methodology used in performing a one-sample t-test. An interval is established based upon the estimated centers of the distributions (sample means), their respective standard error of the means, and a selected reliability coefficient to reflect how confident we wish to be in our final decision (Eq. 7.4).

$$\begin{matrix} \textit{Population} \\ \textit{Difference} \end{matrix} = \begin{matrix} \textit{Sample} \\ \textit{Difference} \end{matrix} \pm \begin{matrix} \textit{Reliability} \\ \textit{Coefficient} \end{matrix} \times \begin{matrix} \textit{Standard} \\ \textit{Error} \end{matrix}$$

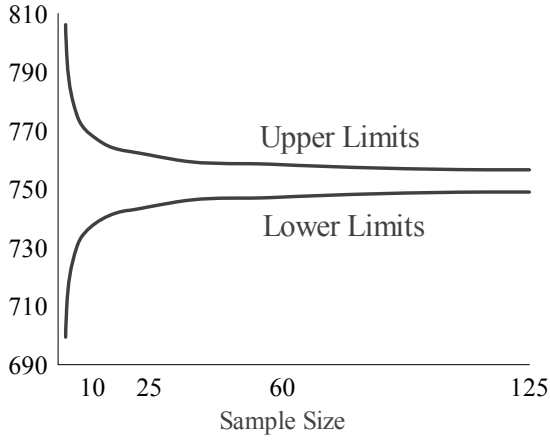


Figure 9.4 Effect of sample size on width of confidence intervals.

The statistical formula compares the central tendencies of two different samples and based on the results determines whether their respective populations are presumed equal or not. For the resulting confidence interval, the researcher looks for the presence or absence of zero within the interval. If they are equal, H_0: $\mu_1 - \mu_2 = 0$, then a zero difference must fall within the confidence interval. If they are not equal, H_1: $\mu_1 - \mu_2 \neq 0$, then zero does not fall within the estimated population interval and the difference cannot be attributed only to random error.

In the one-sample t-test the standard deviation (or variance) was critical to the calculation of the error term in Eq. 7.4. In the two-sample case, the variances should be close together (homogeneity of variance requirement), but more than likely they will not be identical. The simplest way to calculate a central variance term would be to average the two variances:

$$S^2_{average} = \frac{S_1^2 + S_2^2}{2} \qquad \text{Eq. 9.2}$$

Unfortunately the number of observations per level may not be the same; therefore, it is necessary to "pool" these two variances and weigh them by the number of observations per discrete level of the independent variable.

$$S_p^2 = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2} \qquad \text{Eq. 9.3}$$

Using this latter equation, differences in sample sizes are accounted for by producing a **pooled variance** (S_p^2).

The confidence interval for the difference between the population means, based on the sample means, is calculated using the following equation:

$$\mu_1 - \mu_2 = (\overline{X}_1 - \overline{X}_2) \pm t_{n1+n2-2}(1-\alpha/2)\sqrt{\frac{S_p^2}{n_1} + \frac{S_p^2}{n_2}} \qquad \text{Eq. 9.4}$$

Here $\overline{X}_1$ and $\overline{X}_2$ represent the two sample means and n_1 and n_2 are their respective sample sizes. The expression ($\overline{X}_1 - \overline{X}_2$) serves as our best estimate of the true population difference ($\mu_1 - \mu_2$).

The second alternative method for testing the hypothesis is to create a statistical ratio and compare this to the critical value for a particular level of confidence. We can think of this t-test as the ratio:

$$t = \frac{difference\ between\ the\ means}{distribution\ of\ the\ means} \qquad \text{Eq. 9.5}$$

Obviously, if the difference between the samples is zero, the numerator would be zero, the resultant *t*-value would also be zero and the researcher would conclude no significant difference. As the difference between the sample means becomes larger, the numerator increases, the *t*-value increases and there is a greater likelihood that the difference is not due to chance error alone. Looking at the illustrations in Figure 9.5, it is more likely that the groups to the left are significantly different because the numerator will be large; whereas the pair to the right will have a larger denominator because of the large overlap of the spreads of the distributions. But how far to the extreme does the calculated *t*-value in Equation 9.5 need to be in order to be significant? Greater than 1? Or 2? Or 50? The critical value is selected off the Student t-table (Table B5, Appendix B) based on the number of degrees of freedom. This is the same value we previously referred to as the reliability coefficient. In the case of a two-sample t-test the degrees of freedom are $n_1 - 1$ plus $n_2 - 1$, or the total number of observations (N) minus the number of discrete levels of the independent variable (2). This is more commonly written $n_1 + n_2 - 2$.

$$df = n_1 + n_2 - 2 = (n_1 - 1) + (n_2 - 1) = N - 2$$

Notice this was the denominator in our calculation of the pooled variance (Eq. 9.3).

The variance also influences the calculated *t*-value. As observations cluster closer together, it is likely that smaller differences between means may be significant. With respect to Equation 9.5, as the variance becomes smaller the denominator in the ratio becomes smaller and the *t*-value will increase. If the *t*-value is to the extreme of the critical value then the null hypothesis will be rejected in favor of the alternative hypothesis. Once again the pooled variance (Eq. 9.3) is used to calculate this *t*-value:

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{S_P^2}{n_1} + \frac{S_P^2}{n_2}}} \qquad \text{Eq. 9.6}$$

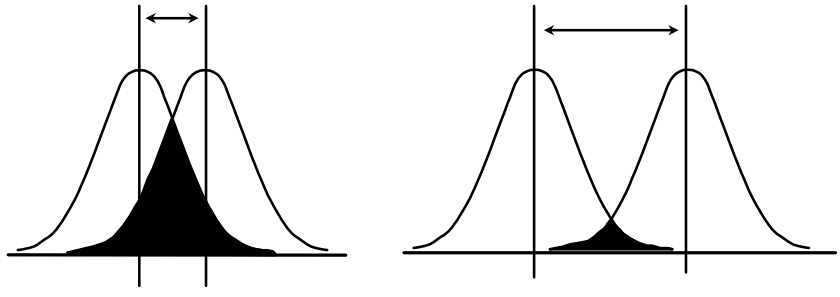


Figure 9.5 Comparison of two different means with similar dispersions.

Note that the numerator is the best guess of the true difference in the population(s) in Eq. 9.4 and the denominator is the standard error term in the same equation.

As discussed in the next section, the t-test can be either one-tailed or two-tailed. For the moment we shall focus only on two-tailed tests:

$$H_0: \mu_1 = \mu_2$$
$$H_1: \mu_1 \neq \mu_2$$

and no prediction will be made whether μ_1 or μ_2 is larger. In this case the decision rule is, with α equal to a set value (usually 0.05):

$$\text{reject } H_0 \text{ if } t > t_{df}(1 - \alpha/2) \text{ or if } t < -t_{df}(1 - \alpha/2)$$

Note that the calculated *t*-value can be either positive or negative depending on which sample mean is considered first. Thus, the resultant *t*-value can be either positive or negative. Again, if the value resulting from Eq. 9.6 is to the extreme (farther from zero to the positive or negative direction) of the critical value, there is sufficient reason to reject the null hypothesis and conclude that this difference cannot be explained by chance error alone.

The use of these two different approaches for using the t-test in hypothesis testing is presented in the following example. An investigator used a study to compare two formulations of a drug to determine the time to maximum concentration (C_{max}). Is there a significant difference between the two formulations (Table 9.2)? The first approach is to establish a confidence interval where the hypotheses are:

$$H_0: \mu_A - \mu_B = 0$$
$$H_1: \mu_A - \mu_B \neq 0$$

The test statistic is Eq. 9.4 and the decision rule is, with $\alpha = 0.05$, reject H_0 if zero does not fall within the confidence interval. The computations involve first calculating the pooled variance and then the confidence interval with 95% confidence:

Table 9.2 C_{max} Values for Two Formulations of the Same Drug

Formulation A						Formulation B					
125	130	135	126	140	135	130	128	127	149	151	130
128	121	123	126	121	133	141	145	132	132	141	129
131	129	120	117	126	127	133	136	138	142	130	122
119	133	125	120	136	122	129	150	148	136	138	140
Mean (ng/ml) 127.00						Mean (ng/ml) 136.54					
Standard deviation 6.14						Standard deviation 8.09					
Subjects 24						Subjects 24					

$$S_p^2 = \frac{23(6.14)^2 + 23(8.09)^2}{24 + 24 - 2} = \frac{2372.40}{46} = 51.57$$

$$\mu_A - \mu_B = (127.00 - 136.54) \pm 2.01\sqrt{\frac{51.57}{24} + \frac{51.57}{24}}$$

$$\mu_A - \mu_B = -9.54 \pm 2.01(2.07) = -9.54 \pm 4.16$$

$$-13.70 < \mu_A - \mu_B < -5.38$$

Thus, since zero is not within the confidence interval, reject H_0 and conclude that there is a significant difference, with formulation A reaching a significantly lower C_{max}. A zero outcome (H_0) is not a possible outcome with 95% confidence.

The second approach is to compare a calculated *t*-value to its corresponding critical value on the t-table (Table B5), where the hypotheses are:

$$H_0: \mu_A = \mu_B$$
$$H_1: \mu_A \neq \mu_B$$

The test statistic is Eq. 9.6 and the decision rule is, with $\alpha = 0.05$, reject H_0 if $t > t_{46}(0.025)$ or $t < -t_{46}(0.025)$. In this case with 46 degrees of freedom, reject H_0 if $t > 2.01$ or $t < -2.01$. The computations are as follows:

$$S_p^2 = \frac{23(6.14)^2 + 23(8.09)^2}{24 + 24 - 2} = 51.57$$

$$t = \frac{127.0 - 136.54}{\sqrt{\frac{51.57}{24} + \frac{51.57}{24}}} = \frac{-9.54}{2.07} = -4.61$$

The results show that the calculated *t*-value is smaller than (to the extreme negative side of) the critical-t of –2.01. Therefore; we would reject H_0, conclude that there is a significant difference, with formulation B reaching a significantly higher C_{max}. In both cases, the results of the statistical test were identical, the rejection of the null hypothesis. With only two formulas being compared, the initial data can be looked at and the results can state that there were was a significant difference between the two formulations, and that with 95% confidence ($\alpha = 0.05$) Formula B has a significantly higher C_{max}.

Computer Generated *p*-values

Most computer software will generate not only a test statistic (e.g., Eq. 9.6) but also an associated *p*-value. As mentioned in Chapter 8, this *p*-value is a *post hoc* representation of the amount of Type I error base on the data in the test. In other words, this is probability of rejecting the null hypothesis when in fact the null hypothesis is true. In most cases this value should be 0.05 or less (95% confidence in the decision to reject the null hypothesis). For example, in the previous comparison of two formulations (Table 9.2) the computer printout would be *t = –4.61, p = 0.0000329*. This can be interpreted, if one rejects the null hypothesis the change of the decision being wrong is less than 0.004%, far less than 5%; therefore, reject the null hypothesis with confidence in the decision.

Corrected Degrees of Freedom for Unequal Variances

As a parametric procedure, the two-sample t-test assumes that the population(s) from which the samples are taken are normally distributed and that they are approximately equal in their dispersion (homogeneity of variance). Tests for normality were discussed in Chapter 6 and tests for homogeneity of variance will be discussed in Chapter 10. As seen previously, the degrees of freedom involved with the two-sample case are calculated as $n_1 + n_2 - 2$. However in certain computer software packages the number of degrees of freedom reported on the output may be less than $n_1 + n_2 - 2$. This is due to a correction factor based on deviations from the ideal situation where the variances are identical and both sample sizes are equal. This correction factor (Satterthwaite, 1946) is referred to as **Welch-Satterthwaite solution** or simply the **Satterthwaite solution**:

$$df = \frac{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)^2}{\frac{\left(\frac{S_1^2}{n_1}\right)^2}{n_1 - 1} + \frac{\left(\frac{S_2^2}{n_2}\right)^2}{n_2 - 1}} \qquad \text{Eq. 9.7}$$

This corrected result, producing a reduced number of degrees of freedom, is used as the reliability coefficient in our confidence interval or the new critical value in the

ratio method for determining significance. For example, if the data presented in Table 9.2 were run on Minitab or Excel for unequal variances (described later) the resultant output would *be t = –4.60, df = 42, p = 0.0000367*. Note that the number of degrees of freedom decreased from 44 to 42. In this case there is a very slight difference in the results because there were equal cell sizes (n = 24) and the difference was due solely to the variances:

$$S_1^2 = (6.14)^2 = 37.70 \quad and \quad S_2^2 = (8.09)^2 = 65.45$$

Equation 9.7 can be used as a check to determine if the degrees of feedom are the same as those calculated using the Satterthwaite solution.

$$df = \frac{\left(\frac{37.70}{24} + \frac{65.45}{24}\right)^2}{\frac{\left(\frac{37.70}{24}\right)^2}{23} + \frac{\left(\frac{65.45}{24}\right)^2}{23}}$$

$$df = \frac{(1.571 + 2.727)^2}{\frac{(1.571)^2}{23} + \frac{(2.727)^2}{23}}$$

$$df = \frac{18.472}{0.107 + 0.323} = \frac{18.472}{0.430} = 42.9 \approx 42$$

Therefore, the adjusted degrees of freedom are the same as those presented in the computer printouts and the reliability coefficient would be adjusted for 42 rather than the original 46 degrees of freedom. For this example, the reliability coefficient or critical value for rejection would still be 2.01. However, when sample sizes get small this correction factor can result in a much larger reliability coefficient.

Basically three factors influence the t-test (and other parametric procedures): 1) normality; 2) similar variances; and 3) sample size. Parametric statistics are robust and moderate violations of these parametric assumptions have little effect in most cases (Cohen, pp. 266, 267). But what if there are violations in normality, homogeneity, and sample size at the same time? This may invalidate the use of the parametric statistic. The one factor that the researcher can control is sample size. Thus every effort should be made to keep the sample sizes the same, allowing for minor deviations from normality and slightly different variances.

One-Sample t-Test Revisited for Critical Values

The one-sample t-test can also use an established critical value as a method for testing the null hypothesis that a sample is taken from a certain population. Using the

previous sample of 30 bottles of cough syrup (Table 5.1), assume that we expect this particular syrup to have a fill volume of 120.0 ml. In this case our expected population center (μ_0) is 120 ml. Is this sample taken from that population?

$$H_0: \mu = \mu_0 = 120.0$$
$$H_1: \mu \neq \mu_0$$

The null hypothesis is that the samples come from a given population and that any difference has arisen simply by chance. The one-sample t-test enables us to determine the likelihood of this hypothesis. The test statistic is:

$$t = \frac{\overline{X} - \mu_0}{\frac{S}{\sqrt{n}}} \qquad \text{Eq. 9.8}$$

The decision rule can be established based on the researcher's desired confidence (acceptable amount of Type I error) in the outcome of the hypothesis testing. With $1 - \alpha$ equal to 0.95 (95% confidence) the decision rule is, reject H_0 if $t > t_{29}(1 - \alpha/2)$ or if $t < -t_{29}(1 - \alpha/2)$, where $t_{29}(1 - \alpha/2)$ is 2.045. For 99% confidence, the decision rule would be, with $\alpha = 0.01$, reject H_0 if $t > +2.756$ or if $t < -2.756$. Therefore if the *t*-value we calculate is to the extreme of 2.045 (positive or negative) H_0 can be rejected with 95% confidence in the decision. If the result is to the extreme of 2.756, H_0 is rejected with 99% confidence. The calculation of the *t*-value or *t*-statistic is:

$$t = \frac{120.05 - 120.00}{\frac{0.84}{\sqrt{30}}} = \frac{0.05}{0.15} = 0.33$$

Similar to both the 95% and 99% confidence interval created in a previous section, where 120.0 fell within the interval, we cannot reject the hypothesis that the sample is equal to the expected population mean of 120 ml. Stated differently, we cannot reject the hypothesis that our sample is taken from a population with a mean of 120 ml.

Matched Pair t-Test (Difference t-Test)

The matched pair, or **paired t-test**, is used when complete independence does not exist between the two samples, two time periods or **repeated measures**. For example, in a pretest-posttest design, where the same individual takes both tests, it is assumed that the results on the posttest will be affected (not independently) by the pretest. The individual actually serves as a control. Therefore the test statistic is not concerned with differences between groups, but actual individual subject differences. The hypotheses are associated with the mean difference in the population based on sample data:

$$H_0: \mu_d = 0$$
$$H_1: \mu_d \neq 0$$

To perform the test a table showing the differences must be created and used to calculate the mean difference and the standard deviation of the difference between the two sample measurements.

Before	After	d (After – Before)	d^2
x_1	x'_1	$d_1 = (x'_1 - x_1)$	d_1^2
x_2	x'_2	$d_2 = (x'_2 - x_2)$	d_2^2
x_3	x'_3	$d_3 = (x'_3 - x_3)$	d_3^2
...	...	...	...
x_n	x'_n	$d_n = (x'_n - x_n)$	d_n^2
		Σd	Σd^2

Each row represents an individual's score or response. The first two columns are the actual outcomes. The third column is the difference between the first two columns per individual. Traditionally the first measure (before) is subtracted from the second measurement (after). Therefore a positive difference represents a larger outcome on the second measure. The mean difference is calculated:

$$\overline{X}_d = \frac{\sum d}{n} \qquad \text{Eq. 9.9}$$

and the standard deviation of the difference is the square root of the variance difference:

$$S_d^2 = \frac{n(\sum d^2) - (\sum d)^2}{n(n-1)} \qquad \text{Eq. 9.10}$$

$$S_d = \sqrt{S_d^2} \qquad \text{Eq. 9.11}$$

The *t*-value calculations are as follows, depending on use of the confidence interval or ratio approach for evaluating the results. Very similar to the one-sample t-test, the confidence interval would be:

$$\mu_d = \overline{X}_d \pm t_{n-1}(\alpha/2) \cdot \frac{S_d}{\sqrt{n}} \qquad \text{Eq. 9.12}$$

Interpreted the same as the two-sample t-test, if zero falls within the confidence interval, a zero outcome is possible and we fail to reject the H_0. Alternatively, if all the possible values in the confidence interval are positive or all are negative, we reject

the null hypothesis and conclude that there is a significant difference.

The second method for hypothesis testing would be to: 1) establish a decision rule based on a critical *t*-value from Tables B5 or B6; 2) calculate a *t*-value based on the ratio of the difference divided by the distribution; and 3) reject the hypothesis under test if the *t*-value that is calculated is more to the extreme than the critical value off the table. Similar to previous tests, our estimator is in the numerator and an error term in the denominator:

$$t = \frac{\overline{X}_d}{\frac{S_d}{\sqrt{n}}} \qquad \text{Eq. 9.13}$$

Like the decision rules for hypothesis testing with the two-sample case, the test can be either one-tailed or two-tailed. In the one-tailed paired t-test, the hypotheses would be either:

$$H_0: \mu_d \leq 0 \quad \text{or} \quad H_0: \mu_d \geq 0$$
$$H_1: \mu_d > 0 \qquad\qquad H_1: \mu_d < 0$$

and the decision rule would be, with $\alpha = 0.05$, reject H_0 if $t > t_{df}(1 - \alpha)$. In the two-tailed test we again split the Type I error between the two tails with our hypotheses being:

$$H_0: \mu_d = 0$$
$$H_1: \mu_d \neq 0$$

the decision rule with $\alpha = 0.05$, is to reject H_0 if $t > t_{df}(1 - \alpha/2)$ or if $t < -t_{df}(1 - \alpha/2)$.

Because we are interested in differences in each individual, with the matched-paired t-test the degrees of freedom (*df*) value is concerned with the number of pairs of individual differences rather than the total number of data points collected.

$$df = n - 1 \text{ (number of pairs)}$$

The following illustrates the use of a one-tailed matched paired t-test. A preliminary study was conducted to determine if a new antihypertensive agent could lower the diastolic blood pressure in normal individuals. Initial clinical results are presented in the second and third columns of Table 9.3. Because this is a one-tailed test (did the new drug lower the blood pressure, indicating a desired direction for the alternate hypothesis), the hypotheses are as follows:

$$H_0: \mu_d \geq 0$$
$$H_1: \mu_d < 0$$

In this case a rise in blood pressure or no change in blood pressure would result in a failure to reject H_0. Only if there was a significant decrease in the blood pressure would we reject H_0 in favor of the alternative hypothesis.

Table 9.3 Diastolic Blood Pressure with a New Antihypertensive

Subject	Before	After	d (after – before)	d^2
1	68	66	–2	4
2	83	80	–3	9
3	72	67	–5	25
4	75	74	–1	1
5	79	70	–9	81
6	71	77	+6	36
7	65	64	–1	1
8	76	70	–6	36
9	78	76	–2	4
10	68	66	–2	4
11	85	81	–4	16
12	74	68	–6	36
		$\sum$ =	–35	253

In this first example we will first establish a critical *t*-value and use the ratio method (Eq. 9.13) for testing the null hypothesis. The decision rule would be, with α = 0.05, reject H_0 if $t < -t_{11}(0.95)$, which is 1.795 in Table B5 (note that this is a one-tailed test; therefore, the critical value comes from the third column, t_{95} the same value is listed in the second column in Table B6). In this case we have set up our experiment to determine if there is a significant decrease in blood pressure and the difference we record is based on the second measure (after) minus the original results (before). Therefore a "good" or "desirable" response would be a negative number. If the ratio we calculate using the t-test is a negative value to the extreme of the critical value we can reject the H_0. Because we are performing a one-tailed test we need to be extremely careful about the signs (positive or negative).

The calculations for the mean difference and standard deviation of the difference are as follows:

$$\overline{X}_d = \frac{\sum d}{n} = \frac{-35}{12} = -2.92$$

$$S_d^2 = \frac{n(\sum d^2) - (\sum d)^2}{n(n-1)} = \frac{12(253) - (-35)^2}{12(11)} = 13.72$$

$$S_d = \sqrt{S_d^2} = \sqrt{13.72} = 3.70$$

The calculation of the *t*-value would be:

$$t = \frac{-2.92}{\frac{3.70}{\sqrt{12}}} = \frac{-2.92}{1.07} = -2.73$$

Therefore, based on a computed *t*-value less than the critical *t*-value of −1.795, the decision is to reject H_0 and conclude that there was a significant decrease in the diastolic blood pressure.

Using this same example, it is possible to calculate a confidence interval with $\alpha =$ 0.05. If zero falls within the confidence interval, then zero difference between the two measures is a possible outcome and the null hypothesis cannot be rejected. From the previous example we know that $\overline{X}_d = -2.92$, $S_d = 3.70$ and $n = 12$. From Table B6 in Appendix B the reliability coefficient for 11 degrees of freedom ($n - 1$) is $t_{11}(1 - \alpha) = 1.795$ at 95% confidence. Calculation of the confidence interval is

$$\mu_d = \overline{X}_d \pm t_{n-1}(\alpha - 1/2)\frac{S_d}{\sqrt{n}}$$

$$\mu_d = -2.92 \pm (1.795)\frac{3.70}{\sqrt{12}} = -2.92 \pm 1.92$$

$$-4.84 < \mu_d < -1.00$$

Since zero does not fall within the interval and in fact all possible outcomes are in the negative direction, it could be concluded with 95% certainty that there was a significant decrease in blood pressure. The results are exactly the same as found when the *t*-ratio was calculated the first time. Based on the confidence interval approach it can be estimated that the true population decrease in diastolic blood pressure is between 1.00 and 4.84 mm/Hg.

Using Excel® or Minitab® for Student t-tests

Excel 2010 has several function (*fx*) options that can be used for t-test applications. Instead of referring to Tables B5 and B6 to determine critical values for the test statistic (or reliability coefficients for confidence intervals) it can be determined using the function **T.INV.2T** for the two-tailed distribution or **T.INV** for a one-tailed distribution. Either function will prompt for the probability (as a decimal) and the degrees of freedom. For older versions of Excel, the **TINV** function will give the two-tailed distribution only. Other Excel 2010 functions allow one to determine the *p*-value for a calculated *t*-statistic: **T.DIST.2T** for a two-tailed probability and **T.DIST.RT** for the one-tailed option. Either function will prompt for the calculated *t*-value and the degrees of freedom. Older versions of Excel included **TDIST** to calculate only the two-tailed probability.

There is a function option to help create the confidence interval for a one-sample t-test. The function is **CONFIDENCE.T** (in Excel 2010) which will create that portion of Eq. 9.1 that includes the reliability coefficient and error term. Excel will

prompted for the "alpha" (amount of Type I error), the sample standard deviation and the sample size. The resultant value needs to be added and subtracted from the sample mean to create the confidence interval.

There are three t-test options as part of the data analysis tools: 1) t-test: two-sample assuming equal variances; 2) t-test: two-sample assuming unequal variances; and 3) t-test: paired two sample for means.

Data ➤ Data Analysis ➤ t-test: Two-Sample Assuming Unequal Variance
Data ➤ Data Analysis ➤ t-test: Two-Sample Assuming Equal Variances
Data ➤ Data Analysis ➤ t-test: Paired Two Sample for Means

The difference between the first two options for the two-sample case is that the application of the Satterthwaite solution to the option for "t-Test: Assuming Unequal Variances". As seen in Figure 9.6, one needs to identify the columns and range in which each level of the independent variable is located (Variable 1 Range and Variable 2 Range); set the "Hypothesized Mean Difference" to zero (H_0: $\mu_1 - \mu_2 = 0$); change the Type I error if 0.05 is not acceptable; and identify where the outcomes should be reported, either starting at a cell on the current page (per this example, D2) or on a new worksheet (by default). Using the data in our previous example (Table 9.2) the results appear in Figure 9.7. This figure represents the results for "t-Test: Two-sample Assuming Equal Variances" and the columns have been expanded for better readability. The means and variance for each level of the independent variable are reported at the top of the results. Near the center are the calculated *t*-statistic and the degrees of freedom. At the bottom are the results for both a one-tailed and two-tailed test. The "t Critical …-tail" would correspond to the value from Tables B5 or B6 and the "P(T<=t) …-tail" is the p-value based on the number of tails.

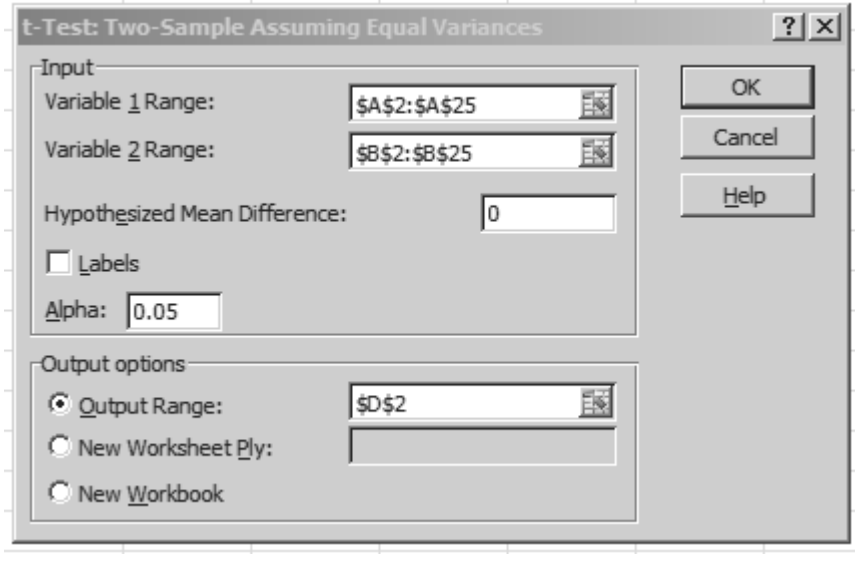


Figure 9.6 Options for a two-sample t-test with Excel.

t-Test: Two-Sample Assuming Equal Variances		
	Variable 1	*Variable 2*
Mean	127	136.5416667
Variance	37.6521739	65.47644928
Observations	24	24
Pooled Variance	51.5643116	
Hypothesized Mean Difference	0	
df	46	
t Stat	-4.6029923	
P(T<=t) one-tail	1.6432E-05	
t Critical one-tail	1.67866041	
P(T<=t) two-tail	3.2864E-05	
t Critical two-tail	2.0128956	

Figure 9.7 Outcome report for a two-sample t-test with Excel.

This example illustrates one disadvantage with Excel – each level of the independent variable is represented as a column (or row), whereas most computer software requires that the data be arranged with each column representing one variable and each row representing an observation. Therefore, data used in other software packages need to be modified to be used in Excel. In the previous example, for the data in Table 9.2, most software would have "formulation" in one column (A or B) and the respective C_{max} value in a second column, as will be illustrated below using Minitab.

For the paired t-test the input requirements are similar to the two-sample example. As seen in Figure 9.6, one column represents the before measurement and second column represents the after measurement. Using this format a positive *t*-value will indicate a more positive result on the post measure. The outputs for the paired results are similar to the two-sample case and presented in Figure 9.8 for data in Table 9.3. The "Pearson correlation" has no relevance to the interpretation of the paired t-test and will be discussed in Chapter 13. It is recommended to use the post-measurement as "Variable 1 Range" and "Variable 2 Range" as the pre-measurement. Using this approach a positive *t*-value will indicate an increase on the post-measurement. For the example illustrated in Figure 9.8, the significant negative *t*-value indicated a significant decrease in the diastolic blood pressure.

Minitab offers a applications for the one-sample, two-sample and paired tests. To access these tests choose "Stat" on the title bar, then "Basic Statistics" and the appropriate t-test:

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	71.5833333	74.5
Variance	33.9015152	37.3636364
Observations	12	12
Pearson Correlation	0.80843835	
Hypothesized Mean Difference	0	
df	11	
t Stat	-2.7277538	
P(T<=t) one-tail	0.00982856	
t Critical one-tail	1.79588482	
P(T<=t) two-tail	0.01965712	
t Critical two-tail	2.20098516	

Figure 9.8 Outcome report for a paired t-test with Excel.

Stat ➤ Basic Statistics ➤ 1-sample t… one sample CI
Stat ➤ Basic Statistics ➤ 2-sample t… two-sample t-test
Stat ➤ Basic Statistics ➤ Paired t… one sample CI

Like most software packages, each column represents a variable and each row an observation. Columns are chosen for Minitab based on whether they independent or dependent variables. Figure 9.9 illustrates the decisions required for a one-sample t-test for the data from Sample B in Table 7.1. *Graphic…* options include a histogram, an individual value plot or a box plot. *Options…* allows one to change in the confidence interval from the default value on 95% or to create a one-tailed interval. The results for a 95% confidence interval are presented in Figure 9.10.

For the two-sample t-test, Minitab automatically applies the Satterthwaite solution and down-regulates the degrees of freedom to adjust for differences in sample sizes or variances. Figure 9.11 illustrated the decisions required for a two-sample t-test for the data in Table 9.2. The "Subscripts" is the column with the independent variable and the "Samples" is the column with the dependent variable. These are selected by double clicking on the variables in the box on the left. *Graphic…* options include an individual value plot or a box plot for each level of the independent variable. *Options…* allows one to change in the confidence interval from the default value on 95%, create a one-tailed interval, or change the predicted difference from the default of zero. The Satterthwaite solution can be overridden by selecting the "Assume equal variances" seen in Figure 9.11. The output for the two-sample t-test for data in Table 9.2 is presented in Figure 9.12. Notice that the results provide both the confidence interval and ratio approaches to the t-test.

For the paired t-test, data is arranged in Minitab similar to Excel, with the pre-

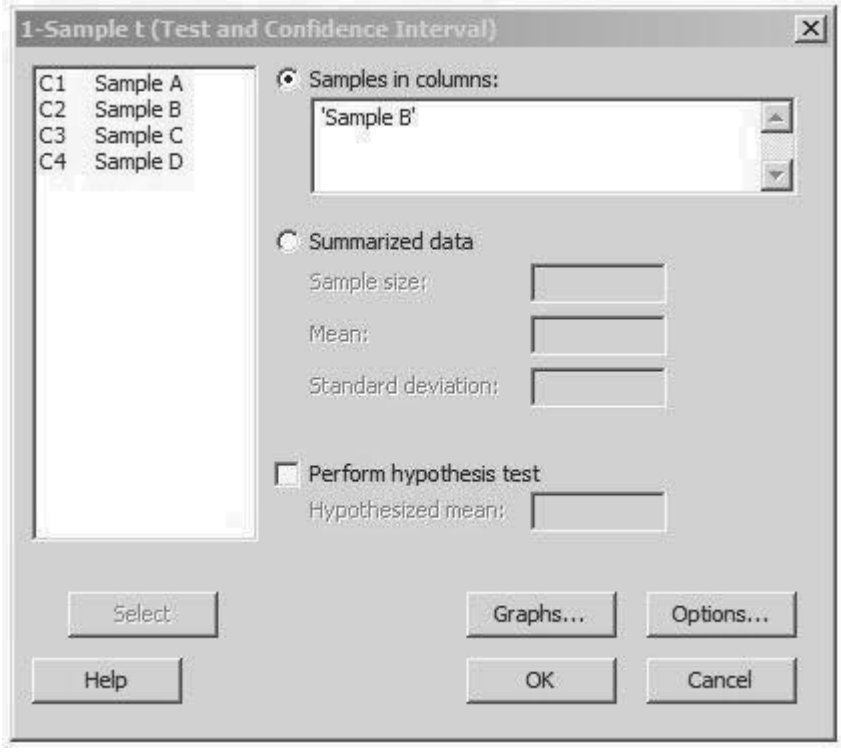


Figure 9.9 Options for one-sample t-test with Minitab.

One-Sample T: Sample B

Variable	N	Mean	StDev	SE Mean	95% CI
Sample B	5	752.80	21.49	9.61	(726.12, 779.48)

Figure 9.10 Outcome report for a one-sample t-test with Minitab.

measurements in one column and post-measurements in a second column. Figure 9.13 illustrated the decisions required for a paired t-test for the data in Table 9.3. The "First Sample" should be the column for the post-measurement and the "Second Sample" should be the column for the pre-measurement. Similar to Excel this will produce a positive *t*-value if there is an increase in the latter measurement. *Graphic*… options include a histogram of the differences, an individual value plot for each level or a box plot of the differences. *Options*… allows one to change the confidence interval from the default value on 95%, create a one-tailed interval, or change the predicted difference from the default of zero. The output for the paired t-test for data

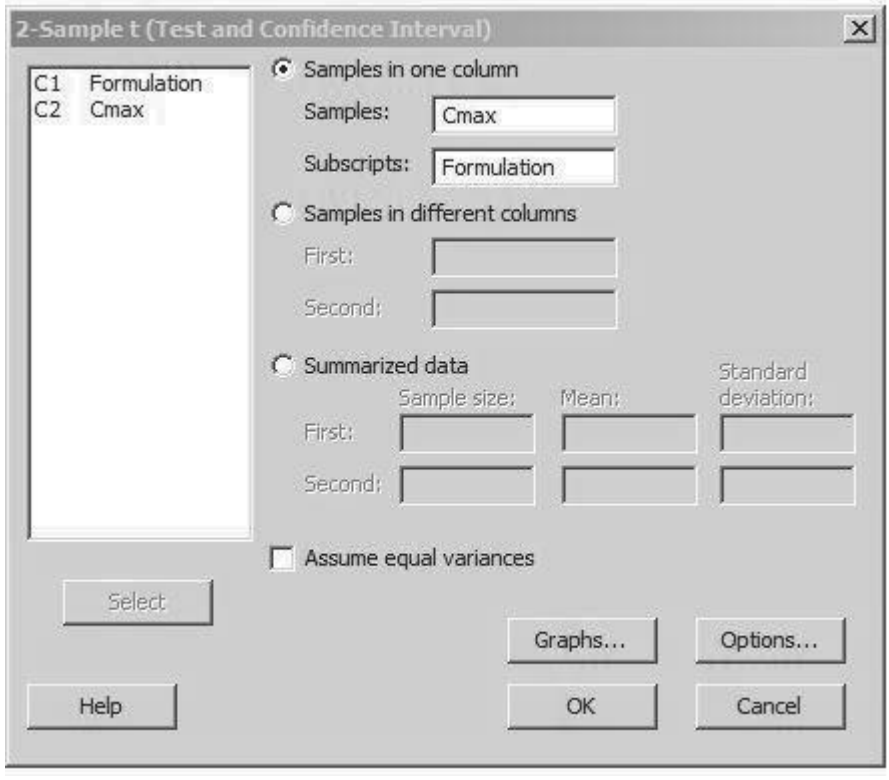


Figure 9.11 Options for one-sample t-test with Minitab.

Two-Sample T-Test and CI: Cmax, Formulation

```
Two-sample T for Cmax

Formulation   N    Mean  StDev  SE Mean
A            24  127.00   6.14      1.3
B            24  136.54   8.09      1.7

Difference = mu (A) - mu (B)
Estimate for difference:  -9.54
95% CI for difference:  (-13.73, -5.36)
T-Test of difference = 0 (vs not =): T-Value = -4.60  P-Value = 0.0(
```

Figure 9.12 Outcome report for a two-sample t-test with Minitab.

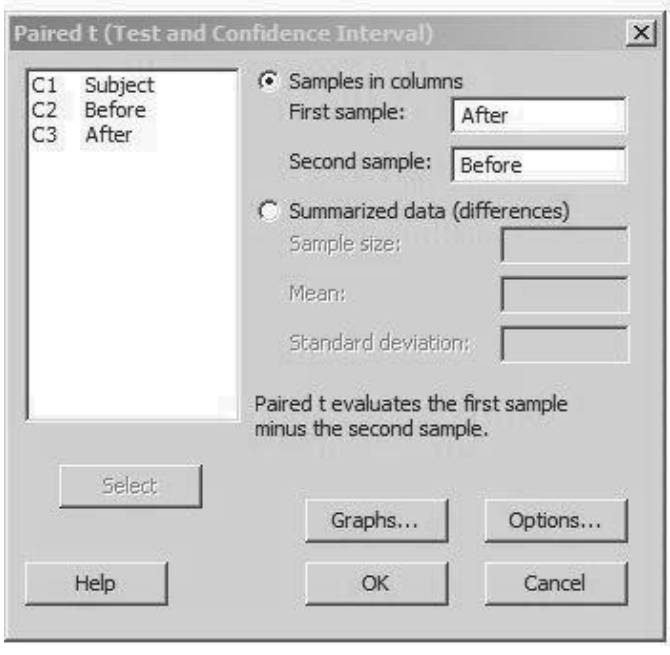


Figure 9.13 Options for a paired t-test with Minitab.

in Table 9.3 is presented in Figure 9.14. Once again note that the results provide both the confidence interval and ratio approaches to the t-test.

Minitab offers some very nice additional features for the t-tests. If one has the mean, standard deviation and sample size already available, they can be simply entered into the "Summarized data" option in Figures 9.9, 9.11, and 9.13. Also, if data happens to be arranged in columns as required by Excel, the two-sample t-test in Minitab can handle this format using the "Samples in different columns" option in Figure 9.11.

Paired T-Test and CI: After, Before

```
Paired T for After - Before

             N   Mean  StDev  SE Mean
After       12  71.58   5.82     1.68
Before      12  74.50   6.11     1.76
Difference  12  -2.92   3.70     1.07

95% CI for mean difference: (-5.27, -0.56)
T-Test of mean difference = 0 (vs not = 0): T-Value = -2.73  P-Value = 0.020
```

Figure 9.14 Outcome report for a paired t-test with Minitab.

Examples in this section were taken from previous data in the chapter and the results (less minor rounding differences) were identical to the results worked out by hand.

References

Cohen, J. (1969). *Statistical Power Analysis for the Behavioral Sciences*. Academic Press, New York.

Salsburg, D. (2002). *The Lady Tasting Tea: How Statistics Revolutionized Science in the Twentieth Century*, Henry Holt and Company, New York, p. 26.

Satterthwaite, F.E. (1946). "An approximate distribution of estimates of variance components," *Biometrics Bulletin* 2:110-114.

Student (1908). "The probable error of a mean," *Biometrika* 6(1):1-25.

Suggested Supplemental Readings

Bolton, S. and Bon, C. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, New York, pp. 120-129.

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Eighth edition, John Wiley and Sons, New York, pp. 175-178.

Snedecor, G.W. and Cochran W.G. (1989). *Statistical Methods*, Iowa State University Press, Ames, IA, pp. 83-105.

Example Problems (Answers are provided in Appendix D)

1. Two groups of physical therapy patients are subjected to two different treatment regimens. At the end of the study period, patients are evaluated on specific criteria to measure the percent of desired range of motion. Do the results listed below indicate a significant difference between the two therapies at the 95% confidence level?

Group 1		Group 2	
78	82	75	91
87	87	88	79
75	65	93	81
88	80	86	86
91		84	89
		71	

Table 9.4 FEV_1 Data

Subject number	Before administration	FEV_1 3 hours after administration
1	3.0	3.1
2	3.6	3.9
3	3.5	3.7
4	3.8	3.8
5	3.3	3.2
6	3.9	3.8
7	3.1	3.4
8	3.2	3.3
9	3.5	3.6
10	3.4	3.4
11	3.5	3.7
12	3.6	3.5

2. Twelve subjects in a clinical trial to evaluate the effectiveness of a new bronchodilator were assessed for changes in their pulmonary function. Forced expiratory volume in one second (FEV_1) measurements were taken before and three hours after drug administration (Table 9.4).

   a. What is $t_{(1-\alpha/2)}$ for $\alpha = 0.05$?
   b. Construct a 95% confidence interval for the difference between population means.
   c. Use a t-test to compare the two groups.

3. Calculate the mean, standard deviation, relative standard deviation, and 95% confidence interval for each of the time periods presented in the following dissolution profile (percentage of label claim):

	Time (minutes)				
Sample	10	20	30	45	60
1	60.3	95.7	97.6	98.6	98.7
2	53.9	95.6	97.5	98.6	98.7
3	70.4	95.1	96.8	97.9	98.0
4	61.7	95.3	97.2	98.0	98.2
5	64.4	92.8	95.0	95.8	96.0
6	59.3	96.3	98.3	99.1	99.2

4. Samples are taken from a specific batch of drug and randomly divided into two groups of tablets. One group is assayed by the manufacturer's own quality control laboratories. The second group of tablets is sent to a contract laboratory for identical analysis. Is there a significant difference between the results generated by the two labs?

Manufacturer	Contract Lab
101.1	97.5
100.6	101.1
98.8	99.1
99.0	98.7
100.8	97.8
98.7	99.5

a. What is $t_{(1-\alpha/2)}$ for $\alpha = 0.05$?
b. Construct a 95% confidence interval for the difference between population means.
c. Use a t-test to compare the two groups.

5. A first-time-in-man clinical trial was conducted to determine the pharmacokinetic parameters for a new calcium channel blocker. The study involved 20 healthy adult males and yielded the following C_{max} data (maximum serum concentration in ng/ml):

715, 728, 735, 716, 706, 715, 712, 717, 731, 709,
722, 701, 698, 741, 723, 718, 726, 716, 720, 721

Compute a 95% confidence interval for the population mean for this pharmacokinetic parameter.

6. Following training on content uniformity testing, comparisons are made between the analytical result of the newly trained chemist with those of a senior chemist. Samples of four different drugs (compressed tablets) are selected from different batches and assayed by both individuals. These results are listed in Table 9.5. Was there a significant difference between the results from these two scientists?

Table 9.5 Comparison of Two Chemists

Sample Drug, Batch	New Chemist	Senior Chemist
A,42	99.8	99.9
A,43	99.6	99.8
A,44	101.5	100.7
B,96	99.5	100.1
B,97	99.2	98.9
C,112	100.8	101.0
C,113	98.7	97.9
D,21	100.1	99.9
D,22	99.0	99.3
D,23	99.1	99.2

7. An examination evaluating cognitive knowledge in basic pharmacology was mailed to a random sample of all pharmacists in a particular state. Those responding were classified as either hospital or community pharmacists. The examination results were:

	Hospital Pharmacists	Community Pharmacists
Mean Score	82.1	79.9
Variance	151.29	210.25
Respondents	129	142

Assuming that these respondents are representative of their particular populations, is there any significant difference between the types of practice based on the examination results?

8. A study was undertaken to determine the cost effectiveness of a new treatment procedure for peritoneal adhesiolysis. Twelve pairs of individuals who did not have complications were used in the study, and each pair was matched on degree of illness, laboratory values, sex, and age. One member of each pair was randomly assigned to receive the conventional treatment, while the other member of the pair received the new therapeutic intervention. Based on the data in Table 9.6, is there sufficient data to conclude at a 5% level of significance that the new therapy is more cost effective than the standard?

9. In a major cooperative of hospitals the average length of stay for kidney transplant patients is 21.6 days. In one particular hospital the average time for 51 patients was only 18.2 days with a standard deviation of 8.3 days. From the data available, is the length of stay at this particular hospital significantly less than expected for all the hospitals in the cooperative?

Table 9.6 Cost in Dollars

Pair	New	Conventional
1	11,813	13,112
2	6,112	8,762
3	13,276	14,762
4	11,335	10,605
5	8,415	6,430
6	12,762	11,990
7	7,501	9,650
8	3,610	7,519
9	9,337	11,754
10	6,538	8,985
11	5,097	4,228
12	10,410	12,667

10

One-Way Analysis of Variance (ANOVA)

Where the t-test was appropriate for the one- or two-sample cases (one or two levels of the discrete independent variable), the F-test or one-way analysis of variance provides an extension to *k* levels of the independent variable. The calculation involves an *analysis of variance* of the individual sample means around a central grand mean. Like the t-test, the dependent variable represents data from a continuous distribution. The analysis of variance is also referred to as the F-test, after R.A. Fisher, a British statistician who developed this test during the 1920s (Salsburg, 2002). This chapter will focus on the one-way analysis of variance (abbreviated with the acronym ANOVA), which involves only one independent discrete variable and one dependent continuous variable.

Hypothesis Testing with the One-Way ANOVA

There are numerous synonyms for the one-way ANOVA including: **univariate ANOVA**, **simple ANOVA**, **single-classification ANOVA**, or **one-factor ANOVA**. The hypotheses associated with the one-way analysis of variance can be expanded to any number (*k*) levels of the discrete independent variable.

$$H_0: \mu_1 = \mu_2 = \mu_3 \ldots = \mu_k$$
$$H_1: H_0 \text{ is false}$$

The null hypothesis states that there are no differences among the population means, and that any fluctuations in the sample means are due to chance variability only.

The ANOVA represents a variety of techniques used to identify and measure sources of variation within a collection of observations, hence the analysis of variance name. The ANOVA has the same assumption as those seen with the t-test: 1) sample sizes are relatively equal; 2) the variances are similar (homogeneity of variance); and 3) the dependent variable is sampled from a normally distributed population. In fact the t-test could be considered a special case of the one-way ANOVA where $k = 2$. Factors that can affect whether differences are statistically significant include: 1) amount of the difference between the sample means; 2) the variances of the dependent variable (wide or narrow dispersion); and 3) the sample size (larger samples provide more reliable information).

Note that the alternative hypothesis does not say that all samples are unequal, nor does it tell where any inequalities exist. The test results merely identify that a difference does occur somewhere among the population means. In order to find where these differences are some form of multiple comparison procedure should be performed if the null hypothesis is rejected (Chapter 11).

The F-Distribution

A full discussion of the derivation of the sampling distribution associated with the analysis of variance is beyond the scope of this text. A more complete description can be found in Kachigan (1991). The simplest approach would be to consider the ratio of variances for two samples randomly selected from a normally distributed population. The ratio of the variances, based on sample sizes of n_1 and n_2 , would be:

$$F = \frac{S_1^2}{S_2^2}$$

Assuming the sample was taken from the same population, the ratio of the variances would be:

$$E(F) = E(\frac{S_1^2}{S_2^2}) = \frac{\sigma^2}{\sigma^2} = 1$$

However, due to the variations in sampling distributions (Chapter 7), some variation from E(F) = 1 would be expected by chance alone due to expected difference between the two sample variances. Based on previous discussions in Chapter 7 it would be expected that the variation of the sampling distribution of S^2 should depend on the sample size n and the larger the sample size, the smaller that variation. Thus, sample size is important to calculating the various F-distributions.

As will be shown in the next section, the F-test will create such a ratio comparing the variation among the levels of the independent variable and the variation within the samples. Curves have been developed that provide values that are likely to be exceeded only 5% or 1% of the time by chance alone (Figure 10.1). Obviously if the calculated F-value is much larger than one and exceeds the critical value indicated below, it is most likely not due to random error. Because of the mathematical manipulations discussed later in this chapter the calculated F-statistic must be positive. Therefore, unlike the t-test, we are only interested in only positive values to the extreme of our critical value. Similar to the t-distribution, the F-distribution is a series of curves, whose shapes differ based on the degrees of freedom. As will be seen later in the chapter, the decision to accept or reject the null hypothesis, based on the shape of the F-distribution, is dependent on both the total sample size and the number of levels associated with the discrete independent variable. As the number of degrees of freedom gets larger, the F-distribution will approach the shape of a normal distribution. A listing of the critical F-values (F_c) is given in Table B7 of Appendix B. Similar to the t-test, Excel can be used to generate the critical value, which will be discussed at the end of this chapter.

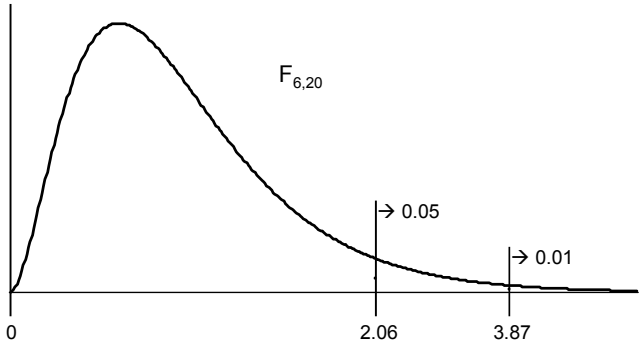


Figure 10.1 Example of an F-distribution.

Test Statistic

The analysis of variance involves determining if the observed sample values belong to the same population, regardless of the level of the discrete variable (group), or whether the observations in at least one of these groups come from a different population.

$$H_0: \mu_1 = \mu_2 = \mu_3 \ldots = \mu_k = \mu$$

To obtain an *F*-value we need two estimates of the population variance. It is necessary to examine the variability (analysis of the variance) of observations within groups as well as between groups. With the t-test, we computed a *t*-statistic by calculating the ratio of the difference between the two means over the distribution of the means (represented by the pooled variance). The *F*-statistic is computed using a simplified ratio similar to the t-test.

$$F = \frac{difference\ \ between\ \ the\ \ means}{standard\ error\ of\ the\ difference\ of\ \ the\ means} \qquad \text{Eq. 10.1}$$

The actual calculation of the *F*-statistic is as follows:

$$F = \frac{MS_B}{MS_W} \qquad \text{Eq. 10.2}$$

This formula shows the overall variability between the samples means (***MS*$_B$** or **mean squared between**) and at the same time it corrects for the dispersion of data points within each sample (***MS*$_W$** or **mean squared within**). The actual calculations for the MS_B and MS_W will be discussed in the following two sections. Obviously, the greater the differences among the sample means (the numerator), the less likely that all the samples were selected from the same population (all the samples represent populations that are the same or are equal). If all the sample means are equal, the

numerator measuring the differences among the means will be zero and the corresponding *F*-statistics also will be zero. As the *F*-statistic increases it becomes likely that a significant difference exists. Like the t-test, it is necessary to determine if the calculated *F*-value is large enough to represent a true difference between the populations sampled or if the difference is merely due to chance error or sampling variation. The decision rule to reject the null hypothesis of equality is stated as follows:

$$\text{with } \alpha = 0.05, \text{ reject } H_0 \text{ if } F > F_{v1,v2}(1 - \alpha)$$

The critical *F*-value is associated with two separate degrees of freedom. The numerator degrees of freedom (v_1) equals $k - 1$ or the number of treatment levels minus one; and the denominator degrees of freedom (v_2) equals $N - k$ or the total number of observations minus the number of treatment levels (k).

An analogy can be made between the F-distribution and the t-distribution. As will be seen in the following sections, the process involves a squaring of the differences between sample means the total mean for all the sample observations. Values for the F-distribution for two levels of the discrete independent variable will be identical to the corresponding t-distribution value, squared. In other words, with only two levels of the independent variable $F_{1,N-2}$ equals $(t_{N-2})^2$, or $(t_{n1+n2-2})^2$, for the same level of confidence $(1 - \alpha)$. This is illustrated in Table 10.1. As might be expected, the outcome for an F-test on data with only two levels of a discrete independent variable will be the same as a t-test if performed on that same information. For example based on the data presented previously in Table 9.2, the result of the t-test was $t = -4.61$, $p < 0.0000329$, whereas the one-way ANOVA performed on the same data would result in $F = 21.19$, $p < 0.0000329$. Each test gives identical *p*-values.

To calculate the *F*-statistic for the decision rule either definitional or computational formulas may be used. With the exception of rounding errors, both methods will produce the same results. In the former case the sample means and standard deviations are used:

$\overline{X}_1, \overline{X}_2, \ldots \overline{X}_k$ = sample means
$S_1^2, S_2^2, \ldots S_k^2$ = sample variance
$n_1, n_2, \ldots n_k$ = sample sizes
N = total number of observations
k = number of discrete levels (treatment levels) of the independent variable

In the computational formula: 1) individual observations; 2) the sum of observations for each level of the discrete independent variable; and 3) the total sum of all observations, are squared and manipulated to produce the same outcome. The analysis of variance is a statistical procedure to analyze the overall dispersion for data in our sample outcomes. The computational method will be described later in this chapter.

Table 10.1 Comparison of Critical Value between t- and F-Distributions

	$\alpha = 0.05$			$\alpha = 0.01$		
df	t_{N-2}	$(t_{N-2})^2$	$F_{1,N-2}$	t_{N-2}	$(t_{N-2})^2$	$F_{1,N-2}$
15	2.131	4.54	4.54	2.946	8.68	8.68
30	2.042	4.17	4.17	2.750	7.56	7.56
60	2.000	4.00	4.00	2.660	7.08	7.08
120	1.979	3.92	3.92	2.617	6.85	6.85
∞	1.960	3.84	3.84	2.576	6.63	6.63

Note: *t*- and *F*-values taken from Tables B5 and B7 in Appendix B, respectively.

ANOVA Definitional Formula

The denominator of the *F*-statistic (Eq. 10.2), the **mean square within** (MS_W), is calculated in the same way as the pooled variance is calculated for the t-test, except expanded to *k* levels instead of only two levels as found in the t-test.

$$MS_W = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2 + (n_3 - 1)S_3^2 + \ldots + (n_k - 1)S_k^2}{N - K} \qquad \text{Eq. 10.3}$$

Note the similarity of this formula and the pooled variance for the t-test (Eq. 9.3). Since no single sample variance is a better measure of dispersion than the other sample variances, our best estimate is to pool the variances and create a single estimate for within variation. The mean square within is often referred to as the **mean-squared error** (MS_E) or **pooled-within-group variance** (S_w^2) and these terms are synonymous.

$$MS_W = MS_E = S_w^2$$

The mean squared within is a measure of random variability or random error among the measured objects and is not the same as the variability of the total set (*N*).

In the t-test, the numerator was the difference between two means (Eq. 9.6), which was easily calculated by subtracting one mean from the other. But how do we calculate a measure of difference when there are more than two means? In the ANOVA, there are *k* different means; therefore a measure is calculated to represent the variability among the different means. This measure of dispersion of the means is calculated similarly to a previous dispersion term, the variance (Eq. 5.3). First, the center (the grand mean) for all sample observations is calculated. Then the squared differences between each sample mean and the grand central mean are calculated. This measures an analysis of the variance between the individual sample means and the total center for all the sample observations. The **grand mean** or **pooled mean** is computed:

$$\overline{X}_G = \frac{(n_1 \overline{X}_1) + (n_2 \overline{X}_2) + (n_3 \overline{X}_3) + \ldots + (n_k \overline{X}_k)}{N} \qquad \text{Eq. 10.4}$$

This grand mean represents a weighted combination of all the sample means and an approximation of the center for all the individual sample observations. From it, the mean squared between (MS_B) is calculated similar to a sample variance (Eq. 5.3) by squaring the difference between each sample mean and the grand mean, and multiplying by the number of observations associated with each sample mean; this is then divided by the numerator degrees of freedom:

$$MS_B = \frac{n_1(\overline{X}_1 - \overline{X}_G)^2 + n_2(\overline{X}_2 - \overline{X}_G)^2 + \ldots + n_k(\overline{X}_k - \overline{X}_G)^2}{K - 1} \qquad \text{Eq. 10.5}$$

Finally the *F*-statistic is based on the ratio of the difference between the means over the distribution of their data points (Eq. 10.2):

$$F = \frac{MS_B}{MS_W}$$

In both the F-test and the t-test, the numerator of the final ratio considers differences between the means and the denominator takes into account how data are distributed around these means. The greater the spread of the sample observations, the larger the denominator, the smaller the calculated statistic and thus a lesser likelihood of rejecting H_0. The greater the differences between the means, the larger the numerator, the larger the calculated statistic, and the greater the likelihood of rejecting H_0 in favor of H_1. In other words, as the centers (means) get further apart the calculated *F*-value will increase and there is a greater likelihood that the difference will be significant. Conversely, as the dispersion becomes larger, the calculated *F*-value will decrease and the observed difference will more than likely be caused by random error.

To illustrate this method of determining the *F*-statistic, assume that during the manufacturing of a specific enteric-coated tablet, samples were periodically selected from production lines at three different facilities. Weights were taken for 15 tablets and their average weights are listed in Table 10.2. The research question would be: is there any significant difference in weights of the tablets among the three facilities? The hypotheses would be:

H_0: $\mu_{\text{facility A}} = \mu_{\text{facility B}} = \mu_{\text{facility C}}$
H_1: H_0 is false

The decision rule is with $\alpha = 0.05$, reject H_0 if $F > F_{2,42}(0.95) = 3.23$. This value is approximated from Table B7 in Appendix B, where 2 is selected from the first column ($k - 1$) and 42 approximated from the second column ($N - k$) and the value is selected from the fourth column, ($1 - \alpha = 0.95$) is 3.24 (an interpolation between 3.23 for 40 *df* and 3.15 for 60 *df*). The computations are as follows:

Table 10.2 Average Weights in Enteric Coated Tablets (in mg)

Facility A		Facility B		Facility C	
277.3	278.4	271.6	275.5	275.5	272.3
280.3	272.9	274.8	274.0	274.2	273.4
279.1	274.7	271.2	274.9	267.5	275.1
275.2	276.8	277.6	269.2	274.2	273.7
273.6	269.1	274.5	283.2	270.5	268.7
276.7	276.3	275.7	280.6	284.4	275.0
281.7	273.1	276.1	274.6	275.6	268.3
278.7		275.9		277.1	
Mean = 276.26		Mean = 275.29		Mean = 273.70	
S.D. = 3.27		S.D. = 3.46		S.D. = 4.16	

$$MS_W = \frac{(n_A - 1)S_A^2 + (n_B - 1)S_B^2 + (n_C - 1)S_C^2}{N - K}$$

$$MS_W = \frac{14(3.27)^2 + 14(3.46)^2 + 14(4.16)^2}{42} = 13.32$$

$$\overline{X}_G = \frac{(n_A \overline{X}_A) + (n_B \overline{X}_B) + (n_C \overline{X}_C)}{N}$$

$$\overline{X}_G = \frac{15(276.26) + 15(275.29) + 15(273.70)}{45} = 275.08$$

$$MS_B = \frac{n_A(\overline{X}_A - \overline{X}_G)^2 + n_B(\overline{X}_B - \overline{X}_G)^2 + n_C(\overline{X}_C - \overline{X}_G)^2}{K - 1}$$

$$MS_B = \frac{15(276.26 - 275.08)^2 + 15(275.29 - 275.08)^2 + 15(273.70 - 275.08)^2}{2}$$

$$MS_B = 25.06$$

$$F = \frac{MS_B}{MS_W} = \frac{25.06}{13.32} = 1.88$$

Thus based on the test results, the decision is with $F < 3.23$, do not reject H_0, and conclude that there is inadequate information to show a significant difference between the three facilities.

ANOVA Computational Formula

The computation technique is an alternative short cut, which arrives at the same results as the definitional method, except the formulas involve the raw data, and the means and standard deviations are neither calculated nor needed in the equations. Using this technique the MS_W and MS_B (also known as the **mean sum of squares**) are arrived at by two steps. First the sums of the squared deviations are obtained and then these sums are divided by their respective degrees of freedom (i.e., numerator or denominator degrees of freedom). Figure 10.2 illustrates the layout for data treated by the computational formula. This type of mathematical notation will be used with similar formulas in future chapters. In the notation scheme, x_{jk} refers to the jth observation in the kth level of the discrete independent variable, where k varies from 1 to k (the number of groups in the analysis), and j varies from 1 to n_j (the number of observations in the kth group). In addition, the sums for each of the columns are added together ($\sum x_T$) to represent the sum total for all the observations (N_K).

A series of intermediate equations are calculated. Intermediate I is the sum of all the squared individual observations.

$$I = \sum_{k=1}^{K} \sum_{i=1}^{n} x_{jk}^2 = (x_{a1})^2 + (x_{a2})^2 + \ldots + (x_{kn})^2 \qquad \text{Eq. 10.6}$$

Intermediate II is the square of the total sum of all observations, divided by the total number of observations.

$$II = \frac{\left[\sum_{k=1}^{K} \sum_{i=1}^{n} x_{jk}\right]^2}{N_K} = \frac{(\sum x_T)^2}{N_K} \qquad \text{Eq. 10.7}$$

Treatments (levels)

A	B	C	…	K
x_{a1}	x_{b1}	x_{c1}	…	x_{k1}
x_{a2}	x_{b2}	x_{c2}	…	x_{k2}
x_{a3}	x_{b3}	x_{c3}	…	x_{k3}
…	…	…	…	…
x_{an}	x_{bn}	x_{cn}	…	x_{kn}
———	———	———	—	———
$\sum x_A$	$\sum x_B$	$\sum x_C$	...	$\sum x_K$
Observations per level = n_A	n_B	n_C	...	n_K

$\sum x_T$ = total sum of observations

Figure 10.2 Data format for the ANOVA computational formula.

Intermediate *III* involves summing each column (level of the discrete variable), squaring that sum, and dividing by the number of observations in the column. Then the results for each column are summed.

$$III = \sum_{k=1}^{K} \frac{\left[\sum_{i=1}^{n} x_{jk}\right]^2}{n_K} = \frac{(\sum x_A)^2}{n_A} + \frac{(\sum x_B)^2}{n_B} + \ldots + \frac{(\sum x_K)^2}{n_k} \qquad \text{Eq. 10.8}$$

These intermediate equations are used to determine the various sums of squares that appear in a traditional ANOVA table:

$$SS_B = III - II \qquad \text{Eq. 10.9}$$

$$SS_W = I - III \qquad \text{Eq. 10.10}$$

$$SS_T = I - II \qquad \text{Eq. 10.11}$$

Note that the sum of squared deviations for the within groups (SS_W) and between groups (SS_B) should add to the total sum of the squares (SS_T) and this relationship can serve as a quick check of our mathematical calculations.

$$SS_B + SS_W = SS_T$$

The ANOVA table is used to calculate the *F*-statistic. Each sum of squares is divided by their respective degrees of freedom and the resultant mean squares are used in the formula present for determining the *F*-statistic (Eq. 10.2):

Source	Degrees of Freedom	Sum of Squares	Mean Square	F
Between Groups	k – 1	III – II	$\frac{III - II}{k - 1}$	$\frac{MS_B}{MS_W}$
Within Groups	N – k	I – III	$\frac{I - III}{N - k}$	
Total	N – 1	I – II		

This method can be applied to the same problem that was used for the definitional formula. The hypotheses, test statistic, decision rule, and critical value ($F_{critical} = 3.23$) remain the same for the data presented in Table 10.2. In Table 10.3 the same data is presented, but includes the sums of the various columns. The mathematics for the computational formula are as follows:

Table 10.3 Average Weights of Enteric-Coated Tablets (in mg)

Facility A	Facility B	Facility C
277.3	271.6	275.5
280.3	274.8	274.2
279.1	271.2	267.5
…	…	…
273.1	274.6	268.3
$\sum x_A = 4143.9$	$\sum x_B = 4129.4$	$\sum x_C = 4105.5$
	$\sum\sum x = 12378.8$	

$$I = \sum\sum x_{jk}^2 = (277.3)^2 + (280.3)^2 + \ldots (268.3)^2 = 3{,}405{,}824.58$$

$$II = \frac{\left[\sum\sum x_{jk}\right]^2}{N_k} = \frac{(12378.8)^2}{45} = 3{,}405{,}215.32$$

$$III = \sum \frac{\left[\sum x_{jk}\right]^2}{n_k} = \frac{(4143.9)^2}{15} + \frac{(4129.4)^2}{15} + \frac{(4105.5)^2}{15} = 3{,}405{,}265.45$$

$$SS_B = III - II = 3{,}405{,}265.45 - 3{,}405{,}215.32 = 50.13$$

$$SS_W = I - III = 3{,}405{,}824.58 - 3{,}405{,}265.45 = 559.13$$

$$SS_T = I - II = 3{,}405{,}824.58 - 3{,}405{,}215.32 = 609.26$$

$$SS_B + SS_W = SS_T \qquad 609.26 = 559.13 + 50.13$$

The ANOVA table for this example would be:

Source	df	SS	MS	F
Between	2	50.13	25.07	1.88
Within	42	559.13	13.31	
Total	44	609.26		

The decision rule is the same, with $F < 3.23$, do not reject H_0. Note that the results are identical to those using the definitional formula, with possible minor rounding differences in the mean square column.

A second example of a one-way analysis of variance, seen below, is a case where C_{max} measurements (maximum concentrations in micrograms per milliliter) were

found for four different formulations of a particular drug.[1] The researcher wished to determine if there was a significant difference in the C_{max} for the definitional formulations.

C_{max} in mcg/ml:	Mean	S.D.	n
Formulation A	123.2	12.8	20
Formulation B	105.6	11.6	20
Formulation C	116.4	14.6	19
Formulation D	113.5	10.0	18

In this case the hypotheses are:

H_0: $\mu_A = \mu_B = \mu_C = \mu_D$
H_1: H_0 is false

The hypothesis under test is that the four formulas of the study drug produce the same C_{max}, on the average. If this is rejected then the alternate hypothesis is accepted, namely that some difference exists somewhere among the four formulations. Using Eq. 10.2, our decision rule is, with $\alpha = 0.05$, reject H_0 if $F > F_{3,73}(0.95) = 2.74$. This critical value comes from Table B7 in Appendix B, with $k - 1$ or 3 in the first column, $N - k$ or 73 approximated in the second column and 2.74 interpolated from the fourth column (between 60 and 120 *df*) at 95% confidence.

The computations using the definitional formula would be:

$$MS_W = \frac{(n_A - 1)S_A^2 + (n_B - 1)S_B^2 + (n_C - 1)S_C^2 + (n_D - 1)S_D^2}{N - K}$$

$$MS_W = \frac{19(12.8)^2 + 19(11.6)^2 + 18(14.6)^2 + 17(10.0)^2}{73} = 153.51$$

$$\overline{X}_G = \frac{(n_A \overline{X}_A) + (n_B \overline{X}_B) + (n_C \overline{X}_C) + (n_D \overline{X}_D)}{N}$$

$$\overline{\overline{X}_G} = \frac{20(123.2) + 20(105.6) + 19(116.4) + 18(113.5)}{77} = 114.68$$

[1] It should be noted that in most cases distributions of C_{max} data would be positively skewed and a lognormal transformation be required. However, for our purposes we will assume that the sample data approximates a normal distribution. Also note that the variances, squares of the standard deviations, are similar and we can assume homogeneity of variances. Specific tests for homogeneity are presented in the last section of this chapter.

$$MS_B = \frac{n_A(\overline{X}_A - \overline{X}_G)^2 + n_B(\overline{X}_B - \overline{X}_G)^2 + n_C(\overline{X}_C - \overline{X}_G)^2 + n_D(\overline{X}_D - \overline{X}_G)^2}{K-1}$$

$$MS_B = \frac{20(123.2 - 114.68)^2 + 20(105.6 - 114.68)^2 + \ldots 18(113.5 - 114.68)^2}{3}$$

$$MS_B = 1060.67$$

$$F = \frac{MS_B}{MS_W} = \frac{1060.67}{153.51} = 6.91$$

The decision based on the sample data is, with $F > 2.74$, reject H_0 and conclude there is a difference between the various formulations.

This last example shows an important feature of the analysis of variance. In this particular case, H_0 was rejected and therefore $\mu_A = \mu_B = \mu_C = \mu_D$ is not true. However, the results of the statistical test do *not* tell us where the difference or differences among the four populations occur. Looking at the data it appears that Formulation A has a C_{max} that is significantly longer than the other formulations. Yet, at the same time Formulation B has a significantly shorter C_{max}. In fact, all four formulations could be significantly different from each other. The *F*-value that was calculated does not provide an answer to where the significant differences exist. In order to determine this, some type of *post hoc* or multiple comparisons procedure needs to be performed (Chapter 11).

Randomized Block Design

The one-way analysis of variance has been presented as a logical extension of the t-test to more than two levels of the independent variable and the **randomized block design** can be thought of as an expansion of the paired t-test to three or more measures of the same subject or sample. Also known as the **randomized complete block design**, it represents a two-dimensional design for repeated measures with one observation per cell.

The randomized block design was developed in the 1920s by R. A. Fisher, to evaluate methods for improving agricultural experiments (Fisher, 1926). To eliminate variability between different locations of fields, his research design first divided the land into blocks. The area within each block were assumed to be relatively homogeneous. Then each of the blocks was further subdivided into plots and each plot within a given block received one of the treatments under consideration. Therefore, only one plot within each block received a specific treatment and each block contained plots that represented all the treatments.

Using this design, subjects are assigned to blocks in order to reduce variability within each treatment level. The randomized block design can be used for a variety of situations where there is a need for homogeneous blocks. The observations or subjects within each block are more homogeneous than subjects within the different blocks. For example, assume that the age of volunteers may influence the study results and

Table 10.4 Randomized Block Design

Age	Treatment 1	Treatment 2	Treatment 3
21-25	1 volunteer	1 volunteer	1 volunteer
26-30	1 volunteer	1 volunteer	1 volunteer
31-35	1 volunteer	1 volunteer	1 volunteer
...	...	...	...
61-65	1 volunteer	1 volunteer	1 volunteer

the researcher wants to include all possible age groups with each of the possible treatment levels. Volunteers are divided into groups based on age (e.g., 21-25, 26-30, 31-35, etc.), then one subject from each age group is randomly selected to receive each treatment (Table 10.4). In this randomized block design, each age group represents one block and there is only one observation per cell (called **experimental units**). Like Fisher's agricultural experiments, each treatment is administered to each block and each block receives every treatment. The rows represent the blocking effect and the columns show the treatment effect.

As a second example, with three treatment levels (three assay methods), assume that instead of 24 tablets randomly sampled from one production run, we sample from 8 different runs. Then taking samples from each run, we have the same analyst evaluate each on the batches using the three assay methods. In this case we assume that each of our individual production runs is more homogeneous than total mixing of all 24 samples across the 8 runs. As seen in Figure 10.3, three samples in each row comprise a block from the same production run. Note there is still only one observation per cell. Differences between the means for the columns reflect treatment effects (in this case the difference between the three methods) and differences between the mean for each row reflect the differences between the production runs.

As seen in Figure 10.3 the independent variables are 1) the treatment levels that appear in the columns (main effect) and 2) the blocks seen in the rows that are sub-levels of the data. The assumptions are that: 1) there has been random independent sampling; 2) at each treatment level, the outcomes are normally distributed and variances for groups at different treatment levels are similar (homogeneity of variance); and 3) block and treatment effects are additive (no interaction between the treatments and blocks). The hypotheses are as follows:

H_0: $\mu_A = \mu_B$ for two treatment levels
H_1: $\mu_A \neq \mu_B$

H_0: $\mu_A = \mu_B = \ldots \mu_K$ for three or more treatment levels
H_1: H_0 is false

As seen in the hypotheses, the main interest is in treatment effects and the blocking is used to eliminate any extraneous source of variation. The decision rule is, with $\alpha = 0.05$, reject H_0 if $F > F_{k-1,j-1}(1 - \alpha)$. The critical F-value is based on $k - 1$ treatment levels as the numerator degrees of freedom, and $j - 1$ blocks as the denominator

	M_1	M_2		M_k	Sum by Block	Block Means
Block (batch) b_1	x_{11}	x_{12}	…	x_{1k}	$\sum x_{b1}$	$\overline{X}_{b1}$
Block (batch) b_2	x_{21}	x_{22}	…	x_{2k}	$\sum x_{b2}$	$\overline{X}_{b2}$
Block (batch) b_3	x_{31}	x_{32}	…	x_{3k}	$\sum x_{b3}$	$\overline{X}_{b3}$
Block (batch) b_4	x_{41}	x_{42}	…	x_{4k}	$\sum x_{b4}$	$\overline{X}_{b4}$
…	…	…	…	…	…	…
Block (batch) b_j	x_{j1}	x_{j2}	…	X_{jk}	$\sum x_{bj}$	$\overline{X}_{bj}$
Sum by column	$\sum x_{t1}$	$\sum x_{t2}$	…	$\sum x_{tk}$	$\sum\sum x_{jk}$	
Treatment means	$\overline{X}_1$	$\overline{X}_2$	…	$\overline{X}_k$		

Figure 10.3 Data format for a randomized block design.

degrees of freedom. The data is presented as follows:

	Treatment Levels			
Blocks	K_1	K_2	...	K_k
B_1	x_{11}	x_{21}	...	x_{k1}
B_2	x_{12}	x_{22}	...	x_{k2}
...	...	...	...	...
B_j	x_{1j}	x_{2j}	...	x_{kj}

The formula and ANOVA table are similar to those involved in the computational formulas for the one-way ANOVA. In this case there are four intermediate calculations, including one that measures the variability of blocks (III_R) as well as one that measures the variability of the methods or treatment effect (III_C). The total sum of squares for the randomized block design is composed of the sums of squares attributed to the treatments, the blocks, and random error. Similar to the computational formula for the one-way ANOVA, Intermediate *I* is the sum of all the squared individual observations.

$$I = \sum_{k=1}^{K} \sum_{j=1}^{J} x_{kj}^2 \qquad \text{Eq. 10.12}$$

Intermediate II is the square of the total sum of all observations, divided by the product of the number of treatments (*K*) times the number of blocks (*J*).

$$II = \frac{\left[\sum_{k=1}^{K}\sum_{j=1}^{J} x_{kj}\right]^2}{kj} \quad \text{Eq. 10.13}$$

Intermediate III_R for the block effect is calculated by adding up all the sums (second to the last column in Figure 10.3) for each block and dividing by the number of treatment levels.

$$III_R = \frac{\sum_{k=1}^{K}\left[\sum_{j=1}^{J} x_{kj}\right]^2}{k} \quad \text{Eq. 10.14}$$

Intermediate III_C for the treatment effect is calculated by adding up all the sums second to the last row in Figure 10.3) for each treatment and dividing by the number of blocks.

$$III_C = \frac{\sum_{j=1}^{J}\left[\sum_{k=1}^{K} x_{kj}\right]^2}{j} \quad \text{Eq. 10.15}$$

The intermediate results are used to calculate each of these various sum of squares:

$$SS_{Total} = SS_T = I - II \quad \text{Eq. 10.16}$$

$$SS_{Blocks} = SS_B = III_R - II \quad \text{Eq. 10.17}$$

$$SS_{Treatment} = SS_{Rx} = III_C - II \quad \text{Eq. 10.18}$$

$$SS_{Error} = SS_{Residual} = SS_T - SS_B - SS_{Rx} \quad \text{Eq. 10.19}$$

An ANOVA table is constructed and each sum of squares is divided by its corresponding degrees of freedom to produce a mean square (Figure 10.4).

The *F*-value is calculated by dividing the mean square for the treatment effect by the mean square error (also referred to as the **mean square residual**):

$$F = \frac{MS_{Rx}}{MS_R} \quad \text{Eq. 10.20}$$

If the calculated *F*-value exceeds the critical value (F_c) for $k - 1$ and $j - 1$ degrees of freedom, H_0 is rejected and it is assumed that there is a significant difference between the treatment effects.

Source	df	SS	MS	F
Treatment	$k - 1$	SS_{Rx}	$\frac{SS_{Rx}}{k - 1}$	$\frac{MS_{Rx}}{MS_R}$
Blocks	$j - 1$	SS_B	$\frac{SS_B}{j - 1}$	
Residual	$(k - 1)(j - 1)$	SS_R	$\frac{SS_R}{(k - 1)(j - 1)}$	
Total	$N - 1$	SS_T		

Figure 10.4 ANOVA Table for a randomized block design.

One of the most common uses for the randomized block design involves cross-over clinical drug trials. **Crossover studies**, are experimental designs in which each patient receives two or more treatments that are being evaluated. The order in which patients receive the various treatments is decided through a random assignment process (for example, if only treatments A and B are being evaluated, half the patients would be randomly assigned to receive A first, the other half would receive A second). This design is in contrast to parallel studies and self-controlled studies. In **parallel studies**, two or more treatments are evaluated concurrently in separate, randomly assigned, groups of patients. An example of a parallel study would be the first question in the problem set in Chapter 9, where physical therapy patients were assigned (presumably by a randomized process) to two different treatment regimens and evaluated (using a two-sample t-test) for outcomes as measured by range of motion. A **self-controlled study**, is one in which only one treatment is evaluated and the same patients are evaluated during treatment and at least one period when no treatment is present. The second question in the problem set for Chapter 9 offers an example of a self-controlled study in which the same patients were measured before and after treatment with a new bronchodilator and their responses evaluated using a paired t-test.

The major advantage of the crossover design is that each patients serves as his or her own control, which eliminates subject-to-subject variability in response to the treatments being evaluated. The term "randomized" in the title of this design refers to the order in which patients are assigned to the various treatments. With each patient serving as a block in the design there is increased precision, because of decreased random error and a more accurate estimate of true treatment differences. Major disadvantages with crossover experiments are that: 1) the patient may change over time (the disease state becomes worse, affecting later measurements); 2) with increased time there is a chance for subjects to withdraw or drop out of the study, which results in decreased sample size; 3) there may be a carryover effect of the first treatment affecting subsequent treatments; and 4) the first treatment may introduce permanent physiological changes affecting later measurements. These latter two problems can be evaluated using a two-way analysis of variance design, discussed in

Table 10.5 Diastolic Blood Pressure with a New Antihypertensive

Blocks (Subject)	Treatment 1 (Before)	Treatment 2 (After)	∑	Mean
1	68	66	134	67
2	83	80	163	81.5
3	72	67	139	69.5
4	75	74	149	74.5
5	79	70	149	74.5
6	71	77	148	74
7	65	64	129	64.5
8	76	70	146	73
9	78	76	154	77
10	68	66	134	67
11	85	81	166	83
12	74	68	142	71
∑ =	894	859	1753	
Mean =	74.50	71.58		

Chapter 12, where two independent variables (treatment and order of treatment) can be assessed concurrently. Additional information about these types of experimental designs are presented by Bolton (2004) and Freidman and colleagues (1985).

As mentioned previously, a paired t-test could be considered a special case of the randomized block design with only two treatment levels. For example, the data appearing in the first three columns of Table 9.3 could be considered a randomized design (Table 10.5). Each subject represents one of twelve blocks, with two treatment measures (before and after). In this particular case the null hypothesis states that there is no difference between the two treatment periods (before versus after):

$$H_0: \mu_B = \mu_A$$
$$H_1: \mu_B \neq \mu_A$$

The decision rule is with $\alpha = 0.05$, reject H_0 if $F > F_{1,11}(.95)$, which is 4.90 (interpolated from Table B7). The calculations are as follows:

$$I = \sum_{k=1}^{K} \sum_{j=1}^{J} x_{kj}^2$$

$$I = (68)^2 + (83)^2 + (72)^2 + \ldots (68)^2 = 128{,}877$$

$$II = \frac{\left[\sum_{k=1}^{K}\sum_{j=1}^{J} x_{kj}\right]^2}{KJ}$$

$$II = \frac{(1753)^2}{24} = 128{,}042.0417$$

$$III_R = \frac{\sum_{k=1}^{K}\left[\sum_{j=1}^{J} x_{kj}\right]^2}{K}$$

$$III_R = \frac{(134)^2 + (163)^2 + \ldots (142)^2}{2} = 128{,}750.5$$

$$III_C = \frac{\sum_{j=1}^{J}\left[\sum_{k=1}^{K} x_{kj}\right]^2}{J}$$

$$III_C = \frac{(894)^2 + (859)^2}{12} = 128{,}093.0833$$

$$SS_{Total} = SS_T = I - II$$

$$SS_T = 128{,}877 - 128{,}042.0417 = 834.9583$$

$$SS_{Blocks} = SS_B = III_R - II$$

$$SS_B = 128{,}750.5 - 128{,}042.0417 = 708.4583$$

$$SS_{Treatment} = SS_{Rx} = III_C - II$$

$$SS_{Rx} = 128{,}093.0833 - 128{,}042.0417 = 51.0416$$

$$SS_{Error} = SS_{Residual} = SS_T - SS_B - SS_{Rx}$$

$$SS_{Residual} = 834.9583 - 708.4583 - 51.0416 = 75.4584$$

The ANOVA table is:

Source	df	SS	MS	F
Treatment	1	51.0416	51.0416	7.44
Blocks	11	708.4583	64.4053	
Residual	11	75.4584	6.8599	
Total	23	834.9583		

With the calculated *F*-value greater than the critical value of 4.90, the decision is to reject H_0 and conclude that there is a significant difference between the before and after measurements with the after measure of diastolic blood pressure significantly lower than that before therapy. These results are exactly the same as observed in the paired t-test example in the previous chapter (both test results with a $p < 0.02$).[2] The utility of this method is that it can be expanded to more than just two levels of treatment.

As will be discussed later, Excel refers to this type of test as "ANOVA: two factor without replication" and reports an *F*-value and corresponding p-value for the blocks as well as the main treatment effect.

Homogeneity of Variance

It is important that we address the issue of homoscedasticity. One of the criteria required to perform any parametric procedure is that the dispersion within the different levels of the discrete independent variable be approximately equal. The reason that homogeneity of variance is important is that the error term denominator of the *F*-ratio (MS_W) is an average for variances as the different levels of the independent variable weighted by the size of each group. When these individual variances differ greatly this average becomes a useless summary for these measures of dispersions. As mentioned in Chapter 9 a simple rule of thumb is that the ratio of largest to smallest group variances should be 2.0 or less. Because of the robustness of the F-distribution, differences with variances can be tolerated if sample sizes are equal (Cochran, 1947; Box, 1954). However, for samples that are unequal in size, a marked difference in variances can affect the statistical outcomes.

Several tests are also available to determine if there is a lack of homogeneity. The simplest is **Hartley's F-max test**. Using this test the following hypotheses of equal variances are tested:

$$H_0: \sigma_1^2 = \sigma_2^2 = \sigma_3^2 \ldots = \sigma_k^2$$
$$H_1: H_0 \text{ is false}$$

The test statistic is a simple ratio between the largest and smallest variances:

---

[2] Using Excel® the *p*-value can be calculated for $t = 2.73$ using the T.DIST.2T function ($p = 0.01958$) as well as the *p*-value for $F = 7.44$ using the F.DIST.RT function ($p = 0.01966$). The minor difference is due to rounding before the final *t*- and *F*-values were reported.

$$F_{max} = \frac{S^2_{largest}}{S^2_{smallest}} \qquad \text{Eq. 10.21}$$

The resultant F_{max} value is compared to a critical value from Table B8 (Appendix B) for k levels of the discrete independent variable and $n - 1$ degrees of freedom, based on n observations per level of the independent variable (equal cell size). If F_{max} exceeds the critical value, H_0 is rejected and the researcher cannot assume that there is homogeneity. For example, consider the previous example comparing the weights of tablets from three different facilities (Table 10.2). The largest variance is from facility C at 17.31 (4.16^2) and the smallest from Facility A is 10.69 (3.27^2). Can the investigator assume that there is homogeneity of variance?

H_0: $\sigma_A^2 = \sigma_B^2 = \sigma_C^2$
H_1: H_0 is false

With $\alpha = 0.05$, H_0 would be rejected if F_{max} exceeds the critical $F_{3,14}$, which is approximately 3.75. Calculation of the test statistic is:

$$F_{max} = \frac{S^2_{largest}}{S^2_{smallest}} = \frac{17.31}{10.69} = 1.62$$

With F_{max} less than 3.75 the researcher would fail to reject the null hypothesis with 95% confidence and would assume that the sample variances are all equal.

A second procedure that can be used for unequal cell sizes (differing numbers of observations per level of the independent variable) would be **Cochran's C test**, which compares the ratio of the largest sample variance with the sum of all variances:

$$C = \frac{S^2_{largest}}{\Sigma S^2_k} \qquad \text{Eq. 10.22}$$

Once again a table of critical values is required (Table B9, Appendix B). The calculated C ratio is compared to a critical value from Table B9 for k levels of the independent variable in the samples and $n - 1$ observations per sample. If C exceeds the critical value, H_0 is rejected and the researcher cannot assume that there is homogeneity. Using the same example as above, with $\alpha = 0.05$, H_0 would be rejected if C exceeds the critical C-value, which is approximately 0.5666. Calculation of the test statistic is:

$$C = \frac{S^2_{largest}}{\Sigma S^2_k} = \frac{(4.16)^2}{(3.27)^2 + (3.46)^2 + (4.16)^2} = \frac{17.31}{39.97} = 0.4331$$

With C less than 0.5666 the exact same result occurs as was found with the Hartley F_{max} results.

If the cell size differs slightly, the largest of the *n*'s can be used to determine the degrees of freedom. Consider the second ANOVA example with four different formulations (A, B, C, and D) and cell sizes of 20, 20, 19, and 18, respectively. In this case $n = 20$, $n - 1 = 19$ and the critical values for C by interpolation would be 0.4355. The test statistic would be:

$$C = \frac{(14.6)^2}{(12.8)^2 + (11.6)^2 + (14.6)^2 + (10.0)^2} = 0.3486$$

In both cases, the statistics are less than the critical values; the researcher fails to reject H_0 and assume that there is homogeneity of variance.

Another alternative procedure that involves more complex calculations is **Bartlett's test**, which is an older test based on a chi-square test (Chapter 16) with $(k - 1)$ degrees of freedom (Barlett, 1937). This test is described in Kirk's book (1968). **Levene's test** and **Brown and Forsythe's test** are two other tests for homogeneity that might be found on computer software packages. The Brown and Forsythe's test is based on Levene's test, but is more robust when groups are unequal in size. However, because the F-test is so robust regarding violations of the assumption of homogeneity of variance, in most cases these tests of homogeneity are usually not required if equal sample sizes are maintained.

Using Excel® or Minitab® for One-Way ANOVAs

Excel 2010 has several function (*fx*) options that are very similar to those used for t-test applications in the previous chapter. Instead of referring to Table B7 to determine critical values for the test statistic they can be determined using the function **F.INV.RT**. For older versions of Excel this command was **FINV**. Either function will prompt for the probability (as a decimal), the numerator degrees of freedom (Deg_freedom1) and the denominator degrees of freedom (Deg_freedom2). Caution should be noted here. Excel 2010 has the command **F.INV** and this command will identify the location for a certain probability on the LEFT end of the curve. Other Excel functions allow one to determine the *p*-value for a calculated *t*-statistic; **F.DIST.RT** (for Excel 2010) or **FDIST** (for older versions). Either function will prompt for the calculated *F*-value, the numerator and denominator degrees of freedom. Once again caution is needed because **F.DIST** in Excel 2010 will do the calculation for the LEFT side of the distribution.

The one-way ANOVA is available as part of the Excel data analysis tools:

Data ➤ Data Analysis ➤ Anova: Single Factor

Similar to the t-test, each level of the independent variable is represented by a different column (or row). As seen in Figure 10.5, one needs to identify the columns and range in which each level of the independent variable is located ("Input Range:"); if representing data by column or row, the amount of acceptable Type I error ("Alpha:"); and identify where the outcomes should be reported, either starting at a cell on the current page (per this example, E2) or on a new worksheet (by default).

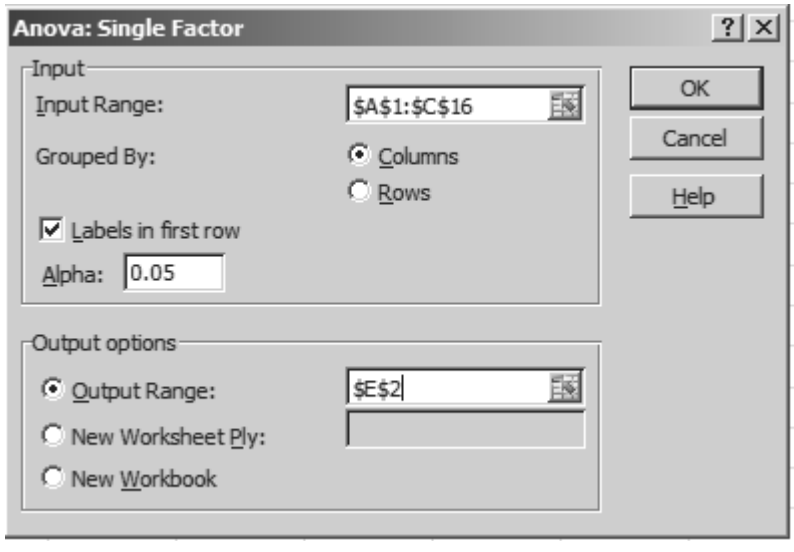


Figure 10.5 Options for the one-sample ANOVA with Excel.

Using the data in our previous example (Table 10.2) the results appear in Figure 10.6. The means and variance for each level of the independent variable are reported at the top of the results. The ANOVA table is reported next. The last three columns of the table present: 1) the *F*-statistic, 2) the associated *p*-value, and 3) the critical value required to reject the null hypotheses with the type I error selected in Figure 10.5.

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Facility A	15	4143.9	276.26	10.66114
Facility B	15	4129.4	275.2933	11.99495
Facility C	15	4105.5	273.7	17.28143

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	50.13378	2	25.06689	1.882958	0.164759	3.219942
Within Groups	559.1253	42	13.31251			
Total	609.2591	44				

Figure 10.6 Outcome report for the one-way ANOVA with Excel.

It is possible to use Excel to evaluate a complete randomized block design. As noted earlier Excel refers to this as two-factor ANOVA:

Data ➤ Data Analysis ➤ Anova: Two-Factor Without Replicates

The data is arranged on the Excel spreadsheet with each column representing a level of the independent variable (e.g., treatment level) and each row representing a block. There is only one observation per cell. As seen in Figure 10.7, the columns and range are required (Input Range:) along with the amount of acceptable Type I error ("Alpha:"); and the user must identify where the outcomes should be reported, either starting at a cell on the current page (per this example, E2) or on a new worksheet (by default). Using the data in our previous example (Table 10.5) the results appear in Figure 10.8. Note that *F*-statistics and *p*-values are reported for both the independent variable and the block effect. The treatment effect is reported second as the "columns" outcome.

Minitab offers the one-way ANOVA under "Stat" on the title bar:

Stat ➤ ANOVA ➤ One-way…

Like most software packages, each column represents a variable and each row an observation. Columns are chosen for Minitab based on whether they independent or dependent variables. Figure 10.9 illustrates the decisions required for a one-way ANOVA for the data from Table 10.2. The dependent variable is labeled "Response" and the independent variable is the "Factor". These are selected by double clicking on the variables in the box on the left. The confidence level can be changed from the default 1 - α of 95% if desired. *Graphs*... option includes individual value plots or box plots for each level of the independent variable. The *Comparisons*... option will be discussed in the next chapter. The results of the analysis are presented in Figure 10.10. The top portion is the traditional ANOVA table with the *F*-statistic and

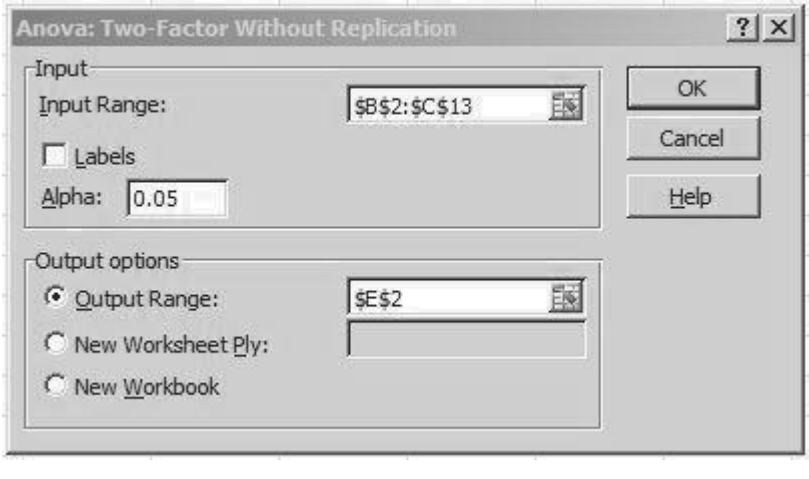


Figure 10.7 Options for a complete randomized block design with Excel.

Anova: Two-Factor Without Replication

SUMMARY	Count	Sum	Average	Variance
Row 1	2	134	67	2
Row 2	2	163	81.5	4.5
Row 3	2	139	69.5	12.5
Row 4	2	149	74.5	0.5
Row 5	2	149	74.5	40.5
Row 6	2	148	74	18
Row 7	2	129	64.5	0.5
Row 8	2	146	73	18
Row 9	2	154	77	2
Row 10	2	134	67	2
Row 11	2	166	83	8
Row 12	2	142	71	18
Column 1	12	894	74.5	37.36364
Column 2	12	859	71.58333	33.90152

ANOVA

ource of Variatio	SS	df	MS	F	P-value	F crit
Rows	708.4583	11	64.4053	9.388736	0.000422	2.81793
Columns	51.04167	1	51.04167	7.440641	0.019657	4.844336
Error	75.45833	11	6.859848			
Total	834.9583	23				

Figure 10.8 Outcome report for a complete randomized block design with Excel.

associated p-value on the right side. Near the bottom is an illustration of the 95% confidence interval for each level of the independent variable.

If data happens to be arranged in Minitab using the prescribed manner for Excel (each column represents one level of the independent) there is an alternative method for performing the test:

Stat ➤ ANOVA ➤ One-way (Unstacked)…

Here the columns that represent the various levels of the independent variable are double clicked from the box on left side and added to the "Responses (in separate columns)" location.. Similar choices for the "One-way…" are available.

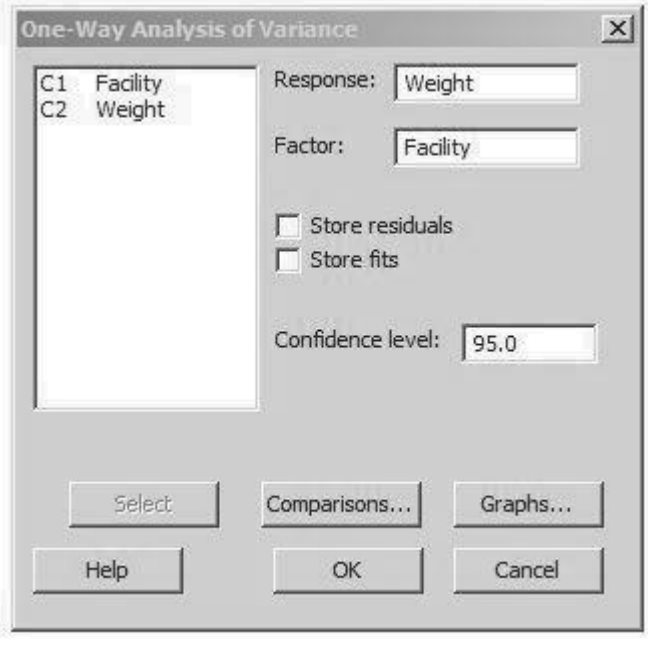


Figure 10.9 Options for the one-way ANOVA with Minitab.

One-way ANOVA: Weight versus Facility

Source	DF	SS	MS	F	P
Facility	2	50.1	25.1	1.88	0.165
Error	42	559.1	13.3		
Total	44	609.3			

S = 3.649 R-Sq = 8.23% R-Sq(adj) = 3.86%

Level	N	Mean	StDev
A	15	276.26	3.27
B	15	275.29	3.46
C	15	273.70	4.16

Individual 95% CIs For Mean Based on Pooled StDev

```
    -+---------+---------+---------+--------
     |                   (-----------*-----------)
     |              (-----------*----------)
     |    (-----------*-----------)
     -+---------+---------+---------+--------
    272.0     273.6     275.2     276.8
```

Pooled StDev = 3.65

Figure 10.10 Outcome report for the one-way ANOVA with Minitab.

Minitab offers both Bartlett's and Levene's tests for assessing homogeneity of variance. Both tests are located under the ANOVA option:

Stat ➤ ANOVA ➤ Test for equal variances…

As with the one-way ANOVA, "Response" represents the dependent variable and

"Factor" is the independent variable. Both tests will be automatically run and results reported graphically and in text format (Figure 10.11). Even though these tests were not discussed in this chapter, their interpretation would be similar to Hartley's and Cochran's tests. The important aspect is the *p*-value reported by either test. If greater than 0.05 the null hypothesis of equal variances cannot be rejected. As seen in Figure 10.11, neither one was significant and the hand calculated results for Hartley's F-max and Cochran's C test, the hypothesis of equal variances cannot be rejected.

The example in this section was taken from previous data in the chapter and the results (minor rounding differences) were identical to the results worked out by hand.

References

Bartlett, M.S. (1937). "Properties of sufficient and statistical tests," *Proceedings of Royal Society of London, Series A* 160:280-282.

Bolton, S. and Bon, C. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, Inc., New York, pp. 311-372.

Box, G.E.P. (1954). "Some theorems on quadratic forms applied in the study of analysis of variance problems," *Annals of Statistics* 25:290-302.

Cochran, W.G. (1947). "Some consequences when the assumptions of analysis of variance are not satisfied," *Biometrics* 3:22-38.

Fisher, R.A. (1926). "The arrangement of field experiments," *Journal of Ministry of Agriculture* 33:503-513.

Friedman, L.M., Furberg, C.D., and DeMets, D.L. (1985). *Fundamentals of Clinical Trials*, PSG Publishing Company, Inc., Littleton, MA, pp. 33-47.

Test for Equal Variances: Weight versus Facility

95% Bonferroni confidence intervals for standard deviations

Facility	N	Lower	StDev	Upper
A	15	2.24092	3.26514	5.76178
B	15	2.37697	3.46337	6.11159
C	15	2.85309	4.15709	7.33576

Bartlett's Test (Normal Distribution)
Test statistic = 0.89, p-value = 0.642

Levene's Test (Any Continuous Distribution)
Test statistic = 0.16, p-value = 0.852

Figure 10.11 Outcome report for tests of homogeneity with Minitab.

Kachigan, S.K. (1991). *Multivariate Statistical Analysis*, Second edition, Radius Press, New York, pp. 195-197.

Kirk, R.E. (1968). *Experimental Design: Procedures for the Behavioral Sciences*, Brooks/Cole, Belmont, CA, pp. 61,62.

Salsburg, D. (2002). *The Lady Tasting Tea: How Statistics Revolutionized Science in the Twentieth Century*. Henry Holt and Company, New York, pp. 48-50.

Suggested Supplemental Readings

Bolton, S. and Bon, C. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, Inc., New York, pp. 215-232.

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Eighth edition, John Wiley and Sons, New York, pp. 303-368.

Kirk, R.E. (1968). *Experimental Design: Procedures for the Behavioral Sciences*, Brooks/Cole, Belmont, CA, pp. 104-109, 131-134.

Example Problems (Answers are provided in Appendix D)

1. In a collaborative trial, four laboratories were sent samples from the same batch of a pharmaceutical product and requested to perform ten assays and report the results based on percentage of a labeled amount of the drug (Table 10.6). Were there any significant differences based on the laboratory performing the analysis?

Table 10.6 Data from Four Different Laboratories

Lab (A)	Lab (B)	Lab (C)	Lab (D)
100.0	99.5	99.6	99.8
99.8	100.0	99.3	100.5
99.5	99.3	99.5	100.0
100.1	99.9	99.1	100.1
99.7	100.3	99.7	99.4
99.9	99.5	99.6	99.6
100.4	99.6	99.4	100.2
100.0	98.9	99.5	99.9
99.7	99.8	99.5	100.4
99.9	100.1	99.9	100.1
$\sum$ = 999.0	996.9	995.1	1000.0
Mean = 99.90	99.69	99.51	100.00
S.D. = 0.25	0.41	0.22	0.34

Table 10.7 Viscosity of Different Batches of a Product

	Viscosity Batch A	Viscosity Batch B	Viscosity Batch C	
	10.23	10.24	10.25	
	10.33	10.28	10.20	
	10.28	10.20	10.21	
	10.27	10.21	10.18	
	10.30	10.26	10.22	
$\sum =$	51.41	51.19	51.06	$\sum\sum = 153.66$

2. In the previous example, perform a test to determine if there is homogeneity of variance.

3. Acme Chemical and Dye received from the same raw material supplier three batches of oil from three different production sites. Samples were drawn from drums at each location and compared to determine if the viscosity was the same for each batch (Table 10.7). Are the viscosities the same regardless of the batch?

4. During a clinical trial, Acme Chemical wants to compare two possible generic formulations to the currently available brand product (reference standard). Based on the following results (Table 10.8), is there a significant difference between the two Acme formulations and the reference standard?

Table 10.8 Original Data for Two Different Formulations

	Plasma Elimination Half-Life (in minutes)		
	Formulation A	Formulation B	Reference Standard
Subject 001	206	207	208
Subject 002	212	218	217
Subject 003	203	199	204
Subject 004	211	210	213
Subject 005	205	209	209
Subject 006	209	205	209
Subject 007	217	213	225
Subject 008	197	203	196
Subject 009	208	207	212
Subject 010	199	195	202
Subject 011	208	208	210
Subject 012	214	222	219

Table 10.9 Days in the Hospital

Physician A	Physician B	Physician C
9	10	8
12	6	9
10	7	12
7	10	10
11	11	14
13	9	10
8	9	8
13	11	15
Mean = 10.38	Mean = 9.13	Mean = 10.75
S.D. = 2.26	S.D. = 1.81	S.D. = 2.66

5. Three physicians were selected for a study to evaluate the length of stay for patients undergoing a major surgical procedure. All these procedures occurred in the same hospital and were without complications. Eight records were randomly selected from patients treated over the past 12 months (Table 10.9). Was there a significant difference, by physician, in the length of stay for these patients?

6. To evaluate the responsiveness of individuals receiving various commercially available benzodiazepines, volunteers were administered these drugs and subjected to a computer simulated driving test. Twelve volunteers were randomly divided into four groups, each receiving one of three benzodiazepines or a placebo. At two week intervals they were crossed over to other agents and retested until each volunteer had received each active drug and the placebo. Driving abilities were measured 2 hours after the drug administration (at approximately the C_{max} for the benzodiazepines). The higher the score, the greater the number of driving errors. The results are listed below:

Benzo (A)	Benzo (B)	Benzo (C)	Placebo
58	62	53	50
54	55	45	51
52	58	48	53
62	56	46	57
51	60	58	61
55	48	61	49
45	73	52	50
63	57	51	60
56	64	55	40
57	51	48	47
50	68	62	46
60	69	49	43

7. Replicate measures are made on samples from various batches of a specific biological product. The researcher is concerned that the first measure may influence the outcome on the second measure. Using a complete randomized block design, is there independence (no effect) between the first and second replicate measures?

	Treatment (% recovered)	
	Replicate 1	Replicate 2
Batch A	93.502	92.319
Batch C	91.177	92.230
Batch D	87.304	87.496
Batch D2	81.275	80.564
Batch G	79.865	79.259
Batch G2	81.722	80.931

11

Multiple Comparison Tests

As discussed in the previous chapter, rejection of the null hypothesis in the one-way analysis of variance simply proves that some significant difference exists between at least two levels of the discrete independent variable. Unfortunately the ANOVA does not identify the exact location of the difference(s). Multiple comparison tests can be used to reevaluate the data for a *significant* ANOVA and identify where the difference(s) exist while maintaining an overall Type I error rate (α) at the same level as that used to test the original null hypothesis for the one-way ANOVA. Assuming an analysis of variance was conducted with $\alpha = 0.05$ and the H_0 was rejected, then the multiple comparison tests will keep the error rate constant at 0.05.

Error Associated with Multiple t-Tests

Sometimes researchers performing multiple t-tests between various two levels of the independent variable err (called pair-wise combinations). It should be noted that the use of the term "pair-wise" refers to comparisons involving two levels of the discrete independent variable and should not be confused with "paired" tests, which involve repeated measures (e.g., paired t-test). By using **multiple t-tests** the researcher actually compounds the Type I error rate. This compounding of the error is referred to as the **experimentwise error rate**. For example, if there are three levels for the independent variable, there are three possible comparisons:

$$\binom{3}{2} = \frac{3!}{2!1!} = 3$$

which are A versus B, B versus C, and A versus C. An alternative formula for the number pair-wise comparisons is simply using the *k* number of levels of the independent variable:

$${}_kC_2 = \frac{k(k-1)(k-2)!}{2!(k-2)!} = \frac{k(k-1)}{2} \qquad \text{Eq. 11.1}$$

Table 11.1 Experimentwise Error Rates for Multiple Paired t-Tests after a Significant ANOVA

Number of Groups (Discrete levels)	Number of Possible Paired Comparisons	Level of Significance Used in Each t-Test	
		0.05	0.01
2	1	0.05	0.01
3	3	0.143	0.030
4	6	0.265	0.059
5	10	0.401	0.096
6	15	0.536	0.140
7	21	0.659	0.190
8	28	0.762	0.245

In the previous example with $k = 3$, the results would be identical:

$$_3C_2 = \frac{3(2)}{2} = 3$$

A simple way of thinking about this compounding error rate would be that if each t-test were conducted with $\alpha = 0.05$, then the error rate would be three comparisons times 0.05, or a 0.15 error rate. Seen in Table 11.1, as the number of levels of the independent variable increases and the number of intersample comparisons (e.g., pair-wise comparisons) increases at a rapid rate, thus greatly increasing the level of α. The actual calculation for compounding the error or experimentwise error rate is:

$$\alpha_{ew} = 1 - (1 - \alpha)^C \qquad \text{Eq. 11.2}$$

where C is the total number of possible independent t-tests comparing only two levels of the discrete independent variable. Table 11.1 lists the experimental error rate for various pair-wise combinations. The third column is for the 95% confidence level and the fourth for the 99% confidence level. One could also think of these comparison as a "family" of possible pair-wise comparisons. These tests are used to ensure that the probability is held constant for the family's multiple comparisons. Thus, a synonym for experimentwise error rate is **familywise error rate** (FWE = α_{ew}).

Overview of Multiple Comparison Tests

As will be seen, there are many multiple comparison tests. Multiple comparison procedures can be divided into *a priori* and *post hoc* tests (planned and unplanned tests). This chapter will present most of the multiple comparison tests in the following order: 1) planned pair-wise comparisons; 2) *post hoc* pair-wise tests; and 3) complex comparisons using Scheffé's procedures.

A second way to divide these tests is into: 1) single-step methods and 2) stepwise, sequential methods (Table 11.2). In the former case, there are simultaneous confidence intervals that allow directional decisions. Tests in the latter group are

Table 11.2. Multiple Comparison Tests

Single-Step Methods	Stepwise (Step-Down) Methods
Bonferroni	Student-Newman-Keul (SNK)
Sidák	REGWQ
Dunnett	REGWF
Fisher LSD	Duncan
Tukey HSD	Tukey's-b
Tukey-Kramer	Bonferroni-Holm
Scheffé	Sidák-Holm
Hochberg's GF2	
Gabriel	

limited to hypotheses testing, but are usually more powerful. Thus, if the primary goal is not hypothesis testing or confidence intervals are not needed, the stepwise methods are preferable. Stepwise tests usually involve a range test. Most of these multiple comparison tests listed in Table 11.2 will be discussed in this chapter.

Certain multiple comparison tests are defined as "exact tests." These exact tests are procedures where the experimentwise error rate is exactly equal to α for balanced as well as unbalanced one-way designs (balanced designs involve an equal number of observations per level of the independent variable). Other tests, such as the REGWQ, REGWF, SNK, and Duncan tests are recommend for balanced designs only.

The standard error term used for most multiple comparison tests is based on modifications of the following:

$$SE = \sqrt{MS_E}\sqrt{\frac{1}{n_1}+\frac{1}{n_2}} \qquad \text{Eq. 11.3}$$

where the MS_E (which is the same as the MS_W) is taken from the original ANOVA table.

The one-way ANOVA is a robust test and can tolerate some deviation from the parameter of equal variances. However, most of the commonly used *post hoc* procedures require equal variances (Tukey HSD, Fisher LSD, Student-Newman-Keul, Duncan); other more obscure tests do not require this assumption (Games-Howell, Dunnett's T3, Dunnett's C, and Tamhane's T2 tests).

Because there are a variety of multiple comparison tests to choose from, it is important to understand these tests and choose the most appropriate one. The test should not be picked at random, and more importantly, it should not be chosen based on the results of the various tests (for example, looking at the results for many different multiple comparison tests and picking the one that gives researcher's desired outcome). Just like any other statistical test the comparisons test should be chosen before the initial ANOVA is computed. Different situations require the use of

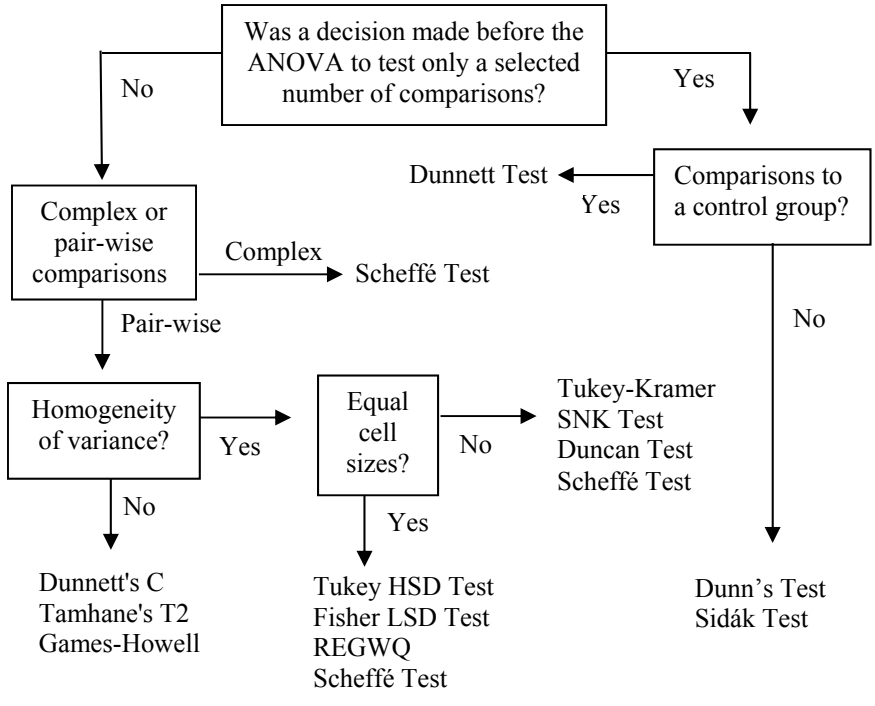


Figure 11.1 Algorithm for choosing multiple comparison procedures.

different multiple comparison tests and there does not appear to be agreement on a "best" procedure to use routinely. Figure 11.1 provide a rough algorithm for selecting a multiple comparison test.

The *q*-Statistic

The *q*-statistic (also known as the *q* range statistic or **Studentized range statistic**) is commonly used in coefficients for multiple comparison tests (planned and *post hoc*). As the number of comparisons between group increases, there is an expected increase in variability and the researcher should compensate for this by using a more conservative test; if not, the likelihood of Type I errors increases considerably. The *q*-statistic provides this more conservative approach. Both the *q*- and *t*-statistics use the difference between means in the numerator. However, the *q*-statistic uses the standard error of the mean in the denominator, whereas the *t*-statistic uses the standard error of the difference between the means. Thus, instead of measuring the difference between two means, like the *t*-statistic, the *q*-statistic tests the probability that the largest and smallest sample means were sampled from the same population. Similar to using the *t*-statistic, if the computed *q*-statistic is not as large as the critical *q*-value from a table, then the researcher cannot reject the null

hypothesis that the groups do not differ at the given alpha significance level. It follows that if the null hypothesis is not rejected comparing the largest and smallest sample means, then all intermediate means representing the other levels of the discrete independent variable are also drawn from the same population. The general formula for the *q*-statistic is:

$$q = \frac{\overline{X}_A - \overline{X}_B}{SE} \qquad \text{Eq. 11.4}$$

The SE term is defined differently for various multiple comparison procedures, the more popular of these will be discussed below. Also, the *q*-value can be used as a reliability coefficient to build a confidence interval:

$$\mu_A - \mu_B = (\overline{X}_A - \overline{X}_B) \pm (q_{critical})(SE) \qquad \text{Eq. 11.5}$$

The q-critical can be found in the Studentized range distribution in Table B10 in Appendix B and is usually defined as $q_{1-\alpha,k-1,N-k}$ where $k-1$ and $N-k$ are the degrees of freedom from the ANOVA table for the within and between effects. Certain multiple comparison statistics define these numerator and denominator degrees of freedom differently and these will be noted below.

One can think of the Studentized range test as a traditional t-test, where the critical values have been adjusted based on the number of sample means being compared. It replaces the traditional one-way analysis of variance with a test that compares only the largest and the smallest means in the experiment. The *q*-statistic is basically an adjusted t-test between the largest and smallest means.

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{S_p^2}{n_1} + \frac{S_p^2}{n_2}}} = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{2S_p^2}{n}}} = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{2MS_E}{n}}} \qquad \text{Eq. 11.6}$$

Above is an expansion of the *t*-statistic: 1) for equal sample sizes and 2) replacing the pooled variance for the MS_{error}, which are the same. The *q*-statistic is the same with the removal of the square root of two in the denominator:

$$q = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{MS_E}{n}}} \qquad \text{Eq. 11.7}$$

Planned Multiple Comparisons

The student t-test can be used to compare only two levels of the independent variable and is only recommended when the researcher has a single **planned comparison,** based on *a priori* theory, established before running the initial analysis

Table 11.3 Bonferroni Adjustment for Maintaining an Experimental Error Rate of 0.05

Number of Discrete Levels	Number of Possible Paired Comparisons	Bonferroni Adjustment	Estimate Critical Value for Infinite Degrees of Freedom
2	1	0.0500	1.960
3	3	0.0167	2.394
4	6	0.0083	2.638
5	10	0.0050	2.807
6	15	0.0037	2.935
7	21	0.0024	3.038
8	28	0.0018	3.124
9	36	0.0014	3.197
10	45	0.0011	3.261

of variance. Bonferroni, Sidák, Dunn, and Dunnett's tests are planned multiple comparison tests, not *post hoc* procedures since decisions are made prior to calculating the original analysis of variance. Following the rejection of the global null hypothesis for the one-way ANOVA, a *post hoc* procedure should be used to identify specific significant differences. These *post hoc* methods are described following the *a priori* methods described below. Most results will be expressed as confidence intervals similar to the previous example (Eq. 11.5).

Bonferroni Adjustment

The Bonferroni adjustment (or **Bonferroni test**) is the simplest multiple comparison test and involves multiple t-tests. In this procedure the experimentwise error rate is kept constant (usually 0.05) by dividing the Type I error by the total number of possible or planned pair-wise comparisons (*C*).

$$\alpha' = \frac{\alpha}{C} \qquad \text{Eq. 11.8}$$

Experimentwise error rate is not exactly equal to α, but is less than α in most situations. Unfortunately, the Bonferroni test may be too conservative and not have enough power to detect significant differences. Plus, tables of critical *t*-values may be hard to find for the required α'. For example, with three levels of an independent variable, $C = 3$ and $\alpha' = 0.0167$ (if the original ANOVA was tested at 0.05). Table 11.3 lists various adjustments for an infinite number of observations. These were calculated under the assumption that the *t*-value is at infinity and uses the standardized normal distribution and *z*-values for the various adjusted *p*-values (α). Notice how the critical value increases as the number of levels of the discrete independent variable increases, thus controlling increased experimentwise error rate.

What if there are less than an infinite number of observations? Other tables are available in various textbooks for smaller sample sizes and smaller α values than those in the third column of Table 11.3. As seen in Table B5 (Appendix B), for larger sample sizes the reliability coefficient is very close to the *t*-value at infinity (1.96). For example, at 80 degrees of freedom the critical *t*-value = 2.00. Therefore, Table 11.3 can be used as a rough approximation for the required critical value.[1]

To illustrate both multiple t-tests and Bonferroni's adjustment, consider the first example in the problem set in Chapter 10 where a significant difference was found with a Type I error rate of 0.05, leading to the rejection of the following "global" null hypothesis associated with the original one-way analysis of variance:

$$H_0: \mu_A = \mu_B = \mu_C = \mu_D$$

The data used for this example were:

Concentration in mcg/ml:	Mean	S.D.	n
Formulation A	123.2	12.8	20
Formulation B	105.6	11.6	20
Formulation C	116.4	14.6	19
Formulation D	113.5	10.0	18

Since there are four levels of the discrete independent variable and six possible pair-wise comparisons, the Bonferroni's adjustment of the α would be 0.008 and a *very rough approximation* for the reliability coefficient would be 2.65 from Table 11.3. Confidence intervals can be created using the following formula (Eq. 9.4):

$$\mu_1 - \mu_2 = (\overline{X}_1 - \overline{X}_2) \pm 2.65\sqrt{\frac{S_P^2}{n_1} + \frac{S_P^2}{n_2}}$$

If we compare the results for all six pair-wise t-tests and six tests with Bonferroni's adjustment, there are more significant findings with the multiple t-test due to the experimentwise error (Table 11.4).

Performing unadjusted multiple t-tests is one of the major errors found in the literature (Glantz, 1980). When the independent variable has more than two discrete levels, an ANOVA followed by an appropriate multiple comparison procedure is the correct test, not multiple t-tests. As seen in Table 11.4, when multiple independent t-tests are applied to the same set of data, it becomes increasingly likely that a significant outcome will result by chance alone.

[1] The *t*-values can be obtained using Microsoft Excel by determining α' (α/C) and using the function T.INV (α', df).

Table 11.4 Results with Multiple t-Tests and the Bonferroni Adjustment

Pairing	Multiple t-Tests Confidence Interval	Bonferroni Adjustments Confidence Interval
$\overline{X}_A - \overline{X}_B$	$+9.79<\mu_A - \mu_B<+25.41$ *	$+7.38<\mu_A - \mu_B<+27.82$ *
$\overline{X}_A - \overline{X}_C$	$-2.07<\mu_A - \mu_C<+15.674$	$-0.24<\mu_A - \mu_C<+13.84$
$\overline{X}_A - \overline{X}_D$	$+2.11<\mu_A - \mu_D<+17.29$ *	$+3.29<\mu_A - \mu_D<+16.11$ *
$\overline{X}_B - \overline{X}_C$	$-19.31<\mu_B - \mu_C<-2.29$ *	$-21.95<\mu_B - \mu_C<+0.35$
$\overline{X}_B - \overline{X}_D$	$-15.04<\mu_B - \mu_D<-0.76$ *	$-17.26<\mu_B - \mu_D<+1.46$
$\overline{X}_C - \overline{X}_D$	$-5.46<\mu_C - \mu_D<+11.26$	$-8.06<\mu_C - \mu_D<+13.86$

* Significant at $p < 0.05$.

Sidák Test

The Sidák test (or **Dunn-Sidák test**) is a variation of the Bonferroni test using a t-test for pair-wise multiple comparisons. For this test, the Type I error rate is modified to slightly smaller adjusted *p*-values than for the Bonferroni test. The Sidák procedure is slightly more powerful than the Bonferroni procedure and guarantees to control for experimentwise error when there are independent comparisons (orthogonal contrasts).

$$\alpha' = 1 - (1 - \alpha)^{1/C} \qquad \text{Eq. 11.9}$$

Once again the problem of identifying appropriate tables limits the usefulness of this procedure (in the previous example C = 3 and $\alpha' = 0.01695$).

Dunn's Multiple Comparisons

Dunn's procedure (also called a **Bonferroni t statistic, Bonferroni corrected test,** or **Fisher protected LSD test**) calculates mean differences for all pair-wise comparisons and compares these differences to a critical value extracted from a table. As an extension of the Bonferroni adjustment it is recommended for multiple planned comparisons, if the number of pair-wise comparisons is not large. As seen in Table 11.1, as larger numbers of comparisons are made, one is increasing the likelihood of a Type I error. Thus, as the number of comparisons increase, a more stringent α level must be used to maintain an overall experimentwise Type I error rate consistent with the Type I error rate in the original analysis of variance. For most of the multiple comparison tests we will continue using the same example from Chapter 10, where it was found that a significant difference existed somewhere between the following means:

Concentration in mcg/ml:	Mean	n	
Formulation A	123.2	20	$MS_W = MS_E = 153.51$
Formulation B	105.6	20	
Formulation C	116.4	19	$\nu_2 = N–K = 73$
Formulation D	113.5	18	

The total number of possible pair-wise comparisons is:

$$C = \binom{4}{2} = \frac{4!}{2!2!} = 6$$

The absolute difference for each pair-wise comparison is computed:

$$\left| \overline{X}_A - \overline{X}_B \right| = 17.6 \qquad \left| \overline{X}_B - \overline{X}_C \right| = 10.8$$
$$\left| \overline{X}_A - \overline{X}_C \right| = 6.8 \qquad \left| \overline{X}_B - \overline{X}_D \right| = 7.9$$
$$\left| \overline{X}_A - \overline{X}_D \right| = 9.7 \qquad \left| \overline{X}_C - \overline{X}_D \right| = 2.9$$

A value is extracted from the table of Dunn's percentage points (Table B11, Appendix B). This value takes into consideration: 1) the total number of possible pair-wise comparisons (C); 2) the original denominator degrees of freedom ($N - k$) for the ANOVA; and 3) the Type I error rate used in the original ANOVA (e.g., $\alpha = 0.05$). As seen in Table B11, the first column is the number of possible combinations, the Type I error rate is in the second column, and the remaining columns relate to the $N - k$ degrees of freedom. In this particular example the table value is

$$t'D_{\alpha;C;N-K} = t'D_{.05;6;73} \approx 2.72$$

This number is then inserted into the calculation of a critical Dunn's value:

$$d = t' D_{\alpha/2;C;N-K} \sqrt{MS_E \cdot \left(\frac{1}{n_1} + \frac{1}{n_2}\right)} \qquad \text{Eq. 11.10}$$

If the absolute mean difference is greater than the calculated d-value there is a significant difference between the two means. The calculation of the d-value for the first pair-wise comparison is:

$$d = (2.72)\sqrt{153.51 \cdot \left(\frac{1}{20} + \frac{1}{20}\right)} = (2.72)(3.92) = 10.66$$

Our decision, with $\left| \overline{X}_A - \overline{X}_B \right|$ difference greater than the calculated d-value of 10.66 is to reject $\mu_A = \mu_B$ and conclude that there is a significant difference between these two population means.

An alternative method is to create a confidence interval similar to the t-test:

$$\mu_1 - \mu_2 = (\overline{X}_1 - \overline{X}_2) \pm t' D_{\alpha/2;C;N-K} \sqrt{MS_E \cdot (\frac{1}{n_1} + \frac{1}{n_2})} \qquad \text{Eq. 11.11}$$

Notice how this equation is exactly the same in layout as all previous confidence intervals (estimate ± reliability coefficient × error term). For the first pair-wise comparison:

$$\mu_A - \mu_B = (123.2 - 105.6) \pm 2.72 \sqrt{153.51 \ (\frac{1}{20} + \frac{1}{20})}$$

$$\mu_A - \mu_B = (17.6) \pm 10.66$$

$$6.94 < \mu_A - \mu_B < 28.26$$

Since zero does not fall within the interval, there is a significant difference between Formulations A and B. Note the same results occurred with the Bonferroni adjustment. However, two important features appear with Dunn's procedure: 1) the table of critical values allows for better corrections for smaller sample sizes and 2) by using the MS_E the entire variance from the original ANOVA is considered rather than only the pooled variance for the pair-wise comparison.

Using the original method for the calculation of the *d*-value, the *d*-value for this second pair-wise comparison is:

$$d = (2.72) \sqrt{153.51 \ (\frac{1}{20} + \frac{1}{19})} = (2.72)(3.97) = 10.80$$

Here the decision, with $| \overline{X}_A - \overline{X}_C |$ greater than 10.80, we fail to reject $\mu_A - \mu_C$, thus we fail to find that there is a significant difference between these two levels. Similarly the confidence interval is:

$$\mu_A - \mu_C = (123.2 - 116.4) \pm 2.72 \sqrt{153.51 \cdot (\frac{1}{20} + \frac{1}{19})}$$

$$-4.00 < \mu_A - \mu_B < 17.60$$

With zero within the confidence interval, the same results are obtained and we cannot conclude that there is a difference. The Dunn's test is not recommended when the investigator plans to perform all possible pair-wise comparisons, but for this example all possible pair-wise comparisons will be tested.

Table 11.5 presents a summary of all pair-wise comparisons. We can conclude that Formulation B has a significantly lower maximum concentration than Formulation A, and that there appear to be no other significant pair-wise comparisons.

Table 11.5 Results of Dunn's Multiple Comparisons

Pairing	Confidence Interval	Results
$\overline{X}_A - \overline{X}_B$	$+6.94 < \mu_A - \mu_B < +28.26$	Significant
$\overline{X}_A - \overline{X}_C$	$-4.00 < \mu_A - \mu_C < +17.60$	
$\overline{X}_A - \overline{X}_D$	$-1.25 < \mu_A - \mu_D < +20.65$	
$\overline{X}_B - \overline{X}_C$	$-21.64 < \mu_B - \mu_C < +0.04$	
$\overline{X}_B - \overline{X}_D$	$-18.85 < \mu_B - \mu_D < +3.05$	
$\overline{X}_C - \overline{X}_D$	$-8.18 < \mu_C - \mu_D < +13.98$	

Dunnett's Test

The last planned multiple comparison test is used when various treatment groups are compared to a single control group. This test was developed by C.W. Dunnett and is based on a modification of the *q*-statistic (1955). It is an exact test (the experimentwise error rated exactly equal to α) for both balanced and unbalanced one-way designs. It generally has better power than alternative tests. Significance can be tested using the following ratio based on the *q*-statistic:

$$q = \frac{\overline{X}_C - \overline{X}_i}{\sqrt{MS_E\left(\frac{1}{n_C} + \frac{1}{n_i}\right)}} \qquad \text{Eq. 11.12}$$

where $\overline{X}_i$ is the sample mean for one experimental groups, the $\overline{X}_C$ is the mean for the control group and *n*'s are the sample sizes for the experimental and control groups. For this test the null hypothesis would be that each experimental group equals the control group.

$$H_0: \mu_I = \mu_C$$
$$H_1: \mu_I \neq \mu_C$$

All the experimental groups and the control group are placed in order based on the magnitude of their means. Then a range (*p*) is determined for the number of inclusive means between the experimental group being considered and the control group. For example, consider the following means ranked from smallest to largest:

$$\overline{X}_5 \quad \overline{X}_1 \quad \overline{X}_C \quad \overline{X}_3 \quad \overline{X}_4 \quad \overline{X}_2$$

Table 11.6 Approximate Sample Sizes for the Control Group in a Dunnett's Test Based on Number of Samples in the Other Treatment Levels

Sample size in Other Treatment Groups	k-Levels of Independent including Control Group						
	3	4	5	6	7	8	10
10	14	17	20	22	24	26	30
15	21	25	30	33	36	39	45
20	28	34	40	44	48	52	60
25	35	43	50	55	60	65	75
30	42	51	60	66	72	78	90
50	70	85	100	110	120	130	150
100	140	170	200	220	240	260	300

In this example, if group 5 were to be compared to the control, the *p*-range would be three (three means are included in the range between $\overline{X}_5$ and $\overline{X}_C$). Similarly, if the second group ($\overline{X}_2$) were compared to the control, the *p*-range would be four. This *p*-value is used in the decision rule:

$$\text{Reject } H_0 \text{ if } q > q_{\alpha,p,N-k}$$

The critical *q*-values are found in Table B12 (Appendix B). This table represents values for two-tailed tests only; tables are available in other texts for one-tailed Dunnett tests (Zar, 2010).

An alternative approach would be to create a confidence interval:

$$\mu_C - \mu_i = \left(\overline{X}_C - \overline{X}_i\right) \pm q_{\alpha,p,N-k}\sqrt{MS_E\left(\frac{1}{n_C}+\frac{1}{n_i}\right)} \qquad \text{Eq.11.13}$$

The interpretation of this test would be similar to the two-sample t-test confidence interval. If zero falls with in the confidence interval, the null hypothesis cannot be rejected. If all the values in the interval are positive or negative values, the null hypothesis is rejected.

For these comparisons, the control group should have more observations than the other comparison groups. It is recommended that the ideal size for the control groups should be approximately $\sqrt{k-1}$ times larger than the sample sizes for the experimental groups. Table 11.6 lists a comparison of the number of observations required in the control group for various numbers of observations in each treatment group.

As an example of Dunnett's test, consider a study to evaluate the responsiveness of individuals receiving various commercially available benzodiazepines. Volunteers were administered these drugs and subjected to a computer-simulated driving test. Volunteers are randomly assigned to three treatment groups receiving different benzodiazepines (n_i = 12) and a control group receiving a placebo (n = 24). In this

Table 11.7 Data for a Dunnett Example

	Drug A	Drug B	Drug D	Placebo (Control)	
	57	60	53	50	57
	53	56	45	51	58
	51	56	48	53	49
	61	54	46	52	50
	50	58	58	61	55
	54	50	61	49	40
	46	69	52	50	47
	62	55	51	60	46
	55	62	55	45	43
	56	53	48	47	50
	49	66	62	48	51
	59	64	49	43	53
Mean =	54.42	58.58	52.33	50.33	

study the higher the score, the greater the number of driving errors. The results of the study are presented in Table 11.7. Performing an ANOVA on this data, it was determined that there was a significant difference and the null hypotheses of $\mu_A = \mu_B = \mu_D = \mu_{Placebo}$ was rejected (decision rule: with $\alpha = 0.05$, reject H_0 if $F > F_{.05,3,56}(0.95) \approx 2.77$):

$$F = \frac{MS_B}{MS_W} = \frac{190.6}{28.93} = 6.59$$

The order of the different sample means is:

$$\overline{X}_B \quad < \quad \overline{X}_A \quad < \quad \overline{X}_D \quad < \quad \overline{X}_C$$

With the $MS_E = MS_W = 28.93$ and $q_{\alpha/2,p,N-k} = q_{.05,3,56} = 2.27$, the comparison for benzodiazepine A to the control group using Dunnett's test and subsequent confidence interval is as follows:

$$\mu_C - \mu_A = (50.33 - 54.42) \pm 2.27\sqrt{28.93\left(\frac{1}{24} + \frac{1}{12}\right)}$$

$$\mu_C - \mu_A = -4.09 \pm 4.32$$

$$-8.41 < \mu_C - \mu_A < +0.23$$

With zero within the interval, there is no significant difference between benzodiazepine A and the control. Similar results are seen with benzodiazepine D

where $p = 2$:

$$\mu_C - \mu_D = (50.33 - 52.33) \pm 2.00\sqrt{28.93\left(\frac{1}{24} + \frac{1}{12}\right)}$$

$$-5.80 < \mu_C - \mu_A < +1.80$$

But there is a significant difference between benzodiazepine B and the control group ($p = 4$):

$$\mu_C - \mu_B = (50.33 - 58.58) \pm 2.41\sqrt{28.93\left(\frac{1}{24} + \frac{1}{12}\right)}$$

$$-12.83 < \mu_C - \mu_A < -3.67$$

Post Hoc Procedures

Post hoc procedures or ***a posteriori*** **tests** are used when the researcher is interested in evaluating differences, but not limited to those specified in advance (required for the Bonferroni, Dunn, or Dunnett's tests). Many of these types of tests are based on a *q*-statistic. As seen in Chapter 10 there are two underlying assumptions associated with the one-way analysis of variance, namely, population normality and homogeneity of variance. Homogeneity of variance is the more serious assumption. However, the ANOVA is a robust statistic and can tolerate minor deviations from the ideal. Equal sample sizes should be the goal to maximum power and robustness of the ANOVA. These same rules apply to *post hoc* procedures.

Tukey HSD Test

The Tukey HSD test (honestly significant difference test) is a *post hoc* procedure that can be used for all pair-wise comparisons between levels of the discrete independent variable. The Tukey HSD test is also referred to as **HDS test** or the **Tukey test**. It is based on the Studentized range distribution (*q*-statistic) and is preferred when the number of groups is large since it is a conservative pair-wise comparison test. Large numbers of groups threaten to inflate the Type I error rate. It is recommended to use the Tukey HSD test if it is required to test all pair-wise comparisons of the means and present confidence intervals. When all pair-wise comparisons are being tested, the Tukey HSD test is more powerful than the Dunn test. Therefore, the Dunn test would be recommended for a small partial set of pair-wise comparisons and the Tukey test would be employed when all pair-wise comparisons are considered *a posteriori*. The Tukey HSD test is limited to only pair-wise comparisons and preferably equal sample sizes (balanced design).

For each pair-wise comparison the following hypotheses are tested.

$$H_0: \mu_A = \mu_B$$
$$H_1: \mu_A \neq \mu_B$$

Using the following statistic

$$q = \frac{\overline{X}_A - \overline{X}_B}{\sqrt{\frac{MS_E}{n}}} \qquad \text{Eq. 11.14}$$

the decision rule is

$$\text{With } \alpha = .05, \text{ reject } H_0 \text{ if } q > q_{\alpha,k,N-k} \text{ or } q < -q_{\alpha,k,N-k}$$

Where α is usually consistent with the value used for the original one-way analysis of variance, k is the number of levels of the independent variable and $N - k$ represents the denominator degrees of freedom from the ANOVA table. The q-value is obtained from Table B10 (Appendix B).

The **Tukey-Kramer test** is a modification of the formula to accommodate for unequal cell sizes:

$$q = \frac{\overline{X}_A - \overline{X}_B}{\sqrt{\frac{MS_E}{2}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}} \qquad \text{Eq. 11.15}$$

Both these formulas (Eqs. 11.14 and 11.15) can be modified to create confidence intervals:

$$\mu_A - \mu_B = (\overline{X}_A - \overline{X}_B) \pm (q_{\alpha,k,N-k})\sqrt{\frac{MS_E}{n}} \qquad \text{Eq. 11.16}$$

$$\mu_A - \mu_B = (\overline{X}_A - \overline{X}_B) \pm (q_{\alpha,k,N-k})\sqrt{\frac{MS_E}{2}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)} \qquad \text{Eq. 11.17}$$

Interpretation of the results would be similar to those used for confidence intervals involving two-sample t-tests; if zero is within the interval there is no significant difference and if zero is outside the interval there is a significant difference between the population means based on the two sample means being tested.

Using the previous example with the four formulations, the critical value from Table B10 is 3.73 for q with k = 4 number of means and $N - k$ = 73 degrees of freedom with 95% confidence ($1 - \alpha$). A comparison between Formulas A and D would be computed as follows:

Table 11.8 Results of the Tukey-Kramer Test Comparisons

Pairing	Confidence Interval	Results
$\overline{X}_A - \overline{X}_B$	$+7.27 < \mu_A - \mu_B < +27.93$	Significant
$\overline{X}_A - \overline{X}_C$	$-3.68 < \mu_A - \mu_C < +17.28$	
$\overline{X}_A - \overline{X}_D$	$-0.92 < \mu_A - \mu_D < +20.32$	
$\overline{X}_B - \overline{X}_C$	$-21.28 < \mu_B - \mu_C < -0.32$	Significant
$\overline{X}_B - \overline{X}_D$	$-18.53 < \mu_B - \mu_D < +2.73$	
$\overline{X}_C - \overline{X}_D$	$-7.84 < \mu_C - \mu_D < +13.64$	

$$q = \frac{123.2 - 113.5}{\sqrt{\frac{153.51}{2}\left(\frac{1}{20} + \frac{1}{18}\right)}} = \frac{9.7}{2.85} = 3.40$$

Since 3.40 is less than the critical value of 3.73 we would fail to reject the hypothesis that $\mu_A = \mu_D$ and conclude that no difference could be found between these two formulas. Similar results would obtained creating a confidence interval:

$$\mu_A - \mu_D = (123.2 - 113.5) \pm 3.73 \cdot \sqrt{\frac{153.51}{2}\left(\frac{1}{20} + \frac{1}{18}\right)}$$

$$\mu_A - \mu_D = (9.7) \pm 10.62$$

$$-0.92 < \mu_A - \mu_D < 20.32$$

Since zero falls within the interval, the decision is that there is no significant difference between Formulations A and D. A summary of all possible pair-wise comparisons using the Tukey-Kramer test is presented in Table 11.8.

It is possible to reject H_0 with the original ANOVA and the Tukey tests fail to detect a pair-wise difference. This is due to the fact that the ANOVA is a more powerful test than multiple comparison tests. In this case repeating the study with a larger sample size would tend to result in a greater likelihood of identifying a significant difference using one of the multiple comparison tests. Alternatively, tests like Scheffé could be used to make more complex comparisons.

Two other *post hoc* procedures are modifications of the Tukey HSD test based on the *q*-statistic. The first is the **Tukey's wholly significant difference (WSD) test** (also called the **Tukey WDS** or **Tukey-b** test)**.** It is a less conservative stepwise procedure. The critical value of Tukey WSD is the average of the corresponding

values for the Tukey's HSD test and the Newman-Keuls test. The second procedure is **Games-Howell test** (also known as the Games and Howell's modification of Tukey's HSD or **GH test**). This pair-wise test is designed for unequal variances and/or unequal sample sizes. The GH test is a relatively liberal *post hoc* procedure and can be too liberal when the sample size is small and it is recommended that individual levels of the independent variable have sample sizes greater than five. Discussions of these tests can be found in Toothaker's book on multiple comparisons (Toothaker, 1991).

Student Newman-Keuls Test

The Student Newman-Keul test (also referred to as the **Newman-Keuls test** or **SNK test**) is a stepwise, multiple range *post hoc* procedure, based on the *q*-statistic, which compares every mean with every other mean in a pair-wise fashion. Notice the formula is identical to that used for the Tukey and Tukey-Kramer tests. For balanced designs the formula is:

$$q = \frac{\overline{X}_A - \overline{X}_B}{\sqrt{\frac{MS_E}{n}}} \qquad \text{Eq. 11.18}$$

For unbalanced designs use:

$$q = \frac{\overline{X}_A - \overline{X}_B}{\sqrt{\frac{MS_E}{2}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}} \qquad \text{Eq. 11.19}$$

The Tukey HSD and Student Newman-Keuls tests are run exactly the same except the Tukey test maintains a constant *k*-value for the number of levels of the independent variable and with the SNK test the value is based on the number of steps inclusive of the means being compared. The Tukey test is more conservative, but it is an exact test and will keep alpha at 0.05 for all pair-wise comparisons, regardless of the number of means in the study.

Sample means are first rank-ordered in ascending or descending order. The number of means between two means being compared (including those two means) become the range. In these cases, the critical value is $q_{\alpha,p,N-k}$, where *p* is the range. The *p*-range is sometimes referred to as "steps." The difference in this process compared to the HSD test is that the critical values for the Tukey test remain constant for all comparisons but the critical values for the SNK differ based on the size of the stepwise differences. SNK tends to be less conservative than the Tukey test and will result in the identification of more significantly different pair-wise comparisons than Tukey. Also, it should be used cautiously for unbalanced cases.

The calculated *q*-statistic is compared to the critical values listed in Table B10 of Appendix B. The denominator degrees of freedom used are similar to the previous procedures and are the same as the original denominator degrees of freedom for the F-test ($N - k$). One additional piece of information is required to read the critical value,

namely the distance in steps (or range). For example, listed below are means used previously, but reordered from the highest to the lowest mean:

Formulation A	123.2
Formulation C	116.4
Formulation D	113.5
Formulation B	105.6

The step difference between Formulation A and Formulation C is two ($p = 2$) and the number of steps between Formulation A and Formulation B is four ($p = 4$). This difference is used to select the appropriate column from Table B10. As seen in Table B10, the first column is the denominator degrees of freedom ($N - k$), the Type I error rate is in the second column, and the remaining columns relate to the number of mean steps. In the above example if we were to select the critical value for a comparison between Formulations A and D the step difference would be three and the $N - k$ degrees of freedom is 73, giving a critical value for $\alpha = 0.05$ of approximately 3.39. The decision rule is with $\alpha = 0.05$, reject the H_0: $\mu_A = \mu_D$ if $q > q_{.05,3,73} \approx 3.39$ and the computation is:

$$q = \frac{123.2 - 113.5}{\sqrt{\frac{153.51}{2}\left(\frac{1}{20} + \frac{1}{18}\right)}} = \frac{9.7}{2.85} = 3.40$$

In this case, q is greater than the critical q-value; therefore, we would reject the H_0 that they are equal. Step-down procedures (such as the SNK test) do not provide confidence intervals, but just divide pair-wise differences into possible overlapping groups.

Similarly, the comparison between Formulations B and D would involve only a two "step difference"; therefore, the decision rule for this comparison is, with $\alpha = 0.05$, reject H_0: $\mu_B = \mu_D$ if $q > q_{.05,2,73} \approx 2.82$ and the computation is:

$$q = \frac{113.5 - 105.6}{\sqrt{\frac{153.51}{2}\left(\frac{1}{20} + \frac{1}{18}\right)}} = \frac{7.9}{2.85} = 2.77$$

Here the calculated q-value is less than the critical q-value, therefore we cannot reject the hypothesis that the formulations are equal. A summary of all possible pair-wise comparisons using the Newman-Keuls test is presented in Table 11.9.

A modification of the SNK test is the **Ryan-Einot-Gabriel-Welch range test**, which is abbreviated as the **REGWQ test** or **Ryan test**. In this adjustment the critical values decrease as "stretch" size decreases (the range from highest to lowest mean in the set being considered). It is based on the q-distribution. Like the Newman-Keuls test, the Ryan test is a step-down procedure and is not recommended for unbalanced design, but is a more conservative test for balanced designs. In this case the family-

Table 11.9 Results of Newman-Keuls' Comparisons

Pairing	q-Statistic	Critical Value	Results
$\overline{X}_A - \overline{X}_B$	6.35	3.73	Significant
$\overline{X}_A - \overline{X}_C$	2.24	2.82	
$\overline{X}_A - \overline{X}_D$	3.41	3.39	Significant
$\overline{X}_B - \overline{X}_C$	3.85	3.39	Significant
$\overline{X}_B - \overline{X}_D$	2.78	2.82	
$\overline{X}_C - \overline{X}_D$	1.01	2.82	

wise error rate does not exceed alpha and the REGWQ is generally considered more powerful than the Tukey test. The **Ryan test F** (or **REGWF**) is a further modification of the SNK test, but based on the F-distribution and it is more computationally intense and more powerful than the REGWQ. More information about these tests can be found in Toothaker's book (Toothaker, 1991).

Fisher LSD Test

The Fisher LSD (least significant difference) test is a *post hoc* procedure based on the *t*-statistic and not a range test (*q*-statistic). Developed by R.A. Fisher, this test is also referred to as the **LSD test** or **protected t-test**. The process compares all possible pair-wise means after a significant F-test rejects the null hypothesis that all levels of the independent variable are equal. The Fisher LSD can handle all pair-wise comparisons and equal sample sizes are not required.

$$\mu_1 - \mu_2 = \left(\overline{X}_1 - \overline{X}_2\right) \pm t_{1-\alpha/2,N-k} \sqrt{\frac{MS_E}{n_1} + \frac{MS_E}{n_2}} \qquad \text{Eq. 11.20}$$

This method is quick, though a less rigorous *post hoc* procedure and has some control over the experimentwise error rate. The problem with this approach is that it can lead to a greater experimentwise error rate if most population means are equal but only one or two are different. Homogeneity of variance is typically assumed for Fisher's LSD. Even though the LSD test can handle unpaired contrasts, it is not to be recommended for multiple comparisons. The Scheffé test is suited for multiple contrasts (complex comparisons).

Similar to previous examples, using this test, a comparison of Formulations A and D produces the following:

Table 11.10 Results of Fisher LSD Pair-wise Comparisons

Pairing	Confidence Interval	Results
$\overline{X}_A - \overline{X}_B$	$+9.80 < \mu_A - \mu_B < +25.40$	Significant
$\overline{X}_A - \overline{X}_C$	$-1.10 < \mu_A - \mu_C < +14.70$	
$\overline{X}_A - \overline{X}_D$	$+1.69 < \mu_A - \mu_D < +17.71$	Significant
$\overline{X}_B - \overline{X}_C$	$-2.90 < \mu_B - \mu_C < +18.70$	
$\overline{X}_B - \overline{X}_D$	$-0.11 < \mu_B - \mu_D < +15.91$	
$\overline{X}_C - \overline{X}_D$	$-5.20 < \mu_C - \mu_D < +11.00$	

$$\mu_A - \mu_D = (123.2 - 113.5) \pm 1.99\sqrt{\frac{153.51}{20} + \frac{153.51}{18}}$$

$$\mu_A - \mu_D = 9.7 \pm 8.01$$

$$+1.69 < \mu_A - \mu_D < +17.71$$

Similar to previous methods, there is a significant difference because zero is not within the confidence interval, and cannot be a possible outcome. Results for all pair-wise *post hoc* comparisons using the Fisher LSD test are presented in Table 11.10.

As seen in the above example and Table 11.10, the LSD test is the most liberal of the *post hoc* tests while controlling the experimentwise Type I error rate at a selected level (typically 5%). In contrast to the HSD test, the LSD intervals are narrower than the HSD intervals, making it easier to find a significant difference. Thus the LSD test is a less conservative *post hoc* procedure than the HSD test. The **Fisher-Hayter test** is a modification of the LSD test designed to control for the liberal α significance level seen with the LSD test. It can be used when all pair-wise comparisons are done *post hoc*, but unfortunately the power may be low for fewer pair-wise comparisons.

Scheffé Procedure

Scheffé's procedure for pair-wise and multiple comparisons offers several advantages over the previous methods: 1) this procedure allows not only pair-wise, but also complex comparisons; 2) Scheffé's procedure guarantees finding a significant comparison if there was a significant *F*-value in the original ANOVA; and 3) the Type I error rate remains constant with the error rate used in the original ANOVA for both pair-wise and complex comparisons. Regarding the second point, results that might not be logical or interpretable, a hypothetical example may be useful for illustrative purposes. Assume that pharmacists are administered a cognitive

test to assess their knowledge of some therapeutic class of medication. The findings listed below, result in a significant one-way ANOVA for the four levels of a discrete independent variable (note that the levels are mutually exclusive and exhaustive).

Level	Years of Experience	Mean Score
A	10 or less	94.8
B	11-20	85.1
C	21-30	91.3
D	More than 30	87.9

However, no significant pair-wise comparisons could be found and when each experience level was compared to all the other three levels there were no significant differences. The only statistically significant difference was between levels A and C combined and levels B and D combined, with levels B and D significantly lower. How can these results be explained logically? Do pharmacists have a mental dormancy during their second decade of practice, but awaken during the third decade? What about support for Mark Twain's adage "When I was a boy of fourteen, my father was so ignorant I could hardly stand to have the old man around. But when I got to be twenty-one, I was astonished by how much he'd learned in seven years"; do parents appear to become smarter as a child passes into adulthood? Could it be during the second decade that many of the pharmacists had teenage sons or daughters and really were not very bright, but the pharmacists become smarter as their children enter adulthood and the real world? Whatever the reason, a logical assessment of the finding is difficult, if not impossible, and makes interpretation difficult.

The first step is to establish a Scheffé value, which is expressed as follows:

$$(Scheffe\ value)^2 = S^2 = (K-1)(F_{K-1,N-K}(1-\alpha))$$

This procedure does not require any additional tables. The Scheffé value is nothing more than the critical *F*-value used in the original ANOVA multiplied by the numerator degrees of freedom ($k - 1$). The Scheffé value is used in the following to create a confidence interval:

$$\psi_i = \hat{\psi}_i \pm \sqrt{S^2 \cdot Var(\hat{\psi}_i)} \qquad \text{Eq. 11.21}$$

where ψ_i (psi) is the estimated population difference and $\hat{\psi}_i$ (psi hat) is the sample difference. This $\hat{\psi}_i$ can represent either a pair-wise or complex comparison:

$$\hat{\psi}_i = \overline{X}_1 - \overline{X}_2 \quad (pair\text{–}wise\ comparison)$$

$$\hat{\psi}_i = \overline{X}_1 - 1/2(\overline{X}_2 + \overline{X}_3) \quad (complex\ comparison)$$

The measure of the standard error term for this equation is slightly more complex than the previous two methods:

$$Var(\hat{\psi}_i) = MS_E \cdot \Sigma \frac{a_k^2}{n_k} \qquad \text{Eq. 11.22}$$

In this formula, a_k represents the prefix to each of the mean values.

$$\hat{\psi}_i = (a_1)\overline{X}_1 + (a_2)\overline{X}_2 + \ldots (a_n)\overline{X}_n$$

For example, consider the following simple pair-wise example:

$$\hat{\psi}_i = \overline{X}_1 - \overline{X}_2$$

This also can be written as:

$$\hat{\psi}_i = (+1)\overline{X}_1 + (-1)\overline{X}_2$$

where the two a_ks equal +1 and −1. A second example, involving a complex comparison is:

$$\hat{\psi}_i = \overline{X}_1 - 1/2(\overline{X}_2 + \overline{X}_3)$$

Once again the formula can be rewritten as:

$$\hat{\psi}_i = (+1)\overline{X}_1 + (-1/2)\overline{X}_2 + (-1/2)\overline{X}_3$$

where the three a_ks equal +1, −1/2, and −1/2. As a quick check, for all comparisons the sum of the absolute a_ks ($\Sigma|a_k|$) must equal 2. In each example the a_k is squared and divided by the number of observations associated with the sample mean and the sum of these ratios is multiplied by MS_E, or MS_W, taken from the ANOVA table in the original analysis of variance (Eq. 11.22). If zero does not fall within the confidence interval produced by the formula (Eq. 11.21), it is assumed that there is a significant difference in the comparison being made. Conversely, if zero falls in the interval no significant difference is found.

Using the same example for the previous *post hoc* procedures, where do the significant pair-wise differences exist between the various formulations?

$$(Scheffe\ \ value)^2 = S^2 = (3)(F_{3,73}(0.95)) = 3(2.74) = 8.22$$

The first pair-wise comparison is between Formulations A and B, where:

$$\psi_1 = Formulation\ A\ versus\ B \qquad \hat{\psi}_1 = 17.6$$

The computation is as follows:

Table 11.11 Results of Scheffé's Pair-wise Comparisons

Pairing	Confidence Interval	Results
$\overline{X}_A - \overline{X}_B$	$+6.37 < \mu_A - \mu_B < +28.83$	Significant
$\overline{X}_A - \overline{X}_C$	$-4.58 < \mu_A - \mu_C < +18.18$	
$\overline{X}_A - \overline{X}_D$	$-1.84 < \mu_A - \mu_D < +21.24$	
$\overline{X}_B - \overline{X}_C$	$-22.18 < \mu_B - \mu_C < +0.58$	
$\overline{X}_B - \overline{X}_D$	$-19.44 < \mu_B - \mu_D < +3.64$	
$\overline{X}_C - \overline{X}_D$	$-8.78 < \mu_C - \mu_D < +14.58$	

$$var(\hat{\psi}_1) = 153.51\left[\frac{(+1)^2}{20} + \frac{(-1)^2}{20}\right] = 15.35$$

$$\psi_1 = +17.6 \pm \sqrt{(8.22)(15.35)}$$

$$+6.37 < \psi_1 < +28.83$$

Because zero does not fall in the confidence interval, the decision is to reject H_0, that Formulation A and Formulation B have the same C_{max}, and conclude that a difference exists. The second pair-wise comparison is between Formulation A and C, with:

$$\psi_2 = Formulation\ A\ versus\ \ C \qquad \hat{\psi}_2 = 6.8$$

The calculation of the confidence interval is:

$$var(\hat{\psi}_2) = 153.51\left[\frac{(+1)^2}{20} + \frac{(-1)^2}{19}\right] = 15.75$$

$$\psi_2 = +6.8 \pm \sqrt{(8.22)(15.75)}$$

$$-4.58 < \psi_2 < +18.18$$

In this case, with zero inside the confidence interval, the decision is that the null hypothesis that Formulation A has the same C_{max} as Formulation C cannot be rejected.

A summary of all possible pair-wise comparisons using Scheffé's procedure appears in Table 11.11.

Table 11.12 Comparison of Results for Various *Post hoc* Procedures

Pairing	Mean Δ	Bonferroni Adjustment	Dunn's	Newman-Keuls	Scheffé
A vs. B	17.6	Significant	Significant	Significant	Significant
B vs. C	10.8	Significant		Significant	
A vs. D	9.5			Significant	
B vs. D	7.9				
A vs. C	6.8				
C vs. D	2.9				

The Scheffé test is not appropriate for planned comparisons. It should be restricted to *post hoc* comparisons where there are a large number of pair-wise comparisons and mainly for complex comparisons. Interestingly each of the three methods gives slightly different results. From this one example, the Scheffé and Dunn procedures appear to be the most conservative tests and the Newman-Keuls test more liberal (Table 11.12). All three procedures found a significant difference between the two extreme means (Formulations A and B); however, results varied for the other pair-wise comparisons.

Scheffé Procedure for Complex Comparisons

In the above example it was possible, by all four methods used, to identify significant pair-wise differences comparing the means of sample groups and extrapolating those differences to the populations which they represent. But what if all possible pair-wise comparisons were made and no significant differences were found? For example, suppose instead we actually found slightly different data among the four formulations (modified from the previous example):

Concentration in mcg/ml:	Mean	S.D.	n
Formulation A	119.7	11.2	20
Formulation B	110.9	9.9	20
Formulation C	117.7	9.5	19
Formulation D	112.6	8.9	18

In this case the calculated *F*-value (3.48) exceeds the critical *F*-value (2.74). Therefore, the null hypothesis that all formulations are equal is rejected. Unfortunately, none of the Scheffé pair-wise comparisons is significant.

As mentioned previously, the Scheffé test also can be used for complex comparisons. This process begins by comparing individual levels (one formulation), to the average of the other combined levels (average of remaining three formulations) and determining if one is significantly larger or smaller than the rest. For example, the formulation with the smallest C_{max} can be compared to the remaining three groups:

$$\hat{\psi}_7 = 110.9 - 1/3(119.7 + 117.7 + 112.6)$$

with the appropriate a_k being:

$$\hat{\psi}_7 = (+1)\ 110.9 + (-1/3)\ 119.7 + (-1/3)\ 117.7 + (-1/3)\ 112.6 = -5.77$$

The computations for the confidence interval, with the calculated MS_E of 98.86 from the original ANOVA, are:

$$var(\hat{\psi}_7) = 98.86\left[\frac{(+1)^2}{20} + \frac{(-33)^2}{20} + \frac{(-.33)^2}{19} + \frac{(-.33)^2}{18}\right] = 6.65$$

$$\psi_7 = -5.77 \pm \sqrt{(8.22)(6.65)} = -5.77 \pm 7.39$$

$$-13.16 < \psi_7 < +1.62$$

Unfortunately, in this particular example all of the single group comparisons were found to be not significant.

Compared to All Others	Confidence Interval
Formulation A	$-1.45 < \psi_8 < +13.39$
Formulation B	$-13.16 < \psi_8 < +1.62$
Formulation C	$-4.25 < \psi_8 < +10.85$
Formulation D	$-11.19 < \psi_8 < +4.19$

With such results, the next logical step is to compare the two larger results with the two smallest. In this case the complex comparison would appear as follows:

$$\frac{\mu_A + \mu_C}{2} - \frac{\mu_D + \mu_B}{2} = 0$$

and also could be written as follows:

$$+\frac{1}{2}(\mu_A) + \frac{1}{2}(\mu_C) - \frac{1}{2}(\mu_D) - \frac{1}{2}(\mu_B) = 0$$

The +1/2s and −1/2s before each population mean become the a_k in the equation for calculating the variance term:

$$Var(\hat{\psi}_i) = MS_E \cdot \Sigma \frac{a_k^2}{n_k}$$

Notice that in both this and the previous example, the $\Sigma|a_k|$ equals 2.

The calculations for the confidence interval are as follows:

$$\psi_{11} = Formulations\ A\ and\ C\ versus\ Formulations\ D\ and\ B$$

$$\hat{\psi}_{11} = 1/2(119.7 + 117.7) - 1/2(110.9 + 112.6) = 6.95$$

$$var(\hat{\psi}_{11}) = 98.86\left[\frac{(+.5)^2}{20} + \frac{(+.5)^2}{19} + \frac{(-.5)^2}{20} + \frac{(-.5)^2}{18}\right] = 5.15$$

$$\psi_{11} = 6.95 \pm \sqrt{(8.22)(5.15)} = 6.95 \pm 6.51$$

$$+0.44 < \psi_7 < +13.46$$

Here we find a significant difference if we compare the two formulations with the highest sample means to those with the smallest sample means.

If a significant pair-wise comparison is found, then more complex comparisons do not need to be computed unless the researcher wishes to analyze specific combinations. Once again it is assumed that the original analysis of variance was found to be significant, and the null hypothesis that all the means are equal was rejected. In all the tests performed in this section the Type I error (α) remained constant with the original error rate used to test the analysis of variance for *k* levels of the independent variable. While the Scheffé test can evaluate more complex comparisons it does so at the expense of statistical power. Even though the Scheffé test is lower in power it can be used when one wishes to do all or a large number of comparisons.

Unbalanced Designs

Many multiple comparison procedures assume that there are equal sample sizes in the groups being compared. Since multiple comparison tests are robust and can contend with minor violations in this assumption, tests specifically designed for unequal sample sizes (unbalanced) are rare. The Tukey-Kramer test has been discussed previously and represents a modified Tukey HSD test for unbalanced designs. It assumes that there is homogeneity comparing the various sample variances. For unbalanced designs with equal variances, it is recommended to use the Tukey-Kramer test if all pair-wise comparisons of the sample means are tested. This test is not an exact test, but it is conservative for unbalanced one-way ANOVAs and the experimentwise error rate will not exceed alpha. It is less conservative when the designs are only slightly unbalanced, but more conservative when there are large differences in samples sizes. Also, the Scheffé and Student-Neuman-Kuels tests can be adjusted for unbalanced designs. Other procedures specifically designed to handle unequal sample sizes include the **Miller-Winer test**, **Hochberg GT2 test**, and **Gabriel test**.

Lack of Homogeneity

Ideally, the one-way ANOVA would be performed only when the assumption of homogeneity of variances is met. However, because it is a robust statistic it can be employed when there is a deviation from this assumption. When the design involves unequal variances, there are several lesser used *post hoc* procedures including Games-Howell, Dunnett's C, Dunnett's T3, and Tamhane's T2 tests, which have been mentioned previously. None of these is an exact test, but the T2, T3 and C are conservative procedures and the experimentwise error rate will not exceed α. For larger samples that are approximately equal in size (balanced), the T2 is more conservative than T3. Whereas, the T3 is more conservative than C for large samples, while C is more conservative for smaller.

The **Games-Howell test** (GH test) is designed for both unequal variances and unequal sample sizes. It is a pair-wise procedure based on the *q*-distribution. The Games-Howell test may be too liberal when sample sizes are small and is therefore recommended for sample sizes greater than five. The Games-Howell is an extension of the Tukey-Kramer test, more powerful (narrower confidence intervals) than C, T2 or T3, and is recommended over these tests. It is most liberal (experimentwise error rate is likely to exceed alpha) when the sample variances are approximately equal.

Both the **Dunnett's T3 and Dunnett's C** are similar *post hoc* procedures for use when the assumption of homogeneity of variance is not met or questionable. The T3 and C should be used for pair-wise comparisons. The **Tamhane's T2** is a pair-wise procedure based on the Student t-distribution. It uses Sidák test to define the alpha level. Tamhane's T2 is a more conservative *post hoc* comparison for data with unequal variances and is appropriate when variances are unequal and/or when the sample sizes are different. Toothaker's textbook covers most of the *post hoc* procedures discussed in these last two sections (Toothaker, 1991).

Other *Post Hoc* Tests

The **Hsu's MCB** (multiple comparison with the best) test creates confidence intervals for the difference between the mean for each level of the independent variable and the best of the remaining level means (Hsu, 1981). In other words each sample mean is compared to the "best" of the other means. Best is a default or the largest mean for the remaining levels. The test calculates q-values associated with each sample. Hsu's MCB is an exact test for a one-way analysis of variance with levels that have equal sample sizes (balanced). With unbalanced group sizes the experimentwise error rate will be smaller than stated and results will be slightly more conservative confidence intervals. Results are comparable to Tukey LDS and Dunnett's tests when confidence intervals are larger. For comparing all pair-wise comparisons, the Tukey confidence intervals will be wider and the hypothesis tests less powerful for the experimentwise error rate.

The **Bonferroni-Holm** test is a step-down test that does not require any assumptions regarding the population distribution. It can be applied to any pair-wise comparison and is a conservative test (the experimentwise error rate does not exceed alpha). The **Sidák-Holm** procedure is similar to the Bonferroni-Holm method except the differences are not compared to alpha, but to the Sidák adjusted alpha. The Sidák-

Holm test is slightly less conservative than Bonferroni-Holm test.

The **Duncan test**, also referred to as the Duncan new multiple range test, is a multiple range test based on the *q*-statistic. It is a stepwise test for ordered means. The test is not recommended for unbalanced cases (Duncan, 1955).

Using Minitab® for Multiple Comparisons

Minitab offers four applications for determining the location(s) of differences if there is a significant one-way ANOVA. It is recommended to perform the ANOVA first using the analysis described in Chapter 10 without the *Comparisons…* activated:

Stat ➤ ANOVA ➤ One-way…
Stat ➤ ANOVA ➤ One-way (Unstacked)…

If the result is a *p*-value greater than 0.05, there is no reason to apply *post hoc* procedures. However, if there is a significant difference, then the options are available: 1) Tukey's HSD; 2) Fisher's LSD; 3) Dunnett's; and 4) Hsu's MCB tests. To access these options, click on the *Comparisons…* (Figure 10.9) and the choices will be listed (Figure 11.2). In the box to the right of each test the Type I error is indicates (and can be changed) as percent (5 = α = 0.05). One or all of the options can be chosen. For Dunnett's test one of the levels of the independent variable must be identified as the control. For Hsu's test either the smallest or largest mean must be

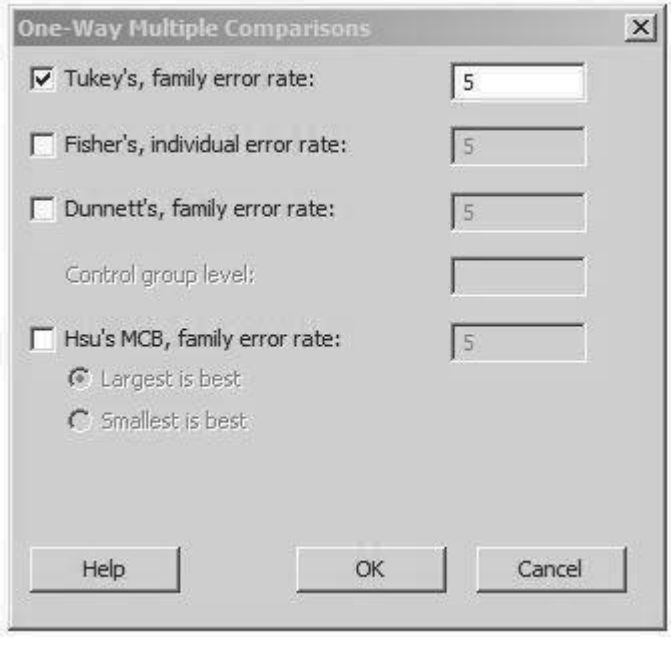


Figure 11.2 Multiple comparison options with Minitab.

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Formulation

Individual confidence level = 98.96%

Formulation = A subtracted from:

Formulation   Lower  Center  Upper  ---------+---------+---------+---------+
B           -27.91  -17.60  -7.29  (------*------)
C           -17.24   -6.80   3.64          (-----*------)
D           -20.29   -9.70   0.89       (-------*------)
                                   ---------+---------+---------+---------+
                                          -15         0        15        30
Formulation = B subtracted from:

Formulation  Lower  Center  Upper  ---------+---------+---------+---------+
C             0.36   10.80  21.24                    (------*------)
D            -2.69    7.90  18.49                 (------*------)
                                   ---------+---------+---------+---------+
                                         -15         0        15        30
Formulation = C subtracted from:

Formulation   Lower  Center  Upper  ---------+---------+---------+---------+
D           -13.62   -2.90   7.82            (------*------)
                                   ---------+---------+---------+---------+
                                          -15         0        15        30
```

Figure 11.3 Results for a Tukey HSD with Minitab.

chosen as "best". Whichever test is chosen, the results will be a presented as confidence intervals that are interpreted similar to previous intervals; if zero falls within the interval the difference is not significant. Figure 11.3 presents the output for the data for the original four formulations used as examples throughout this chapter. Using the Tukey HSD test, note there are significant differences between Formulations A and B and B and C because zero falls outside the confidence interval.

If we wanted to consider Formulation A as the control and use Dunnett's test to compare the other three formulations to the control, the *Comparisons…* choice appears in Figure 11.4 and the outcome is reported in Figure 11.5. In this example Formulations B and D are significantly different because zero difference is outside the 95% confidence interval.

References

Duncan, D.B. (1955). "Multiple range and multiple F tests," *Biometrics* 11:1-42

Dunnett, C.W. (1955.) "A multiple comparison procedure for comparing several treatments with a control," *Journal of the American Statistical Association*, 50:1096-1121.

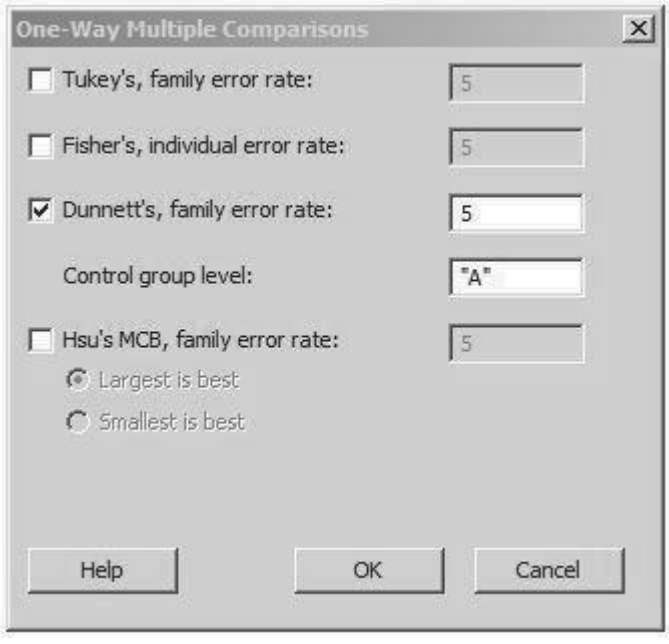


Figure 11.4 Dunnett's selection with Minitab.

```
Dunnett's comparisons with a control

Family error rate = 0.05
Individual error rate = 0.0189

Critical value = 2.40

Control = level (A) of Formulation

Intervals for treatment mean minus control mean

Level    Lower  Center  Upper  ----+---------+---------+---------+-----
B      -27.01  -17.60  -8.19  (-----------*-----------)
C      -16.33   -6.80   2.73               (-----------*----------)
D      -19.37   -9.70  -0.03          (-----------*-----------)
                              ----+---------+---------+---------+-----
                              -24.0     -16.0     -8.0      0.0
```

Figure 11.5 Results for a Dunnett's comparison with Minitab.

Glantz, S.A. (1980). "Biostatistics: How to detect correct and prevent errors in the medical literature," *Circulation* 61(1):1-7.

Hsu, J.C. (1981). "Simultaneous confidence intervals for all distances from the 'best'," *Annals of Statistics* 9:1026-1034.

Toothaker, L.E. (1991). *Multiple Comparisons for Researchers*, Sage Publications, Newbury Park, CA.Zar, J.H. (2010). *Biostatistical Analysis*, Fifth edition, Prentice-Hall, Upper Saddle River, NJ, Table B.6, pp. 733-734.

Suggested Supplemental Readings

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Eighth edition, John Wiley and Sons, New York, pp. 322-324.

Fisher, L.D. and van Belle, G. (1993). *Biostatistics: A Methodology for the Health Sciences*, John Wiley and Sons, New York, pp. 596-661.

Hochberg Y. and Tamhane, A.C. (1987). *Multiple Comparison Procedures*, John Wiley and Sons, New York.

Kirk, R.E. (1968). *Experimental Design: Procedures for the Behavioral Sciences*, Brooks/Cole, Belmont, CA, pp. 69-98.

Toothaker, L.E. (1991). *Multiple Comparisons for Researchers*, Sage Publications, Newbury Park, CA.

Zar, J.H. (2010). *Biostatistical Analysis*, Fifth edition, Prentice-Hall, Upper Saddle River, NJ, pp. 226-244.

Example Problems (Answers are provided in Appendix D)

Based on the information presented, identify where the significant differences exist using the various *post hoc* procedures for the following problems.

1. A prospective study was conducted on 105 patient randomly assigned to one of three HMG-CoA reductase inhibitors for lowering cholesterol levels. After 12 months 94 of the patients were still being followed. Table 11.13 represents the change in total cholesterol reported for the patients (different between pretreatment level and most recent cholesterol level). There was a significant ANOVA ($p < 0.05$) comparing the three agents and rejection of the null hypothesis that $\mu_A = \mu_B = \mu_C$. The ANOVA table shows that the *F*-statistic exceeded the critical *F*-value of 3.111:

Source	DF	SS	MS	F
Between	2	3900.30	1950.15	4.27
Within	91	41604.48	457.19	
Total	93	45505		

Table 11.13 Change in Total Cholesterol Levels (mg/dl) for Patients Treated with Three Different HMG-CoA Reductase Inhibitors

	Drug A		Drug B		Drug C	
	1	−3	−20	−3	−54	−33
	−42	−14	16	−35	−7	−24
	−7	2	3	9	−30	2
	−33	−23	−32	−37	−48	−36
	−2	−45	10	10	9	−14
	8	6	−33	13	12	−23
	15	−36	−24	0	−39	−18
	7	20	−29	−37	−39	1
	−16	21	−35	3	−32	−26
	20	33	−21	−45	−48	−46
	−21	−23	−22	−12	−55	−35
	8	−21	34	−4	−68	−5
	−38	−19	−9	14	7	−12
	−3	−39	13	15	−10	−3
	7	11	−9	7	13	−10
	1				12	−8
					−39	
Mean =	−7.26		−8.67		−21.39	
Standard Deviation =	21.03		20.90		22.13	
n =	31		30		33	

Use the most appropriate multiple comparison test(s), to identify significant difference(s) among these different agents, given the following scenarios.

a. Scenario 1: The researcher decided before the study to compare the newest agent (Drug C) to each of the other drugs (currently on the hospital formulary) if there was a significant ANOVA.
b. Scenario 2: The researcher decided before the study to consider Drug C and the "control" agent and compare each of the other drugs to this product.
c. Scenario 3: After identifying a significant ANOVA the researcher decided to compare the three agents "after the fact" to determine where the difference(s) exist.

2. Problem 3 in Chapter 10 compares the viscosity of a raw material delivered to three different sites.

Hypotheses: H_0: $\mu_A = \mu_B = \mu_C$
H_1: H_0 is false

Decision rule: With $\alpha = 0.05$, reject H_0 if $F > F_{2,12}(0.95) \approx 3.70$.

Data:	Batch A	Batch B	Batch C
Mean =	10.28	10.24	10.21
S.D. =	0.037	0.033	0.026
n =	5	5	5

Results:

$$F = \frac{MS_B}{MS_W} = \frac{0.0063}{0.0010} = 6.3$$

Decision: With F > 3.70, reject H_0, conclude that $\mu_A = \mu_B = \mu_C$ is not true.

Use the appropriate *post hoc* test(s) for results with equal sample sizes.

3. Consider the results presented in Figure 11.4. Five different dissolution apparatuses (testers) are evaluated to determine if there is significant difference in their results based on testing of a single product at 30 minutes. Where were the significant difference(s) among the various dissolution apparatuses in this study?

4. Problem 6 in Chapter 10, which evaluated various benzodiazepines and responses to a computerized simulated driving test, resulted in the rejection of the null hypothesis $\mu_A = \mu_B = \mu_C = \mu_{Placebo}$. Where were the significant differences?

5. Using Problem 6 in Chapter 10 once again, consider the same results, but use the placebo results as a control. How do the three benzodiazepines compare to the control group?

12

Factorial Designs: An Introduction

As presented in Chapter 10, the simple one-way analysis of variance is used to test the effect of one independent discrete variable. When using factorial designs it is possible to control for multiple independent variables and determine their effect on a single dependent continuous variable.

Through random sampling and an appropriate definition of the population, in an ideal world, the researcher should be able to control all variables not of interest to the particular research design. For example in a laboratory, the researcher should be able to control the temperature of the experiment, the quality of the ingredients used (the same batch, the same bottle), the accuracy of the measurements, and numerous other factors that might produce bias in the statistical analyses performed. However, in many research situations, several different factors must be considered at the same time as well as the relationship of these variables to each other. Therefore, the study must be designed to consider two or more independent variables at the same time and their influence on the outcome of the dependent variable.

Factorial Designs

The ANOVA model discussed in Chapter 10 is referred to as a "simple" or "one-way" analysis of variance because only a single independent variable or factor is being assessed. The term **factor** is synonymous with the terms **independent variable, treatment variable**, **predictor variable**, or **experimental variable**. Throughout this chapter the terms *factor* and *independent variable* will be used interchangeably.

Instead of repeating our experiment for each independent variable or factor, we can design a more efficient experiment that evaluates the effects of two or more factors at the same time. These types of designs are referred to as **factorial designs** because each level of one factor is combined with each level of the other factor, or independent variable. The primary advantage of a factorial design is that it allows us to evaluate the effects of more than one independent variable, separately and in combination with each other. As will be seen, these factorial designs can be used to increase the control we have over our experiment by reducing the within-group variance. The factorial designs also offer economic advantages by reducing the total number of subjects or observations, which would be needed if the two main effects were evaluated separately.

To illustrate this situation, consider the following example. Back in the 1990s a school of pharmacy was working on developing a new method of delivering its recently developed Pharm.D. curriculum to B.S. pharmacists desiring to obtain this degree, but unable to take a one- or two-year sabbatical to return to school. Therefore, the school worked with different delivery systems to provide distance learning for the didactic portion of the course work. The primary investigator developed a satisfaction index on a linear scale for the pharmacist to evaluate the convenience, flexibility, and usefulness of the course materials, as well as the user friendliness of the course work. It was assumed that the better the response (maximum score of 10), the more likely that pharmacists would begin the course work and continue to the end of the didactic portion of the program. A pilot study was conducted on a random sample of pharmacists. Two different delivery methods were considered: written monographs (M_1) and computer-based training using CD-ROMs (M_2). However, early in the development of course work there were concerns that institutional (primarily hospital) and ambulatory (mostly retail) pharmacists might possess different learning styles and might react differently with respect to their evaluation of the course materials. Therefore, the pilot study was designed to evaluate two independent variables, the delivery system used, and the pharmacist's practice setting, either institutional (S_1) or ambulatory (S_2). This can be illustrated in the simplest possible experimental design, a two-by-two (2×2) factorial design:

Methods		S_1	S_2
	M_1	A	B
	M_2	C	D

Settings

where A, B, C, and D represent the mean results for the continuous dependent variable (satisfaction index), and rows and columns represent the main factors tested. For example, A represents the responses for pharmacists practicing in institutional settings who receive the written monongraphs; whereas D represents ambulatory pharmacists exposed to computer-based training.

In this design the principal investigator (PI) was interested in evaluating the main effects of both the factors used and the interaction of these two factors. In this case the PI was dealing with three different hypotheses:

H_{01}: $\mu_{M1} = \mu_{M2}$ (Main effect of the delivery method)
H_{02}: $\mu_{S1} = \mu_{S2}$ (Main effect of the practice setting)
H_{03}: $(\mu_{M1,S1} - \mu_{M1,S2}) = (\mu_{M2,S1} - \mu_{M2,S2})$
(Interaction between method and setting)

The first hypotheses (H_{01}) evaluates the main factor for the two methods used for distance learning (M_1, M_2). Are they approximately the same or are they statistically different? The second hypothesis (H_{02}) assesses the influence of the pharmacists' practice setting (S_1, S_2) and what influence settings might have on evaluations of the course materials. These first two hypotheses are called **tests of main effects** and are

similar to separate tests using a one-way analysis of variance. The third hypothesis (H_{03}) evaluates the possibility of relationships between the row and column variables. As discussed below, two independent variables are considered to interact if differences in an outcome for specific levels of one factor are different at two or more levels of the second factor.

Whenever we evaluate the effect of two or more independent variables on a dependent variable, we must be cautious of a possible interaction between these independent variables. The **interaction effect** measures the joint effects of two or more factors on the dependent variable. If the factors are independent of each other, or have no relationship, there will be no interaction. We are interested in detecting interactions because the overall tests of main effects, without considering interactions, may cause us to make statements about our data that are incorrect or misleading. The validity of most multifactorial designs is contingent on an assumption of no interaction effects among the independent variables. One might argue that a more appropriate procedure is to test for any interaction first and if no interaction is detected (e.g., the test is not significant), then perform separate tests for the main effects. However, if interaction exists, it is meaningless to test the main effects or to try to interpret the main effects. The approach used in this book is to evaluate the main effect and interactions in concert as a more efficient and time-saving method. Granted, if the interaction is found to be significant, the results of the evaluation of main effects are without value, because the factors are not independent of each other.

To illustrate the various outcomes, consider the possible outcomes in Figure 12.1 for our experiment with the factors of delivery system and practice setting. Here results are plotted for one main factor (setting) on the x-axis and the second main factor (delivery system) on the y-axis. In Outcome I the results are the same for all four observations; therefore the investigator would fail to reject any of the three hypotheses and conclude that there was no significant effect for either of the main effects and there was no interaction between the two factors. For Outcome II, there is a significant difference between the two delivery methods used ($M_1 > M_2$) and the investigator could reject H_{01}, but would fail to reject the other two hypotheses. The opposite results are seen in Outcome III, where the investigator would find there is a significant difference between the two practice settings ($S_2 > S_1$) and reject H_{02}, but would fail to reject the other two hypotheses. Outcome IV represents a rejection of both H_{01} and H_{02} where $M_1 > M_2$ and $S_2 > S_1$, but there is no significant interaction and H_{03} cannot be rejected.

Outcomes V and VI illustrate two possible interactions. In Outcome V there are significant differences in the main effects and a significant interaction between the two main factors. We can see that the two lines cross and there is a significant interaction between methods and settings. In this example, it appears that institutional pharmacists prefer monographs and ambulatory pharmacists favor the computer-based training. Because of the interaction, it becomes meaningless to evaluate the results of the main effects, because if there was a significant difference between methods of delivery, it may be influenced by the practice setting of the pharmacists. In Outcome VI there is no difference in M_1 based on the practice setting, but there is a difference for M_2. Is this significant? Is there an interaction between the two main effects (factors)? A two-way analysis of variance can determine, with a certain degree of confidence, which hypotheses should be rejected as false.

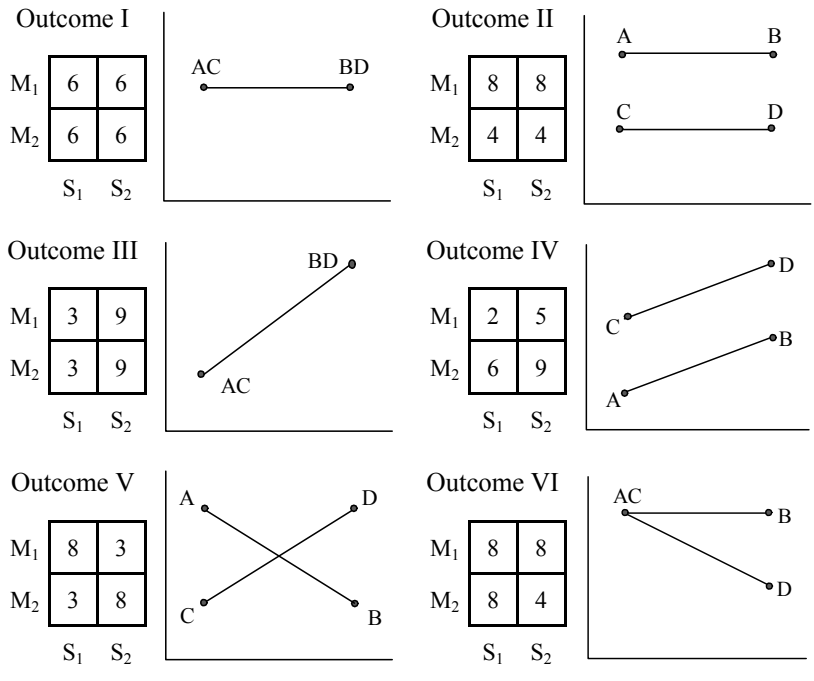


Figure 12.1 Examples of possible outcomes with a 2 × 2 factorial design.

Two-Way Analysis of Variance

In the one-way ANOVA, we were only concerned with one major treatment effect or factor:

$$H_0: \mu_1 = \mu_2 = \mu_3 = ... \mu_k$$
$$H_1: H_0 \text{ is false}$$

However, in a two-way ANOVA, we are interested in the major effects of two variables and their potential interaction.

The number of factors and levels within each factor determine the dimensions of a factorial design. For example, if Factor A consists of three levels and Factor B only two, it would be presented as a 3 × 2 (read three-by-two) factorial design. Areas within the factorial design, where dependent variable outcomes are reported, are called **cells**. In the case of a 4 × 5 factorial design, there are 20 cells (4 × 5 = 20). In factorial designs there must be more than one observation per cell. If there were only one observation per cell, there would be no variance within the cells and therefore a required sum-of-squares error term would not be available. Each of the two major factors is a discrete independent variable and the significance of each factor is measured based on a single continuous dependent variable. The design for a two-way analysis of variance is presented in Figure 12.2. The column factor is represented by

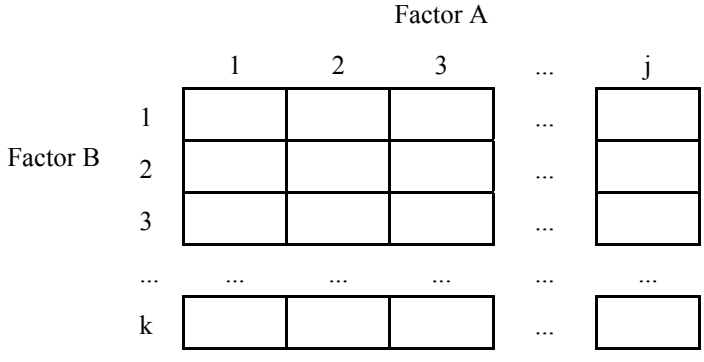


Figure 12.2 Layout for a two-way analysis of variance.

j-levels and the row factor by *k*-levels for each discrete independent variable. As seen in the previous illustration three hypotheses are being tested simultaneously: two testing for the main effects and one for the interaction:

H_{01}: $\mu_{A1} = \mu_{A2} = \mu_{A3} = ... \mu_{AJ}$ (Main effect of Factor A)
H_{02}: $\mu_{B1} = \mu_{B2} = \mu_{B3} = ... \mu_{BK}$ (Main effect of Factor B)
H_{03}: $(\mu_{A1,B1} - \mu_{A1,B2}) = (\mu_{A2,B1} - \mu_{A2,B2}) =$ etc. (Interaction of A and B)

At the same time, there are three mutually exclusive and exhaustive alternative hypotheses to complement each of the null hypotheses:

H_{11}: H_{01} is false
H_{12}: H_{02} is false
H_{13}: H_{03} is false

With the one-way analysis of variance, the degrees of freedom for the critical *F*-value were associated with the number of levels of the independent variable ($k - 1$) and the total number of observations ($N - k$). Because the two-way analysis of variance deals with two independent variables, we may have different critical *F*-values (F_c) associated with each null hypotheses tested and these are directly associated with the number of rows and columns presented in the design matrix. The symbols used are: *j* for the number of levels of the column variable; *k* for the number of levels of the row variable; N_k is the total number of observations; and *n* is the number of observations per cell in the case of equal cell sizes.

There are three separate decision rules, one for each of the two main variables and one for the interaction between the two independent variables. Each hypothesis may be tested with a different *F*-value, but each should be tested at the same α.

$$\text{Reject } H_0 \text{ if } F > F_{v1v2}(1 - \alpha)$$

Where the denominator (ν_2) degrees of freedom is always $j{\cdot}k{\cdot}(n-1)$ and numerator (ν_1) will vary depending upon which null hypothesis is being tested, then:

$$\begin{aligned} &H_{01}: && \nu_1 = j-1 \\ &H_{02}: && \nu_1 = k-1 \\ &H_{03}: && \nu_1 = (j-1)(k-1) \end{aligned}$$

For equal cell sizes (the numbers of observations in each cell of the matrix are equal), the formulas are similar to the computational formulas for the one-way ANOVA computational formulas. For intermediate value I, each observation (x_i) is squared, and then summed.

$$I = \sum_{k=1}^{K} \sum_{j=1}^{J} \sum_{i=1}^{I} x_i^2 \qquad \text{Eq. 12.1}$$

In the case of equal cell sizes the number observations in each cell for $i = 1$ to I for be equal to n then Eq. 12.1 could be written as follows:

$$I = \sum_{k=1}^{K} \sum_{j=1}^{J} \sum_{i=1}^{n} x_i^2$$

However, in a later section of this chapter, equations will be presented for unequal cell sizes and the "i" summation notation will be used for continuity.

In intermediate value II the total sum of all observations is squared and divided by the total number of observations in the data set.

$$II = \frac{\left[\sum_{k=1}^{K} \sum_{j=1}^{J} \sum_{i=1}^{I} x_i \right]^2}{N} \qquad \text{Eq. 12.2}$$

To compute the intermediate value IV, the sum of values for each cell of the matrix is squared and then all these values are summed and finally divided by the number of observations in each cell:

$$IV = \frac{\sum_{k=1}^{K} \sum_{j=1}^{J} \left[\sum_{i=1}^{I} x_i \right]^2}{n} \qquad \text{Eq. 12.3}$$

There are two intermediate III values, one for the main effect of Factor A (columns) and one for the main effect of Factor B (rows). In the former case the sum of all values for each column is totaled and squared. These squared values are then summed

and divided by the product of the number columns multiplied by the number of observations per cell:

$$III_C = \frac{\sum_{j=1}^{J}\left[\sum_{k=1}^{K}\sum_{i=1}^{I} x_i\right]^2}{k \cdot n} \qquad \text{Eq. 12.4}$$

A similar procedure is used for the intermediate III rows, where the sum of all values for each row is totaled and squared. These squared values are then summed and divided by the product of the number of rows multiplied by the number of observations per cell:

$$III_R = \frac{\sum_{k=1}^{K}\left[\sum_{j=1}^{J}\sum_{i=1}^{I} x_i\right]^2}{j \cdot n} \qquad \text{Eq. 12.5}$$

The SS_{total} and SS_{error} are calculated in a similar way to the one-way ANOVA. Note that the former error term SS_W is now referred to as SS_E or SS_{error}.

$$SS_{Error} = SS_E = I - IV \qquad \text{Eq. 12.6}$$

$$SS_{Total} = SS_T = I - II \qquad \text{Eq. 12.7}$$

In the two-way ANOVA, SS_{rows}, $SS_{columns}$ and $SS_{interactions}$ are calculated from the sum of squares formulas III_R and III_C.

$$SS_{(Rows)} = SS_R = III_R - II \qquad \text{Eq. 12.8}$$

$$SS_{Columns} = SS_C = III_C - II \qquad \text{Eq. 12.9}$$

$$SS_{Interaction} = SS_{RC} = IV - III_R - III_C + II \qquad \text{Eq. 12.10}$$

The key difference with this design is that the between-group variance is further divided into the different sources of variation (row variable, column variable, and interaction). A certain amount of variation can be attributed to the row variable and some to the column variable. The remaining left over or **residual variation** is attributable to the "interaction" between these two factors.

The sum-of-squares information is inserted into an ANOVA table (Figure 12.3) where there are three levels for the between mean variability, the main effect of the rows variable, the main effect of the columns variable, and the effect of their interactions. The first column indicates the source of the variance. The second column is the degrees of freedom associated with each source. Note that the total number of

Source	Degrees of Freedom	Sum of Squares	Mean Squares (MS)	F
Between:				
Rows	$k-1$	SS_R	$\frac{SS_R}{k-1}$	$\frac{MS_R}{MS_E}$
Columns	$j-1$	SS_C	$\frac{SS_C}{j-1}$	$\frac{MS_C}{MS_E}$
Interaction	$(k-1)(j-1)$	SS_{RC}	$\frac{SS_{RC}}{(k-1)(j-1)}$	$\frac{MS_{RC}}{MS_E}$
Within:				
Error	$k\cdot j\cdot(n-1)$	SS_E	$\frac{SS_E}{k\cdot j\cdot(n-1)}$	
Total	$N-1$	SS_T		

Figure 12.3 Computations for the ANOVA table for a two-way design.

degrees of freedom is one less than the total number of observations, again to correct for bias ($N - 1$). The third column is the sum of squares calculated by Eqs. 12.6 through 12.10. The fourth column contains the mean-square terms that are calculated by dividing the sum of squares by the corresponding degrees of freedom for each row. Finally, the *F*-values are calculated by dividing each of the mean square between values by the mean-square error. Figure 12.4 represents other symbols that can be used to represent an analysis of variance table. Computer programs, such as Excel or Minitab, present results of factorial design calculations in formats similar to those in Tables 12.1 and 12.2.

The within or error line in the ANOVA table represents the error factor or **residual variance,** which cannot be accounted for by the variability among the row means, column means, or cell means. As will be discussed later, the mean-square error serves as the error term in the fixed effects ANOVA. As seen in Figure 12.3, the denominator of each ratio in the last column is the variance estimate based on the pooled within-groups sum of squared deviations. Once again this within groups variance (MS_E) is a measure of random error or chance differences among the variables.

If one or more of the *F* values calculated in the ANOVA table exceed their parallel critical F_c value defined in the decision rule, the hypothesis or hypotheses will be rejected in favor of the alternative hypothesis. It could be possible for all three null hypotheses to be rejected, meaning that both column and row variables were significantly different and that there was a significant interaction between the two variables. If a significant outcome is not identified for the interaction portion of the ANOVA table then the outcome of the *F*-tests for the two main effects can be interpreted the same way as the *F*-ratio in the one-way ANOVA. This measure of

Source	df	SS	MS	F
Between:				
Rows	$k-1$	SS_R	MS_R	F_R
Columns	$j-1$	SS_C	MS_C	F_C
Interaction	$(k-1)(j-1)$	SS_{RC}	MS_{RC}	F_{RC}
Within:				
Error	$k \cdot j \cdot (n-1)$	SS_E	MS_E	
Total	$N-1$	SS_T		

Figure 12.4 ANOVA table for a two-way design.

interaction is based upon the variability of the cell means. Therefore, when significant interaction occurs, caution must be used in interpreting the significance of the main effects. As mentioned previously, the validity of most factorial designs assume that there is no significant interaction between the independent variables. When interpreting the outcome of the two-way ANOVA, especially if there is a significant interaction, a plotting of the means (similar to Figure 12.1) can be extremely helpful to visualize the outcomes and identify the interaction.

As an example of a two-way ANOVA we will use a previous example associated with a two-sample t-test, where the investigator compared two formulations of the same drug and was interested in determining the maximum concentration (Table 9.2). However, in this case the study involved a two-period crossover study and the researcher wanted to make certain that the order in which the subjects received the formulation did not influence the C_{max} for the formulation received during the second period. The three hypotheses under test were:

$$H_{01}: \mu_{\text{Formula A}} = \mu_{\text{Formula B}}$$
$$H_{02}: \mu_{\text{Order 1}} = \mu_{\text{Order 2}}$$
$$H_{03}: (\mu_{\text{Formula A,First}} - \mu_{\text{Formula B,First}}) = (\mu_{\text{Formula A,Second}} - \mu_{\text{Formula B,Second}})$$

and the decision rules were: 1) with $\alpha = 0.05$ and $n = 12$, reject H_{01} if $F > F_{1,44}(0.95) \approx 4.06$; 2) with $\alpha = 0.05$ and $n = 12$, reject H_{02} if $F > F_{1,44}(0.95) \approx 4.06$; and 3) with $\alpha = 0.05$ and $n = 12$, reject H_{03} if $F > F_{1,44}(0.95) \approx 4.06$. In the case of a 2×2 design all the critical values will be the same because there are identical numerator degrees of freedom ($\nu_1 = 1$).

The data observed by the investigator is presented in Table 12.1. Also included are: 1) the sum of observations for each cell (2×2 design); 2) the sum for each column (formulations A and B); 3) the sum for each row (order in which formulations were received); and 4) the total sum of all the observations. The initial computations of the intermediate values are:

Table 12.1 Sample Data for a Two-way Crossover Clinical Trial (C_{max})

	Formulation A			Formulation B			$\sum_{j=1}^{J}\sum_{i=1}^{I}$	$\sum_{k=1}^{K}\sum_{j=1}^{J}\sum_{i=1}^{I}$
Formula A Received First	125	130	135	149	151	130		
	128	121	123	132	141	129		
	131	129	120	142	130	122		
	119	133	125	136	138	140		
$\sum_{i=1}^{I}$ =	1,519			1,640			3,159	
Formula B Received First	126	140	135	130	128	127		
	126	121	133	141	145	132		
	117	126	127	133	136	138		
	120	136	122	129	150	148		
$\sum_{i=1}^{I}$ =	1,529			1,637			3,166	
$\sum_{k=1}^{K}\sum_{i=1}^{I}$ =	3,048			3,277				6,325

$$I = \sum_{k=1}^{K}\sum_{j=1}^{J}\sum_{i=1}^{I} x_i^2 = (125)^2 + (130)^2 + \ldots (148)^2 = 836{,}917$$

$$III_R = \frac{\sum_{k=1}^{K}\left[\sum_{j=1}^{J}\sum_{i=1}^{I} x_i\right]^2}{j \cdot n} = \frac{(3{,}159)^2 + (3{,}166)^2}{24} = 833{,}451.54$$

$$III_C = \frac{\sum_{j=1}^{J}\left[\sum_{k=1}^{K}\sum_{i=1}^{I} x_i\right]^2}{k \cdot n} = \frac{(3{,}048)^2 + (3{,}277)^2}{24} = 834{,}543.04$$

$$IV = \frac{\sum_{k=1}^{K}\sum_{j=1}^{J}\left[\sum_{i=1}^{I} x_i\right]^2}{n} = \frac{(1{,}519)^2 + \ldots (1{,}637)^2}{12} = 834{,}547.58$$

The sum of squares values required for the ANOVA table are:

$$SS_R = III_R - II = 833{,}451.54 - 833{,}450.52 = 1.02$$

$$SS_C = III_C - II = 834{,}543.04 - 833{,}450.52 = 1{,}092.52$$

$$SS_{RC} = IV - III_R - III_C + II$$

$$SS_{RC} = 834{,}547.58 - 833{,}451.54 - 834{,}543.04 + 833{,}450.52 = 3.52$$

$$SS_E = I - IV = 836{,}917 - 834{,}547.58 = 2{,}369.42$$

$$SS_T = I - II = 836{,}917 - 833{,}450.52 = 3{,}466.48$$

The resultant ANOVA table is as follows:

Source	df	SS	MS	F
Between				
Rows (order)	1	1.02	1.02	0.02
Column (formula)	1	1,092.52	1,092.52	20.29*
Interaction	1	3.52	3.52	0.07
Within (error):	44	2,369.42	53.85	
Total	47	3,466.48		

In this example, with α = 0.05, the decision is to reject H_{02} (* in the table) and conclude that there is a significant difference between the two formulations. Note that this is a valid decision since there is not a significant interaction between the two factors. Also, there is no significant difference based on the order in which the drugs were administered. If the data is visually represented similar to the examples in Figure 12.1, it is possible to see the significance in formulation, the closeness and insignificance of the order in which the drugs were administered, and the lack of any interaction (Figure 12.5).

A second example involving more levels of the independent variable is represented by a pharmaceutical manufacturer wishing to evaluate two automated systems for dissolution testing. Four separate batches of a particular agent were tested using each of the two automated systems and a technician-operated traditional dissolution system. Presented in Table 12.2 are the results of the experiment. Is there a significant difference between the batches or procedure used, or is there a significant interaction between the two factors?

Once again three hypotheses are being tested simultaneously (H_{01} for differences in the method used, H_{02} for differences in the batches tested, and H_{03} for possible interaction between the two factors):

Means in Each Cell

126.6	136.7
127.4	136.4

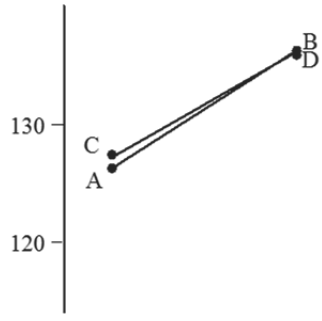


Figure 12.5 Visual representation of clinical trial data.

Table 12.2 Comparison of Methods of Dissolution Testing

		Dissolution Results at 10 Minutes (%) n = 6			
Batch	Statistic	Traditional Method	Automated System I	Automated System II	$\sum\sum x$
A	$\sum x$ =	391	378	310	1079
	$\sum x^2$ =	25,627	23,968	16,189	
	Mean =	65.17	63.00	51.67	
	SD =	5.41	5.55	5.72	
B	$\sum x$ =	369	360	358	1087
	$\sum x^2$ =	22,831	21,734	21,510	
	Mean =	61.50	60.00	59.67	
	SD =	5.24	5.18	5.46	
C	$\sum x$ =	406	362	330	1098
	$\sum x^2$ =	27,612	21,982	18,284	
	Mean =	67.67	60.33	55.00	
	SD =	5.27	5.32	5.18	
D	$\sum x$ =	401	383	345	1129
	$\sum x^2$ =	26,945	24,579	19,993	
	Mean =	66.83	63.83	57.50	
	SD =	5.38	5.11	5.09	
	$\sum\sum x$ =	1567	1483	1343	4393

H_{01}: $\mu_{\text{Traditional}} = \mu_{\text{Automated I}} = \mu_{\text{Automated II}}$
H_{02}: $\mu_{\text{Batch A}} = \mu_{\text{Batch B}} = \mu_{\text{Batch C}} = \mu_{\text{Batch D}}$
H_{03}: $(\mu_{\text{Traditional,Batch A}} - \mu_{\text{Traditional,Batch B}}) \ldots =$
$(\mu_{\text{Automated II,Batch C}} - \mu_{\text{Automated II,Batch D}})$

and the decision rules are, with $\alpha = 0.05$ and $n = 6$: 1) reject H_{01} if $F > F_{2,60}(0.95) = 3.15$; 2) reject H_{02} if $F > F_{3,60}(0.95) = 2.76$; and 3) reject H_{03} if $F > F_{6,60}(0.95) = 2.25$. Here the critical F-values are different because the matric is larger than a 2 × 2 design. The computations are:

$$I = \sum_{k=1}^{K} \sum_{j=1}^{J} \sum_{i=1}^{I} x_i^2$$

$$I = (57\ )^2 + (62\ )^2 + (65\ )^2 + \ldots (44\ )^2 = 271{,}245$$

$$II = \frac{\left[\sum_{k=1}^{K} \sum_{j=1}^{J} \sum_{i=1}^{I} x_i \right]^2}{N}$$

$$II = \frac{(4393\ )^2}{72} = 268{,}034.01\ 39$$

$$III_R = \frac{\sum_{k=1}^{K} \left[\sum_{j=1}^{J} \sum_{i=1}^{I} x_i \right]^2}{j \cdot n}$$

$$III_R = \frac{(1079\ )^2 + (1087\ ) + \ldots (1129\ )^2}{18} = 268{,}114.1667$$

$$III_C = \frac{\sum_{j=1}^{J} \left[\sum_{k=1}^{K} \sum_{i=1}^{I} x_i \right]^2}{k \cdot n}$$

$$III_C = \frac{(1567\ )^2 + (1483\ )^2 + (1343\ )^2}{24} = 269{,}101.1250$$

$$IV = \frac{\sum_{k=1}^{K}\sum_{j=1}^{J}\left[\sum_{i=1}^{I} x_i\right]^2}{n}$$

$$IV = \frac{(391)^2 + (378)^2 + (345)^2 + \ldots (310)^2}{6} = 269{,}514.1667$$

$$SS_R = III_R - II = 268{,}114.1667 - 268{,}034.0139 = 80.1528$$

$$SS_C = III_C - II = 269{,}101.1250 - 268{,}034.0139 = 1067.1111$$

$$SS_{RC} = IV - III_R - III_C + II$$

$$SS_{RC} = 269{,}514.1667 - 268{,}114.1667 - 269{,}101.1250 + 268{,}034.0139$$

$$SS_{RC} = 332.8889$$

$$SS_E = I - IV = 271{,}245 - 269{,}514.1667 = 1739.8333$$

$$SS_T = I - II = 271{,}245 - 268{,}034.0139 = 3210.9861$$

The results of the statistical analysis are presented in an ANOVA table:

Source	df	SS	MS	F
Between				
Rows (batch)	3	80.1528	26.72	0.92
Column (method)	2	1067.1111	533.56	18.40*
Interaction	6	332.8889	55.48	1.91
Within (error):	60	1739.8333	28.99	
Total	71	3219.9861		

There was no significant interaction between the two factors; therefore, our decision is to reject the null hypothesis H_{02} (* in the table) for the main effect of methods used and assume that all three methods of dissolution testing are not all equal. There was no significant difference based on the batches tested.

Computational Formula with Unequal Cell Size

Every attempt should be made to have an equal number of observations in each cell for two main reasons: 1) to have a more robust statistic and 2) most computer software program require equal cell sizes. Unfortunately, sometimes data are lost despite the best intentions of the researcher. When data are available that do not

contain equal cell sizes, the exact same procedure is used except that slightly modified formulas are substituted for Eqs. 12.3 through 12.5. For intermediate value IV, each cell is summed, that value is squared and divided by the number of observations within the cell, and these values for all individual cells are summed:

$$IV = \sum_{k=1}^{K} \sum_{j=1}^{J} \frac{\left[\sum_{i=1}^{I} x_i\right]^2}{n_i} \qquad \text{Eq. 12.11}$$

For the intermediate step involving the rows factor, all values within a row are summed, squared, and then divided by the number of observations within that row (N_R). Finally, all the calculated squared sums for each row are added together:

$$III_R = \sum_{k=1}^{K} \frac{\left[\sum_{j=1}^{J} \sum_{i=1}^{I} x_i\right]^2}{N_R} \qquad \text{Eq. 12.12}$$

The intermediate step for the column is calculated in a similar manner as the III_R except the values in each column and the total number of observations per column (N_C) are used:

$$III_C = \sum_{j=1}^{J} \frac{\left[\sum_{k=1}^{K} \sum_{i=1}^{I} x_i\right]^2}{N_C} \qquad \text{Eq. 12.13}$$

These modified intermediate steps, along with values I and II are then used to calculate the sum of squares value using the same formulas (Eqs. 12.6 through 12.10) used for data with equal cell sizes.

As an example of this application, consider the previous clinical trials example. However in this case, due to dropouts in the study, there were three fewer subjects on the second leg of the clinical trial (Table 12.3). In this case the decision rules remain the same, except the denominator degrees of freedom decreases ($N - k$). With $\alpha =$ 0.05 and $n = 12$: 1) reject H_{01} if $F > F_{1,41}(0.95) \approx 4.08$; 2) reject H_{02} if $F > F_{1,41}(0.95) \approx$ 4.08; and 3) reject H_{03} if $F > F_{1,41}(0.95) \approx 4.08$.

The initial computational steps are:

$$I = \sum_{k=1}^{K} \sum_{j=1}^{J} \sum_{i=1}^{I} x_i^2$$

$$I = (125\ )^2 + (130\ )^2 + (135\ )^2 + \ldots (150\ )^2 + (148\ )^2 = 785{,}392$$

Table 12.3 Sample Data of a Clinical Trial with Unequal Cells (C_{max})

	Formulation A			Formulation B			∑∑	∑∑∑
Formula A Received First	125	130	135	149	151	...		
	128	121	123	132	141	129		
	131	129	120	142	130	122		
	119	133	125	...	138	140		
	∑ =	1,519			1,374		2,893	
Formula B Received First	126	140	135	130	128	127		
	126	121	133	141	145	132		
	117	126	...	133	136	138		
	120	136	122	129	150	148		
	∑ =	1,402			1,637		3,039	
∑∑ =		2,921			3,011			5,932

$$II = \frac{\left[\sum_{k=1}^{K}\sum_{j=1}^{J}\sum_{i=1}^{I} x_i\right]^2}{N}$$

$$II = \frac{(5{,}932)^2}{45} = 781{,}969.42$$

$$III_R = \sum_{k=1}^{K} \frac{\left[\sum_{j=1}^{J}\sum_{i=1}^{I} x_i\right]^2}{N_R}$$

$$III_R = \frac{(2{,}893)^2}{22} + \frac{(3{,}039)^2}{23} = 781{,}973.89$$

$$III_C = \sum_{j=1}^{J} \frac{\left[\sum_{k=1}^{K}\sum_{i=1}^{I} x_i\right]^2}{N_C}$$

$$III_C = \frac{(2{,}921)^2}{23} + \frac{(3{,}011)^2}{22} = 783{,}063.41$$

$$IV = \frac{\sum_{k=1}^{K}\sum_{j=1}^{J}\left[\sum_{i=1}^{I} x_i\right]^2}{n}$$

$$IV = \frac{(1{,}519)^2}{12} + \frac{(1.374)^2}{10} + \frac{(1{,}402)^2}{11} + \frac{(1{,}637)^2}{12} = 783{,}073.03$$

Calculation of the sum of squares:

$$SS_R = 781{,}973.89 - 781{,}969.42 = 4.47$$

$$SS_C = 783{,}063.41 - 781{,}969.42 = 1{,}093.99$$

$$SS_{RC} = 783{,}073.03 - 781{,}973.89 - 783{,}063.41 + 781{,}969.42 = 5.15$$

$$SS_E = 785{,}392 - 783{,}073.03 = 2{,}318.97$$

$$SS_T = 785{,}392 - 781{,}969.42 = 3{,}422.58$$

The ANOVA table from the sum of squares data and appropriate degrees of freedom appears as follows:

Source	df	SS	MS	F
Between				
Rows (order)	1	4.47	4.47	0.08
Column (formula)	1	1,093.99	1,093.99	19.34*
Interaction	1	5.15	5.15	0.09
Within (error):	41	2,318.97	56.56	
Total	44	3,422.58		

There is no significant interaction and the results, with $\alpha = 0.05$, is to reject H_{02} (* in the table) and conclude that there is a significant difference between the two formulations, but there is no significant difference based on the order that the drugs were administered. These results are identical to the ones found when all of the cell sizes were equal.

Post Hoc Procedures

Similar to the one-way ANOVA, if there are significant findings for the tests of main effect in the two-way analysis and no significant interaction effect, *post hoc* procedures must be used to determine where those differences occur. If there are no significant interactions, then the *post hoc* procedures described in Chapter 11 can be performed on significant main effect factors. For example, consider the results of the

analysis of the three methods for dissolution testing presented above. The findings for the method provide the only significant difference ($F_{columns}$ = 18.49). Since there were no effects from the batch factor or a significant interaction, the data can be combined for each method tested.

Dissolution Results at 10 Minutes (%) n = 6

	Traditional Method	Automated System I	Automated System II
$\sum x$ =	1567	1483	1343
$\sum x^2$ =	103,015	92,263	75,976
Mean =	65.29	61.79	55.96
SD =	5.52	5.21	5.99

One-way analysis of this data would produce an F = 17.11 with an MS_E = 31.165. Using Scheffé's procedure, the following results were observed:

Pairing	Confidence Interval	Results
$\overline{X}_T - \overline{X}_I$	$-0.54 < \mu_T - \mu_I < +7.54$	
$\overline{X}_T - \overline{X}_{II}$	$+5.28 < \mu_T - \mu_{II} < +13.37$	Significant
$\overline{X}_I - \overline{X}_{II}$	$+5.28 < \mu_I - \mu_{II} < +9.87$	Significant

Thus, based on the *post hoc* analysis there was no significant difference between the traditional dissolution testing method and the first automated process. However, both of these methods were significantly different from the second automated process.

When there is a significant interaction, *post hoc* analyses are required for each independent variable separately to determine significant differences between or among the levels of each variable. In other words we must perform a one-way ANOVA on each level of the main effect variable(s), which are found to be significant with a two-way ANOVA. For illustrative purposes assume the data previously shown in Table 12.2 was instead found to have the data in Table 12.4. A resultant ANOVA table shows there is also a significant interaction between the batch and method used.

Source	df	SS	MS	F
Column (method)	2	1067.1111	533.56	18.49*
Interaction	6	391.2223	65.20	2.26*

The method for evaluating this data is to divide the total variances for both the significant main effect and the interaction. This is accomplished by creating a sum of squares comparison ($SS_{comparison}$) for each level of the significant main effect (*s in the table). For illustrative purposes we will assume that the column factor is significant:

Table 12.4 Comparison of Methods of Dissolution Testing with New Data

		Dissolution Results at 10 Minutes (%) n = 6			
Batch	Statistic	Traditional	System I	System II	∑∑x
A	∑x =	391	378	345	1114
	Mean =	65.17	63.00	57.50	
B	∑x =	369	360	358	1087
	Mean =	61.50	60.00	59.67	
C	∑x =	406	362	330	1098
	Mean =	67.67	60.33	55.00	
D	∑x =	401	383	310	1094
	Mean =	66.83	63.83	51.67	
∑∑x =		1567	1483	1343	4393

$$\sum SS_{comparison} = SS_C + SS_{RC} \qquad \text{Eq. 12.14}$$

Estimating this $SS_{comparison}$ for each row involves the following equation:

$$SS_{comparison} = \frac{\sum_{j=1}^{J}\left[\sum_{i=1}^{I} x_i\right]^2}{n} - \frac{\left[\sum_{k=1}^{K} x_i\right]^2}{j \cdot n} \qquad \text{Eq. 12.15}$$

where the first part of the equation involves summing each squared cell ∑x and the second portion is the square for the sum for the row divided by the number of levels multiplied by the number of observations per cell. For example with the first row in Table 12.2:

$$SS_{comparison}\ for\ Row\ 1 = \frac{(391)^2 + (378)^2 + (345)^2}{6} - \frac{(1114)^2}{3(6)} = 187.45$$

The results for the second row would be:

$$SS_{comparison}\ for\ Row\ 2 = \frac{(369)^2 + (360)^2 + (358)^2}{6} - \frac{(1087)^2}{3(6)} = 11.45$$

The information from these comparisons is placed in an ANOVA table along with the error measurement (SS_E) from the original two-way ANOVA table. The results from Table 12.5 are presented below (* indicating significant outcomes):

Table 12.5 Common Z_0-values

% Confidence	Z_0
90	3.29
95	3.92
99	5.15
99.5	5.62
99.9	6.60

Source	df	SS	MS	F
Row 1	2	187.45	93.73	3.24*
Row 2	2	11.45	5.73	0.20
Row 3	2	485.33	242.67	8.41*
Row 4	2	774.11	387.06	13.41*
Error	60	1730.83	28.85	

As in previous tests, the sum of squares term is divided by the appropriate degrees of freedom and each of the row mean squares is divided by the MS_E. Each *F*-value would be compared to a critical $F_{(j-1),(J\cdot K\cdot(n-1))}\, F(1-\alpha)$. In this case the critical value is $F_{2,60} = 3.15$, and all but the second row showed significant differences. Note that the sum of SS terms fulfills Eq. 12.15:

$$187.45 + 11.45 + 485.33 + 774.11 = 1067.11 + 391.22$$

This same process can be modified if a significant main effect is identified for the column independent variable.

Repeated Measures Design

A **repeated measures design** is an experimental design in which a dependent variable is measured for each subject at two or more points in time or under different conditions. The design is to control for the variability among subjects, where each subject will serve as his/her own control. When only one independent factor is used in the design it is called a single-factor repeated measure. This design is exactly the same as a randomized complete block design (Chapter 10) where the subjects make up the blocking variable and the *k* points in time or different conditions make up the factor associated with the repeated measures. The same calculations and interpretations presented in the randomized complete block design are used for the repeated measures design. As mentioned previously, Excel defines this test as "ANOVA: Two-Factor Without Replication" in the data analysis options.

Repeatability and Reproducibility

A special application of a two-way analysis of variance is to estimate the repeatability and reproducibility of intra- and interlaboratory studies on test data. The simplest study design is to send samples to each of several different laboratories. Each laboratory follows specific instructions for measuring specific traits of the samples with at least two repeated measures. The **repeatability**, the with-run or within-laboratory precision, is an assessment of the variability of replicate runs for the same sample preparation within a short period of time. It can also be the evaluation of data obtained by one person while repeatedly measuring the same item or sample. A synonymous term for repeatability is **inherent precision**. In contrast the **reproducibility** of a method, the between-laboratory or between-run precision, involves replicated runs at different times, at different locations or by different operators. Both measures are associated with random error in the system.

Sometime these measurements are referred to as **gauge or gage repeatability and reproducibility** (GR&R). Historically this comes from engineering and the use of a gauge to measure thicknesses or widths of manufactured items to insure product consistency.

To complicate the situation, International Conference on Harmonization guidelines (ICH, 1995) define repeatability as the precision under the same operating conditions over a short time interval (**intra-assay precision**) and reproducibility is the precision only between laboratory sites (collaborative studies that are usually applied to standardizing a method). ICH included a third term, **intermediate precision**, as an assessment of within laboratory variability due to different days, different analysts, or different equipment. Thus, intermediate precision and reproducibility are calculated the same way, but defined differently depending on what is being assessed. For example, if we have three different technicians in the same laboratory it is intermediate precision; if the three technicians work at three different laboratories (example below) it is reproducibility among the laboratories.

The two-way ANOVA is one accurate method for quantifying repeatability and reproducibility. In addition, the analysis of variance method can quantify the interaction between repeatability and reproducibility (the variability of the interaction between the analyst/laboratory and the samples). The two-way fixed effects model with replications is calculated in the traditional manner using the analyst/laboratory as the column variable and the samples tested as the row variable. Each sample is tested multiple times by the same observer and these results appear within a given cell (replicate measurements). All samples should be tested an equal number of times by each analyst or laboratory, thus creating equal cell sizes. Repeatability and reproducibility are calculated using values that are found in the two-way ANOVA table (Figure 12.4) where the columns are the analyst (or laboratory) variable and the rows variable represents the samples run by each analyst (or laboratory).

Calculations are based on the degree of certainty the investigator requires. These calculations are dependent on a reliability coefficient (Z_0) that is based in the normal distribution and includes the range of area under the standardized curve. Thus, if one wants to be 95% confident, $Z_0 = 3.92$ (1.96 times 2 or a range from −1.96 to +1.96). Listed in Table 12.5 are various commonly used Z_0-values. In the case of repeatability

and reproducibility, 99% confidence is usually desired and will be used in the following example (Z_0 = 5.15).

When performing a two-way ANOVA there are four sources of variability and since sample data is the best estimate of population variances (σ^2), we will use our sample measures of variance (S^2):

Sources of variability	Variance Component
Analyst (or laboratory)	S_j^2
Sample	S_k^2
Analyst/Sample interaction	S_{jk}^2
Error (repeatability)	S_{error}^2

The total variability in the data is:

$$Total\ variation = Z_0 \cdot S_{Total}$$

The S_{Total} is calculated by taking into consideration the variability attributed to the samples (k) as well as the repeatability and reproducibility:

$$S_{Total} = \sqrt{S_k^2 + S_{repeatibility}^2 + S_{reproducility}^2}$$

The specific calculation for repeatability is as follows:

$$Repeatability = Z_0 \cdot \sqrt{MS_E} \qquad \text{Eq. 12.16}$$

The MS_E is the error term taken from the two-way ANOVA table. Similar values are extracted from the ANOVA table for reproducibility:

$$Reproducibility = Z_0 \cdot \sqrt{\frac{MS_C - MS_{RC}}{k \cdot n}} \qquad \text{Eq. 12.17}$$

where k is the number of samples evaluated by each analyst (or laboratory) and n is the number of repeated trials on each sample. The interaction between variability for the samples and analysts/laboratories is:

$$Interaction = Z_0 \cdot \sqrt{\frac{MS_{RC} - MS_E}{n}} \qquad \text{Eq. 12.18}$$

Note that it is possible to have such small interaction (small MS_{RC}) that the equation may attempt to take the square root of a negative number. Since this is impossible to

Table 12.6 Sample Data for Measures of Repeatability and Reproducibility

	Laboratory A		Laboratory B		Laboratory C	
Sample	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
1	100.4	100.6	100.6	101.0	101.2	100.5
2	102.8	102.4	102.3	102.6	102.4	102.2
3	104.8	105.1	104.5	104.9	104.4	104.7
4	102.6	103.0	102.8	102.4	102.9	103.1
5	103.1	101.3	102.9	102.7	102.5	102.7
6	103.8	103.4	104.2	103.9	104.5	104.1
7	101.4	101.6	101.6	102.0	102.3	102.7
8	100.8	100.4	101.4	100.9	101.8	101.5
9	101.6	101.3	101.2	101.0	101.6	101.2
10	105.1	104.9	105.2	104.9	104.3	104.6

Two-way ANOVA for data

Source	df	SS	MS	F	p
Laboratory (column)	2	0.577	0.289	2.554	0.094
Sample (row)	9	115.741	12.860	113.806	<0.0005
Interaction	18	3.599	0.200	1.770	0.081
Error	30	3.390	0.113		
Total	59	123.307			

calculate (square roots of negatives are imaginary numbers), the interactive effect in this case equals zero. The measurement systems repeatability and reproducibility (*R & R*) is the propagation of the variability for repeatability, reproducibility and the interaction:

$$R\,\&\,R = \sqrt{Repeatability^2 + Reproducibility^2 + Interaction^2} \qquad \text{Eq. 12.19}$$

The samples will have some variability and this is evaluated by:

$$V_P = Z_0 \cdot \sqrt{\frac{MS_R - MS_{RC}}{j \cdot n}} \qquad \text{Eq. 12.20}$$

where j is the number of analysts/laboratories. The total system variation is the propagation of the sample variability and the repeatability/reproducibility variability:

$$V_T = \sqrt{R\,\&\,R^2 + V_P^2} \qquad \text{Eq. 12.21}$$

As an example, consider three different laboratories, analyzing ten different samples and each laboratory performs two assays on each sample (it is assumed that the same analyst performs the tests on the same equipment for each laboratory to remove possible additional variability). The data for this assessment appears in Table 12.6

along with the results of the two-way ANOVA calculations. With 99% confidence in our results (Z_0 = 5.15), the various calculations are as follows:

$$Repeatability = 5.15\sqrt{0.113} = 1.731$$

$$Reproducibility = 5.15\sqrt{\frac{0.289 - 0.200}{10(2)}} = 0.343$$

$$Interaction = 5.15\sqrt{\frac{0.200 - 0.113}{2}} = 1.074$$

$$R\,\&\,R = \sqrt{(1.731)^2 + (0.343)^2 + (1.074)^2} = 2.066$$

$$V_P = 5.15\sqrt{\frac{12.860 - 0.200}{(3)(2)}} = 7.481$$

$$V_T = \sqrt{(2.066)^2 + (7.481)^2} = 7.761$$

How can the above results be interpreted? As noted previously, each type of random variability contributes a certain proportion to the total variability of the system. Therefore, the calculation of the percent each contributes to the total variability is:

$$\%\ Contribution = \left(\frac{Source}{V_T}\right)^2 \times 100 \qquad \text{Eq. 12.22}$$

For this particular example the contributions of the repeatability, reproducibility, and samples are:

$$\%\ repeatibility = \left(\frac{Repeatibility}{V_T}\right)^2 \times 100 = \left(\frac{1.731}{7.761}\right)^2 \times 100 = 4.97\%$$

$$\%\ reproducibility = \left(\frac{reproducibility}{V_T}\right)^2 \times 100 = \left(\frac{0.343}{7.761}\right)^2 \times 100 = 0.20\%$$

$$\%\ R\,\&\,R = \left(\frac{R\,\&\,R}{V_T}\right)^2 \times 100 = \left(\frac{2.066}{7.761}\right)^2 \times 100 = 7.09\%$$

$$\%\ sample = \left(\frac{V_P}{V_T}\right)^2 \times 100 = \left(\frac{7.481}{7.761}\right)^2 \times 100 = 92.91\%$$

Potential guidelines for interpreting the results are as follows: 1) the percent contributed by the repeatability should be 5% or less (if greater than 5%, the measurement system may not be adequate for its intended application); 2) the percent contribution for the R&R term should be less than 30% (if greater than 30% effort should be made to reduce the variability before further analyses are performed). In this particular example, both the repeatability and R&R were acceptable.

Latin Square Designs

As discussed in Chapter 10, the one-way ANOVA (referred to as a **completely randomized block design**) allows the researcher to minimize experimental error by creating relatively homogeneous subgroups by blocking the data. Similarly, the two-way ANOVA described earlier in this chapter is also a completely randomized block design. We could think of the various samples or observations for a single subject as a "block." It is assumed that the only variability within the blocks is due to difference in the levels of the independent variable. Also in Chapter 10 we discussed the **randomized block design** where treatments are repeated once per block in only one direction; we expanded the paired t-test to more than two levels and each subject or unit served as its own control.

An extension of the randomized block design to include two extraneous factors in the same study is called a **Latin square design**. In the Latin square design one possible source of extraneous variation is assigned to the columns of the two-way matrix and the second source of extraneous variation is assigned to the rows. Like the randomized block design the outcome is measured once and only once in each row and each column. Therefore, the number of columns, rows, and treatments are all equal. This design is sometime referred to as **Youden square plan.** The purpose of the design is to control the variation in the experiment. Because of its design, the Latin square is more powerful than either the randomized block design or completely randomized block design.

This design was originally used in agricultural experiments, where fields were divided into units or plots to account for variations in soil quality and other environmental factors. Treatments are assigned at random within the rows and columns (each treatment once per row and once per column). Number of rows and columns must be the same and it is assumed that there is no interaction between the row and column variables. An example of four treatments administered in four rows (I-IV) and four columns (1-4) as illustrated below.

		Column Factor			
		1	2	3	4
Row Factor	I	A	B	C	D
	II	C	D	A	B
	III	D	C	B	A
	IV	B	A	D	C

In this example 1, 2, 3, and 4 represent different patients and I, II, III, and IV represent the order in which the four patients will receive the treatments. For example patient 1 will

receive A first, C second, D third, and B fourth. Whereas, patient 4 will receive D first, B second, A third, and C last. In the Latin square design there are t treatments and t^2 experimental units ($R \times C$). In the above example, $t = 4$ with 16 experimental units. Examples of other possible Latin square designs would include the following permutations:

3 × 3	4 × 4	5 × 5
ABC	ABCD	ABCDE
BCA	BADC	BAECD
CAB	CDBA	CDAEB
	DCAB	DEBAC
		ECDBA

In all cases, each treatment appears only once in each column and once in each row. An advantage of the Latin square design is that it allows the researcher to control for two sources of variation by blocking the variables. One disadvantage is that the number of levels of each blocking variable must equal the number of treatment levels (requiring t^2 number of experimental units). Another disadvantage is that the researcher must assume there is no interaction between the treatment and blocking variables. Also, the smallest possible Latin square is a 3 × 3 design (Mason, 1989, p. 149).

In the Latin square design the hypothesis being tested concerns equality among the levels of the discrete independent variable and in this experimental design we have a three-factor ANOVA, with one fixed and two random factors. In this design there are no replicate measures. Similar to the two-way ANOVA, the Latin square design tests three null hypotheses simultaneously. There is no significant difference among the level of the treatment and no difference for the two extraneous factors in the design:

$$H_{01}: \mu_{C1} = \mu_{C2} \ldots = \mu_{Cj} \quad \text{(extraneous factor in the column)}$$
$$H_{02}: \mu_{R1} = \mu_{R2} \ldots = \mu_{Rk} \quad \text{(extraneous factor in the row)}$$
$$H_{03}: \mu_{T1} = \mu_{T2} \ldots = \mu_{Tn} \quad \text{(treatment variable)}$$

In this design we are primarily concerned with the significance of the third hypothesis (treatment effect) and at the same time that the extraneous variable is not significantly different. The critical value for rejecting each of the null hypotheses is the same since $j = k = t$. In this case the numerator degrees of freedom would be the t and the denominator (or error) degrees of freedom is $(t - 1)(t - 2)$. Thus for Latin square the critical value would be:

$$\text{with } \alpha = 0.05, \text{ reject } H_0 \text{ if } F > F_{t,(t-1)(t-2)}(1 - \alpha)$$

For example, in the case of a 4 × 4 Latin square design shown above the denominator degrees of freedom is $(4 - 1)(3 - 1) = 6$. With a decided 95% level of confidence, the critical value would be $F_{4,6}(0.95) = 4.5337$ (from Table B7). The difficulty of fewer errors is that the degrees of freedom can be corrected by using replicates or repeated measures. Listed in Table 12.7 are critical values for various Latin square designs.

Table 12.7 Critical Values for Latin Square Designs

Design	υ_1,υ_2	$(1-\alpha) = 0.95$	$(1-\alpha) = 0.99$
3 × 3	3,2	19.1642	99.1640
4 × 4	4,6	4.5337	9.1484
5 × 5	5,12	3.1059	5.0644
6 × 6	6,20	2.5990	3.8714
7 × 7	7,30	2.3343	3.3045
8 × 8	8,42	2.1681	2.9681
9 × 9	9,56	2.0519	2.7420
10 × 10	10,72	1.9649	2.5775

This table was created using Microsoft® Excel 2010 function command F.INV(alpha,df_1,df_2).

The Latin square design involves a $t \times t$ matrix design with an equal number of rows and columns and t is the number of treatments. Each cell will contain only one observation and the formulas used are similar to those already used in the two-way ANOVA computational formulas. Intermediates *I* and *II* are similar, except for the fact that there is only one observation per cell (even though it is a $t \times t$ design we will continue to use the previous j and k notations to refer to column and row functions, respectively) .

$$I = \sum_{k=1}^{K} \sum_{j=1}^{J} x_i^2 \qquad \text{Eq. 12.23}$$

$$II = \frac{\left[\sum_{k=1}^{K} \sum_{j=1}^{J} x_i\right]^2}{k \cdot j} \qquad \text{Eq. 12.24}$$

where $k \cdot j$ is the total number of cells within the Latin square design. For intermediate value *I* each value is squared and summed. For intermediate II the values are all summed and then squared before dividing by *N*.

The two intermediates associated with the variability due to the rows and columns are calculated by squaring the sum of each column and summing these results (for III_C) or squaring the sum of each row and summing these results (for III_R). Each of these sums is divided by the number of columns or rows.

$$III_C = \frac{\sum_{j=1}^{J}\left[\sum_{k=1}^{K} x_i\right]^2}{j} \qquad \text{Eq. 12.25}$$

$$III_R = \frac{\sum_{k=1}^{K}\left[\sum_{j=1}^{J} x_i\right]^2}{k} \quad \text{Eq. 12.26}$$

Where j and k are the number of columns and rows, these are always the same in a Latin square design. The last measure takes into account the variability of the treatment effects. In this case the sum for all the results for each individual level of treatment is calculated regardless of the column or row location.

$$III_T = \frac{\left(\sum x_1\right)^2 + \left(\sum x_2\right)^2 + \left(\sum x_3\right)^2 + \ldots \left(\sum x_T\right)^2}{T} \quad \text{Eq. 12.27}$$

where T is the number of levels of the treatment or independent variable. Again, in a Latin square design, $j = k = T$. The sum of squares terms are calculated similar to those for the two-way ANOVA:

$$SS_{Total} = SS_T = I - II \quad \text{Eq. 12.28}$$

$$SS_{Rows} = SS_R = III_R - II \quad \text{Eq. 12.29}$$

$$SS_{Columns} = SS_C = III_C - II \quad \text{Eq. 12.30}$$

$$SS_{Treatment} = III_T - II \quad \text{Eq. 12.31}$$

$$SS_{Error} = I - III_R - III_C - III_T + 2II \quad \text{Eq. 12.32}$$

The sum of squares information is inserted into an ANOVA table (Figure 12.6) where the effect of the treatment is evaluated in contrast to the other variables. Results from the ANOVA table are compared to the critical value defined above.

Source	df	SS	MS	F
Between:				
Rows	$k - 1$	SS_R	MS_R	F_R
Columns	$j - 1$	SS_C	MS_C	F_C
Treatment	$t - 1$	SS_T	MS_T	F_T
Within:				
Error	$(j - 1)(k - 2)$	SS_E	MS_E	
Total	$N - 1$	SS_T		

Figure 12.6 ANOVA table for a Latin square design.

As an example, assume we are conducting a small study on the responses of pharmacy students to a series of case studies and we are considering two possible sources of extraneous variation: 1) the individual students and 2) the order in which the case studies are presented. Based on several questions the students' responses could range from 0 to 100 points. In this example the hypotheses are:

H_{01}: $\mu_{C1} = \mu_{C2} \ldots = \mu_{Cj}$ (no difference based on the student)
H_{02}: $\mu_{R1} = \mu_{R2} \ldots = \mu_{Rk}$ (no difference based on the order of case)
H_{03}: $\mu_{T1} = \mu_{T2} \ldots = \mu_{Tn}$ (no significant difference in case studies)

Here we are primarily concerned with the significance of the case studies themselves (treatment effect) and want to determine if the extraneous factors (students or order) have any effect. The five case studies (A, B, C, D, and E) are presented in the following Latin square design:

		Student				
		1	2	3	4	5
Order	1st	B	E	A	C	D
	2nd	D	A	E	B	C
	3rd	E	B	C	D	A
	4th	A	C	D	E	B
	5th	C	D	B	A	E

For a five-treatment model Latin square design, the denominator degrees of freedom are $(5 - 1)(5 - 2) = 12$, 95% confidence, the critical value would be $F_{5,12(0.95)} = 3.106$ (from Table B7). In this example the first student would receive case study B first, followed by D, E, A, and conclude with case study C. The results are as follows (with the column and row sums included):

		Student					
		1	2	3	4	5	
Order	1st	88	80	80	84	86	418
	2nd	81	81	82	91	86	421
	3rd	86	85	82	77	77	407
	4th	80	81	83	84	87	415
	5th	87	84	83	78	78	410
		422	411	410	414	414	2071

The results for the individual case studies are as follows:

Case Study:	A	B	C	D	E
Σx:	396	434	420	411	410
Mean:	79.2	86.8	84	82.2	82

The calculations for the Latin square design are as follows:

$$I = \sum_{k=1}^{K} \sum_{j=1}^{J} x_i^2 = 88^2 + 80^2 + 80^2 ... + 78^2 = 171{,}879$$

$$II = \frac{\left[\sum_{k=1}^{K} \sum_{j=1}^{J} x_i \right]^2}{k \cdot j} = \frac{2071^2}{25} = 171{,}561.64$$

$$III_C = \frac{\sum_{j=1}^{J} \left[\sum_{k=1}^{K} x_i \right]^2}{j} = \frac{418^2 + 421^2 + 407^2 + 415^2 + 410^2}{5} = 171{,}587.80$$

$$III_R = \frac{\sum_{k=1}^{K} \left[\sum_{j=1}^{J} x_i \right]^2}{k} = \frac{422^2 + 411^2 + 410^2 + 414^2 + 414^2}{5} = 171{,}579.40$$

$$III_T = \frac{\left(\sum x_1\right)^2 + ... \left(\sum x_5\right)^2}{T} = \frac{396^2 + 434^2 + 420^2 + 411^2 + 410^2}{5} = 171{,}718.60$$

$$SS_T = I - II = 171{,}879 - 171{,}561.64 = 317.36$$

$$SS_R = III_R - II = 171{,}579.40 - 171{,}561.64 = 26.16$$

$$SS_C = III_C - II = 171{,}587.80 - 171{,}561.64 = 17.76$$

$$SS_{Treatment} = III_T - II = 171{,}718.60 - 171{,}561.64 = 156.96$$

$$SS_{Error} = I - III_R - III_C - III_T + 2II$$

$$SS_{Error} = 171{,}879 - 171{,}579.40 - 171{,}587.80 - 171{,}718.60 + 2(171{,}561.64)$$

$$SS_{Error} = 116.48$$

Table 12.8 Number of Runs Associated with Various Latin Square Designs

Design	Factors	Runs (experimental units)
3 × 3 Latin Square	3	9
4 × 4 Latin Square	3	16
5 × 5 Latin Square	3	25
6 × 6 Latin Square	3	36
3 × 3 Graeco-Latin Square	4	9
4 × 4 Graeco-Latin Square	4	16
5 × 5 Graeco-Latin Square	4	25
6 × 6 Graeco-Latin Square	4	36
4 × 4 Hyper-Graeco-Latin Square	5	16
5 × 5 Hyper-Graeco-Latin Square	5	25
6 × 6 Hyper-Graeco-Latin Square	5	36

Source	df	SS	MS	F
Between:				
Rows	4	26.16	6.54	0.67
Columns	4	17.76	4.44	0.46
Treatment	4	156.96	39.24	4.44*
Within:				
Error	12	116.48	9.71	
Total	24	317.36		

Neither of the two nuisance variables (student or order) was significant, but the case studies (treatment) were significant (*). Thus, one should be concerned that the responses to the cases studies vary, but the order in which they are administered or the students reacting to the case studies did not have an impact.

Other Designs

The **Graeco-Latin square design** is an extension of the Latin square design and allows for the identification and isolation of three extraneous sources of variation. Greek letters are superimposed on the Latin letters in such a way that each Greek letter occurs once in each column, once in each row, and once with each Latin letter. Another way to think of these designs, is that we are concerned with main factor outcome (or treatment factor) and several nuisance independent or predictor factors. For the previous Latin square design there were two nuisance (predictor) factors. For Graeco-Latin square designs there are three nuisance factors and for **hyper-Graeco-Latin square designs** there are four nuisance predictor factors. The predictor or nuisance variables are the blocking variables. The advantage with the Graeco-Latin design is a reduction in the number of experimental units, as illustrated in Table 12.8. Notice that

with the Graeco-Latin square design you are testing four factors concurrently and with the hyper-Graeco-Latin square design there are five variables tested concurrently.

In many cases a complete randomized block design will require a large number of treatments that may not be economically or practically feasible. The **balanced incomplete block design** includes only a part of the treatments in a block. There will be missing pieces of information but the design must be balanced by the fact that each level of each factor has the same number of observations. Because some of the information is missing at other levels for each factor, the method involves incomplete blocks.

Other types of designs include **fractional factorial designs**, **split plot designs**, and **orthogonal array designs**. Each of these types of designs requires stringent assumptions about the absence of interaction effects. We will not discuss formulas and calculations involved in these multifactor designs because they are tedious and best run on a computer. Details can be found in advanced texts (Kirk, 1968; Mason, 1989).

Fixed, Random, and Mixed Effect Models

As seen with the previous examples of the two-way analysis of variance, the levels of the independent variable were purposefully set by the investigator as part of the research design. Such a design is termed a **fixed effects model** because the levels of the independent variables have been fixed by the researcher. The result of a fixed effect model cannot be generalized to values of the independent variables beyond those selected for the study. Any factor can be considered fixed if the researcher uses the same levels of the independent variable on replications of a study. The fixed effects design is normally used for cost considerations and because studies usually involve only a specific number of levels for the independent variables of interest.

If the levels under investigation are chosen at random from a population then the model used would be called a **random effects model** and results can be generalized to the population from which the samples were selected. Usually, the researcher will randomly select the number of levels that represent that independent variable. It is assumed that the selected levels represent all possible levels of that variable.

Lastly, there can be **mixed effects models** that contain both fixed effect variable(s) and random effects variable(s). An illustration of a mixed random effects model is a general linear regression model where the effects of multiple predictor variables, both continuous and discrete, are evaluated on a single outcome. As will be discussed in Chapter 14 the ANOVA can be used to evaluate an interaction effect, but regression models cannot evaluate interactions.

The computational formulas for all three models are identical except for the numerator used to calculate the F-value in the ANOVA table. In certain situations the mean square interaction is substituted for the traditional mean squares error (MS_E) term. The fixed effect model would be calculated as presented in Figure 12.4. Using the symbols presented in Figure 12.4 the following modifications are required. For the random effects model modifications, where both the row and column variables are random, both the row and column F-statistics are modified as follows:

$$F_R = \frac{MS_R}{MS_{RC}} \quad \text{Eq. 12.33}$$

$$F_C = \frac{MS_C}{MS_{RC}} \quad \text{Eq. 12.34}$$

With the mixed effect, either the row or column variable could be fixed. If the columns are fixed and the rows are random, then equation Eq. 12.34 would be used for the column statistic and the traditional *F*-statistic would be calculated for the row.

$$F_R = \frac{MS_R}{MS_E} \quad \text{Eq. 12.35}$$

Similarly, with the mixed effect model where the row variable is fixed and the column variable is random, Eq. 12.33 would be used for the row effect and the traditional *F*-statistic would be used for the column:

$$F_C = \frac{MS_C}{MS_E} \quad \text{Eq. 12.36}$$

Beyond a Two-Way Factorial Design

There are numerous other ANOVA designs, but they all employ the same logic as the one-way and two-way ANOVAs. One can increase the number of independent variables to create more complex **N-way ANOVA** designs. As we have seen the two-way ANOVA we can evaluate both the main and interaction effects. However, the two-way ANOVA is less sensitive than one-way ANOVA to moderate violations of the assumption of homogeneity and one needs approximately equal variances.

Figure 12.7 represents a three-dimensional schematic comparing three independent variables (a three-way ANOVA). The three independent variables (A, B, and C) are represented by a dimension of the drawing. The shaded cube represents the combined effect of the third level of Factor A (columns), the first level of Factor B (rows), and the second level of Factor C (plains).

The advantage of these multifactor designs is the increased efficiency for comparing different levels of several independent variables or factors at one time instead of conducting several separate single-factor experiments. However, as the number of independent variables increases the number of possible outcomes increases and designs get extremely complicated to interpret, especially the interactions between two or possibly more variables. For example, with a two-way ANOVA there are two tests of the main effect and one interaction to interpret. With the three-way ANOVA these are increased to three tests of the main effect, three two-way interactions, and one three-way interaction. With the three-way ANOVA we would have various numerator and denominator degrees of freedom and resultant *F*-statistics (Figure 12.8). Similar to the two-way ANOVA, the denominator degrees of freedom

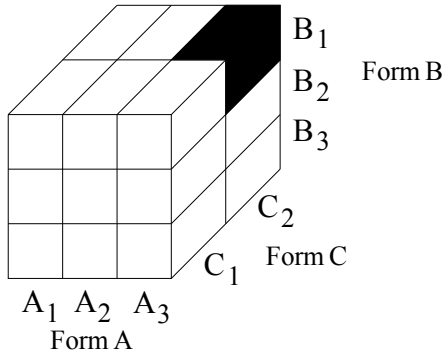


Figure 12.7 Example of a three-way ANOVA.

Source of Variation	ν_1	ν_2	F-statistic
Column factor (A)	$j-1$	$j \cdot k \cdot m \cdot (n-1)$	MS_C/MS_E
Row factor (B)	$k-1$	$j \cdot k \cdot m \cdot (n-1)$	MS_R/MS_E
Plain factor (C)	$m-1$	$j \cdot k \cdot m \cdot (n-1)$	MS_P/MS_E
Interaction (AB)	$(j-1)(k-1)$	$j \cdot k \cdot m \cdot (n-1)$	MS_{RC}/MS_E
Interaction (AC)	$(j-1)(m-1)$	$j \cdot k \cdot m \cdot (n-1)$	MS_{CP}/MS_E
Interaction (CB)	$(k-1)(m-1)$	$j \cdot k \cdot m \cdot (n-1)$	MS_{RP}/MS_E
Interaction (ABC)	$(j-1)(k-1)(m-1)$	$j \cdot k \cdot m \cdot (n-1)$	MS_{RCP}/MS_E

Figure 12.8 Degrees of freedom and F-statistics for a three-way ANOVA.

remain constant. Random and mixed effect models can also be used in the three-way ANOVA design.

This same reasoning can be expanded to a four-way ANOVA where the complexity of the outcomes includes four tests of main effects, six two-way interactions, four three-way interactions, and one four-way interaction (Table 12.9). N-way ANOVAs are also referred to as **MANOVA** or multiple analysis of variance. MANOVAs are intended for large research studies where there are a number of different variables assessed. Multiple one-way ANOVAs can result in a compounding of the error rate when the same data is used repeatedly (Chapter 10). MANOVAs can detect mean differences for a number of different groups and their potential interactions where the Type I error rate remains constant.

Another type of related statistical procedure is the **analysis of covariance** or **ANCOVA**. This procedure is useful for detecting mean differences among three or more groups when the researcher wishes to hold one variable constant. For example, evaluating the patients' knowledge of their particular disease state, controlling the level of education (e.g., less than high school education to graduate degrees). ANCOVA is useful in pharmacoeconomic studies where variables such as age,

Table 12.9 Possible Significant Outcomes in Factorial Designs

	Main Effects	Interactions	
One Factor	A		
Two Factors	A	A × B	
	B		
Three Factors	A	A × B	A × B × C
	B	A × C	
	C	B × C	
Four Factors	A	A × B	A × B × C
	B	A × C	B × C × D
	C	A × D	A × C × D
	D	B × C	
		B × D	A × B × C × D
		C × D	
Five Factors	A	A × B	A × B × C
	B	A × C	A × C × D
	C	A × D	A × D × E
	D	A × E	B × C × D
	E	B × C	B × D × E
		B × D	C × D × E
		B × E	
		C × D	A × B × C × D
		C × E	A × B × C × E
		D × E	B × C × D × E
			A × B × C × D × E

gender, educational background, or income could bias the study results. The measures are typical covariates (Chapter 13) and could also include a measure of people's aptitude, prior experience, or pretest scores.

In addition to MANOVAs and ANCOVAs there is **MANCOVA** (**multivariate analysis of covariance**), which is, combination of ANCOVA and MANOVA designs. The MANCOVA is used when the researcher wishes to detect mean differences among a number of different levels of the independent variable, while holding one or more other variables constant. The MANCOVA is useful for when a variety of levels is evaluated on a number of different measures.

Finally, there are **fractional factorial designs** or **incomplete block designs.** These are experimental designs in which only some of the treatment blocks are included in the statistical analysis. Because of this increased complexity, factorial designs involving more than three factors pose difficulties in the interpretation of the interaction effects. Therefore, most factorial designs are usually limited to three factors. Factorial designs are well beyond the scope of this book. Excellent references

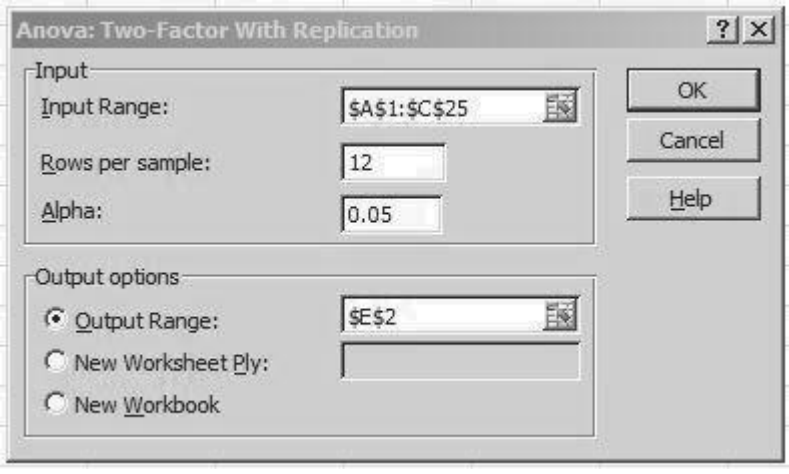


Figure 12.9 Options for a two-way ANOVA with Excel.

for any of the methods described in this last section of the chapter would be Petersen (1985) and Box et al. (1978).

Using Excel® or Minitab® for Two-Way ANOVAs

The two-way ANOVA is available as one of the Excel data analysis tools:

Data ➤ Data Analysis ➤ Anova: Two-Factor With Replication

The layout for data is to place each level of one independent variable in a column and arrange by rows to match the second independent variable. For example, using the data in Table 12.1, there are two levels for the *j*-independent variable. Data for each variable would be in columns B and C (Column A is reserved for the labels for the levels of the *k*-independent variable). For the *k*-independent variable in Table 12.1 there are also only two levels for the second independent variable and twelve observations per level; in this case the first row is for the labels for the *j*-independent variable, the next 12 rows (2-13) would represent the first *k*-level and the following 12 rows (14-25) the second *k*-level. As seen in Figure 12.9, the required information is the located in the data ("Input Range:"), but unlike previous Excel programs, it includes not only the data, but the labels included in Column A and Row 1 ($A1$1 through C25). Additional information needed for Figure 12.9: 1) how many observations per cell ("Rows per sample:"); 2) the amount of acceptable Type I error ("Alpha:"); and 3) identify where the outcomes should be reported, either starting at a cell on the current page (per this example, E2) or on a new worksheet (by default). Using the data in our previous example (Table 12.1), partial results appear in Figure 12.10. On rows prior to the ANOVA table that appears in Figure 12.10 there would be a summary of the descriptive statistics (counts, sums, means and variance), which are not shown here. The *F*-statistic and the associated *p*-value appear for each of the main

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.020833	1	1.020833	0.018973	0.891073	4.061706
Columns	1092.521	1	1092.521	20.30522	4.83E-05	4.061706
Interaction	3.520833	1	3.520833	0.065437	0.799292	4.061706
Within	2367.417	44	53.80492			
Total	3464.479	47				

Figure 12.10 Outcome report for a two-way ANOVA with Excel.

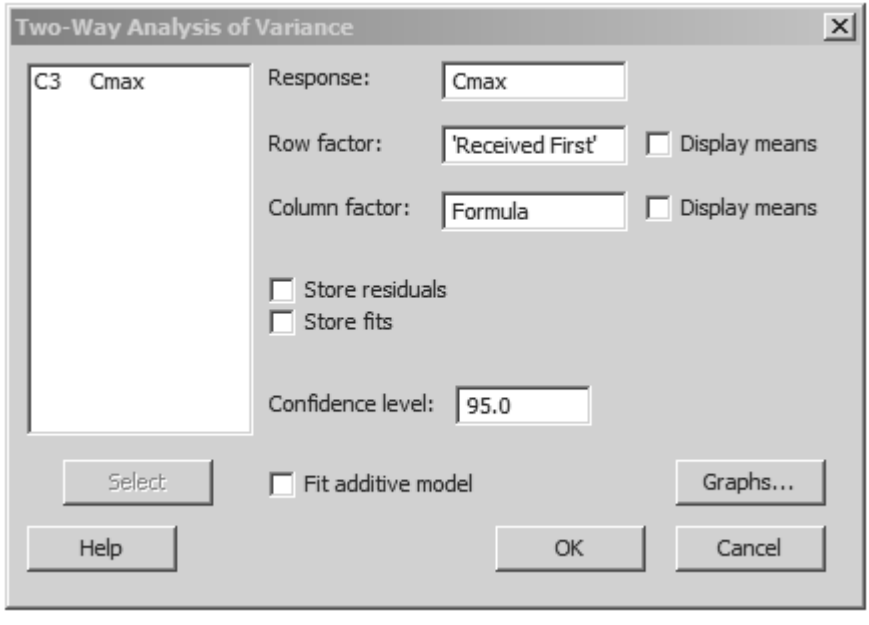


Figure 12.11 Options for the two-way ANOVA with Minitab.

effects and the interaction between the two is reported in the ANOVA table.

Minitab offers the two-way ANOVA under "Stat" on the title bar:

Stat ➤ ANOVA ➤ Two-way…

Similar to the one-way ANOVA, each column represents a variable and each row an observation. Columns are chosen for Minitab based on whether they are independent or dependent variables. Figure 12.11 illustrates the decisions required for a two-way ANOVA for the data from Table 12.1. The dependent variable is labeled

Two-way ANOVA: Cmax versus Received First, Formula

Source	DF	SS	MS	F	P
Received First	1	1.02	1.02	0.02	0.891
Formula	1	1092.52	1092.52	20.31	0.000
Interaction	1	3.52	3.52	0.07	0.799
Error	44	2367.42	53.80		
Total	47	3464.48			

S = 7.335 R-Sq = 31.67% R-Sq(adj) = 27.01%

Figure 12.12 Outcome report for the two-way ANOVA with Minitab.

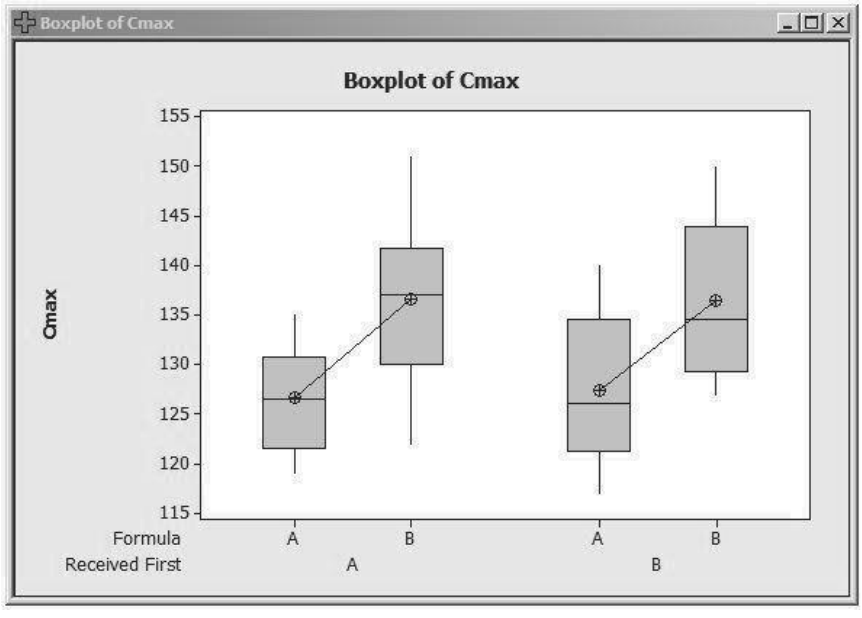


Figure 12.13 Box plot for the two-way ANOVA with Minitab.

"Response:", one independent variable is the "Row Factor:" and the second independent variable the "Column Factor:". These are selected by double clicking on the variables in the box on the left. The confidence level can be changed from the default $1 - \alpha$ of 95% if desired. The *Graphs*... option includes individual value plots or box plots for each level of the independent variable. The results of the analysis are presented in Figure 12.12 and a box-and-whisker plot is presented in Figure 12.13. The ANOVA table is presented with *F*-statistic and associated *p*-value on the right side for each independent variable and the interaction.

It is possible to assess the repeatability and reproducibility for sample data using the "gage" application with Minitab:

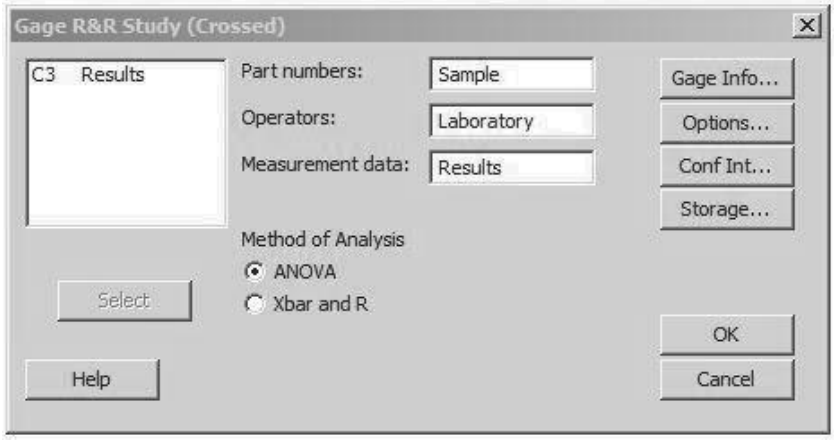


Figure 12.14 Choices for repeatability and reproducibility with Minitab.

Stat ➤ Quality Tools ➤ Gage Study ➤ Gage R&R study (crossed)

Other choices are available under "Gage Study", but the design used in this chapter involves the "crossed" option. To identify fields to perform the test, engineering labels are used (Figure 12.14) where "Part numbers:" is the sample independent variable (rows) and "Operators:" is the independent variable measuring the reproducibility (columns – locations, operators, equipment, etc.). The "Measurement data:" is the dependent variable of interest. Figure 12.14 includes the information in Table 12.6. The results of the analysis are presented in Figure 12.15 and we will focus on the top portion dealing with the percent each factor contributes to the variability in the model. Here some of the terminology is slightly different from that presented in the chapter. The percents contributed for "Repeatability" and "Total" are consistent terms, but the "Reproducibility laboratory" (here laboratory was the variable selected for "Operator") is our reproducibility measure and "Part-to-Part" is the sample variability.

References

Box, G.E., Hunter, W.G. and Hunter, J.S. (1978). *Statistics for Experimenters*, John Wiley and Sons, New York.

"ICH Topic Q2A Validation of Analytical Methods: Definitions and Terminology," *International Conference on Harmonization*, London, England, 1995.

Kirk, R.E. (1968). *Experimental Design: Procedures for the Behavioral Sciences*, Brooks/Cole Publishing, Belmont, CA.

Mason, R.L., Gunst, R.F., and Hess, J.L (1989). *Statistical Design and Analysis of Experiments*, John Wiley and Sons, New York.

Gage R&R

Source	VarComp	%Contribution (of VarComp)
Total Gage R&R	0.16092	7.09
Repeatability	0.11300	4.98
Reproducibility	0.04792	2.11
Laboratory	0.00444	0.20
Laboratory*Sample	0.04348	1.91
Part-To-Part	2.11002	92.91
Total Variation	2.27094	100.00

Source	StdDev (SD)	Study Var (6 * SD)	%Study Var (%SV)
Total Gage R&R	0.40114	2.40687	26.62
Repeatability	0.33615	2.01693	22.31
Reproducibility	0.21890	1.31339	14.53
Laboratory	0.06660	0.39958	4.42
Laboratory*Sample	0.20852	1.25113	13.84
Part-To-Part	1.45259	8.71554	96.39
Total Variation	1.50696	9.04177	100.00

Figure 12.15 Outcome report for GR&R with Minitab.

Petersen, R.G. (1985). *Design and Analysis of Experiments*, Marcel Dekker, New York.

Suggested Supplemental Readings

Havilcek, L.L. and Crain, R.D. (1988). *Practical Statistics for the Physical Sciences*, American Chemical Society, Washington, pp. 255-333.

Kachigan, S.K. (1991). *Multivariate Statistical Analysis*, Radius Press, New York, pp. 203-215.

Kirk, R.E. (1968). *Experimental Design: Procedures for the Behavioral Sciences*, Brooks/Cole Publishing, Belmont, CA, pp. 164-169, 403-420.

Zar, J.H. (2010). *Biostatistical Analysis*, Fifth edition, Prentice-Hall, Upper Saddle River, NJ, pp. 249-284.

Example Problems (Answers are provided in Appendix D)

1. A preformulation department is experimenting with different fillers and various speeds on a tableting machine (Table 12.10). Are there any significant differences in hardness based on the following samples?

Table 12.10 Results of an Experiment Involving Tablet Hardness

	Hardness (kP)							
	Speed of Tableting Machine (1000 units/hour)							
Filler	80		100		120		180	
Lactose	7	8	6	7	5	7	6	7
	5	7	8	8	8	9	8	9
	8	9	6	7	7	7	7	7
	7	7	8	10	9	10	8	9
Microcrystalline Cellulose	7	7	8	9	5	7	7	6
	7	9	6	7	8	8	6	6
	5	7	8	7	5	7	8	7
	8	9	6	7	8	8	9	9
Dicalcium Phosphate	7	5	4	6	6	7	4	6
	5	7	6	7	4	5	9	7
	7	7	5	6	7	7	5	6
	5	8	7	8	5	6	7	6

2. An investigator compares three different indexes for measuring the quality of life of patients with a specific disease state. She randomly selects four hospitals and identifies twelve individuals with approximately the same state of disease. These patients are randomly assigned to each of the indexes and evaluated (note one patient's information was lost due to incomplete information). The results are presented in Table 12.11. Are there any differences based on indexes or hospital used?

Table 12.11 Results of a Study on Patients' Quality of Life

	Quality of Life Index (Scores 0-100)					
	Index 1		Index 2		Index 3	
Hospital A	67	73	85	91	94	95
	61	69	81	87	99	92
Hospital B	81	83	83	...	86	85
	85	80	81	84	89	80
Hospital C	82	77	79	74	81	85
	80	86	80	84	82	77

Table 12.12 Comparison of Sample Results in Various Laboratories

	Laboratory						
	1	2	3	4	5	6	7
Sample 1	80.22	80.49	80.23	80.93	80.20	80.80	80.00
	80.80	80.19	80.58	79.14	80.14	80.35	80.87
	80.38	80.35	80.44	80.46	80.79	80.65	80.99
	80.99	80.12	79.21	80.38	80.45	80.55	80.35
Sample 2	75.94	76.24	76.72	77.99	76.84	76.52	75.95
	75.85	75.22	76.34	76.45	76.10	76.69	76.15
	75.74	76.49	76.08	75.85	76.82	76.8	76.45
	76.45	76.36	76.71	76.21	76.03	75.77	75.87
Sample 3	74.83	75.00	75.77	76.32	76.17	75.30	75.28
	74.98	75.81	75.09	75.96	75.88	75.38	75.79
	75.40	74.21	75.54	75.17	77.36	75.14	75.45
	75.06	74.39	75.33	75.08	75.06	74.39	75.65

3. In a multicenter study, individuals at seven laboratories trained to perform specific analyses using identical equipment from an instrument manufacturer. Following training, three samples were sent to each laboratory and four assays were performed on each sample (Table 12.12). Were good repeatability and reproducibility found in the results from the seven laboratories?

13

Correlation

Both correlation and regression analysis are concerned with continuous variables. Correlation does not require an independent (or predictor) variable, which as we will see in the next chapter, is a requirement for the regression model. With correlation, two or more variables may be compared to determine if there is a relationship and to measure the strength of that relationship. Correlation describes the degree to which two or more variables show interrelationships within a given population. The correlation may be either positive or negative. Correlation results do not explain why the relation occurs, only that such a relationship exists. Unlike linear regression (Chapter 14), covariance and correlation do not define a line, but indicate how close the data is to falling on a straight line. If all the data points are aligned in a straight diagonal, the correlation coefficient would equal a +1.0 or –1.0.

Graphic Representation of Two Continuous Variables

Graphs offer an excellent way of showing relationships between continuous variables on interval or ratio scales. The easiest way to visualize this relationship graphically is by using a **bivariate scatter plot**. Correlation usually involves only dependent or response variables. If one or more variables are under the researcher's control (for example, varying concentrations of a solution or specific speeds for a particular instrument) then the linear regression model would be more appropriate. Traditionally, with either correlation or regression, if an independent variable exists it is plotted on the horizontal *x*-axis of the graph or the **abscissa**. The second or dependent variable is plotted on the vertical *y*-axis or the **ordinate** (Figure 13.1). In the correlation model, both variables are evaluated with equal import, vary at random (both referred to as dependent variables), are assumed to be from a normally distributed population, and may be assigned to either axis.

The first role of correlation is to determine the strength of the relationship between the two variables represented on the *x*-axis and the *y*-axis. The measure of this magnitude is called the correlation coefficient (discussed in the next section). The data required to compute this coefficient are two continuous measurements (x,y) obtained on the same entity (a person, object, or data point) referred to as the **unit of association**. As will be seen, the **correlation coefficient (*r*)** is a well-defined mathematical index that measures the strength of relationships. This index measures

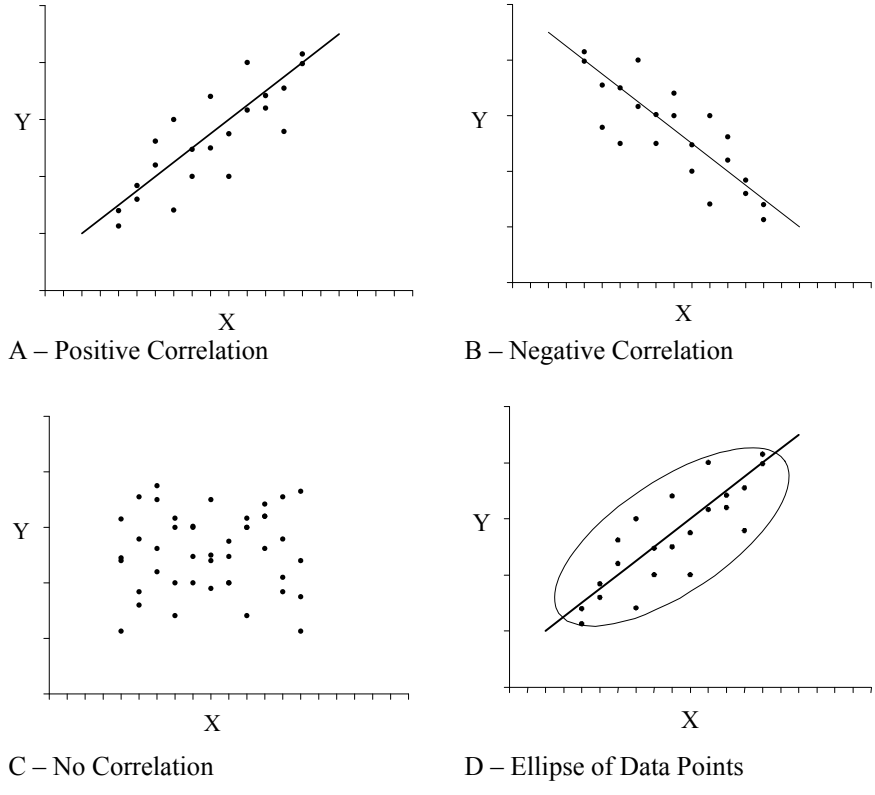


Figure 13.1 Examples of graphic representations of correlation data.

both the magnitude and the direction of the relationships.

+1.0	perfect positive correlation
0.0	no correlation
−1.0	perfect negative correlation

If there is a perfect relationship (a correlation coefficient of +1.00 or −1.00), a straight line can be drawn through all of the data points. The greater the change in *Y* for a constant change in *X*, the steeper the slope of the line. In a less than perfect relationship between two variables, the closer the data points are located on a straight line, the stronger the relationship and greater the correlation coefficient. In contrast, a zero correlation would indicate absolutely no linear relationship between the two variables.

Graph A in Figure 13.1 represents a **positive correlation** where data points with larger *x*-values tend to have corresponding large *y*-values. As seen later, an example of a positive correlation concerns the heights and weights of individuals. As the heights of people increase their weights also tend to increase. Graph B is a **negative**

correlation, where *Y* appears to decrease as values for *X* increase (approaching a perfect negative correlation of −1.00). An example of a negative or **inverse correlation** might be speed versus accuracy. The faster an individual completes a given task, the lower the accuracy; the slower the person's speed, the greater the accuracy of the task. Graph C in Figure 13.1 shows a scattering of points with no correlation or discernible pattern.

More visual information can be presented by drawing a circle or an ellipse to surround the points in the scatter plot (D in Figure 13.1). If the points fall within a circle there is no correlation. If the points fall within an ellipse, the flatter the ellipse the stronger the correlation until the ellipse produces a straight line or a perfect correlation. The orientation of the ellipse indicates the direction of the correlation. An orientation from the lower left to the upper right is positive and from the upper left to the lower right is a negative correlation. Dashed lines can be drawn on the *x*- and *y*-axes to represent the centers of each distribution. These lines divide the scatter plot into **quadrants**. In an absolute 0.00 correlation, each quadrant would have an equal number of data points. As the correlation increases (in the positive or negative direction) the data points will increasingly be found in only two diagonal quadrants. An additional assumption involved with the correlation coefficient is that the two continuous variables possess a **joint normal distribution**. In other words, for any given value on the *x*-axis variable, the *y*-variable is sampled from a population that is normally distributed around some central point. If the populations, from which the samples are selected are not normal, inferential procedures are invalid (Daniel, 2005). In such cases the strength of the relationship can be calculated using an alternative nonparameteric procedure such as Spearman rank correlation (Chapter 21) or a transformation procedure can be used to create an approximate normal distribution (Chapter 6).

Covariance

The simplest approach to discussing correlation is to focus first on only two continuous variables. The **correlational relationship** can be thought of as an association that exists between the values representing two random variables. In this relationship we, as the investigators, have no control over the observed values for either variable.

Covariance is a measure of the strength of association between two continuous variables. It measures the linear relationship between two random variables. The term "linear dependence" is sometime used to refer to covariance which can serve as a measure of dependence between two variables. It provides the goodness of fit for the best possible linear function between two variables. Covariance is calculated as follows for population data:

$$cov(X,Y) = \frac{\sum (x_i - \mu_x)(y_i - \mu_y)}{N} \qquad \text{Eq. 13.1}$$

For sample data it will be slightly larger by dividing by n − 1. When there is a strong comparison, large positive deviations for *x*-values will match with large positive

deviations for *y*-values. At the same time large negatives will match with large negatives. A positive covariance indicates that values above the mean for one variable are associated with above the mean values for a second variable and below the mean values are similarly associated. Conversely, a negative covariance indicates that above the mean values of one variable are associated with below the mean values of the second variable. If two variables are completely unrelated to each other the covariance is zero. Values can range from negative infinity to positive infinity.

It is difficult to compare the covariance between the *x*- and *y*-variables if they differ in magnitude (e.g., comparing patient weights in kilograms to heights in centimeters); therefore some type of **standardization** is required and this will be discussed below. This is accomplished by creating standardized values (subtracting the mean from each value and dividing by the standard deviation). This will result in a mean of 0 and standard deviation of 1. Covariances are useful when applied in the analysis of covariance (ANCOVA) for comparing two or more linear regression lines. The ANCOVA is used to compare two or more linear regression lines.

There are several different methods for calculating measures of correlation; the most widely used is the Pearson product-moment correlation coefficient (*r*).

Pearson Product-Moment Correlation Coefficient

The correlation coefficient assumes that the continuous variables are randomly selected from normally distributed populations. This coefficient is the average of the products for each *x*- and *y*-variable result measured as units in standardized normal distribution. Therefore the correlation coefficient is the sum of the products divided by $n - 1$, or

$$r_{xy} = \frac{\sum z_x z_y}{n - 1} \qquad \text{Eq. 13.2}$$

where z_x and z_y are standard scores for the variables at each data point and n is the sample size or the number of data points (each point representing an *x*- and *y*-value). Ideally, we would know the population mean (μ) and standard deviation (σ) for each variable. This can be a very laborious process and involves computing the mean of each distribution, and then determining the deviation from the mean for each value in terms of a standard score.

$$z_x = \frac{x_i - \mu_x}{\sigma_x} \quad \textit{and} \quad z_y = \frac{y_i - \mu_y}{\sigma_y} \qquad \text{Eq. 13.3}$$

Unfortunately, we usually do not know these parameters for the population; therefore, we must approximate the means and standard deviations using sample information.

A slightly more convenient formula for calculating the association of two variables is the **Pearson r** or the **Pearson product-moment correlation**. This coefficient is the product of the **moments** ($x_i - \mu$) for the two-variable observation.

Table 13.1 Data Layout for Computation of the Pearson Product-Moment Correlation Coefficient – Definitional Formula

x	y	$x - \overline{X}_x$	$Y - \overline{X}_y$	$(x - \overline{X}_x)(y - \overline{X}_y)$	$(x - \overline{X}_x)^2$	$(y - \overline{X}_y)^2$
x_1	y_1	...	...	...	...	...
x_2	y_2	...	...	...	...	...
x_3	y_3	...	...	...	...	...
...	...	...	...	...	...	...
x_n	y_n	...	...	...	...	...
				$\sum(x - \overline{X}_x)(y - \overline{X}_y)$	$\sum(x - \overline{X}_x)^2$	$\sum(y - \overline{X}_y)^2$

The moment deviation $(x_i - \overline{X})$ is the difference between the individual observations and the sample mean for that variable. The covariance is standardized by placing the value in the numerator and dividing it by the deviations associated with each variable. The formula for this correlation coefficient is as follows:

$$r = \frac{\sum(x - \overline{X}_x)(y - \overline{X}_y)}{\sqrt{\sum(x - \overline{X}_x)^2 (y - \overline{X}_y)^2}} \qquad \text{Eq. 13.4}$$

These calculations involve a determination of how values deviate from their respective sample means: how each *x*-value deviates from the mean for the *x*-variable ($\overline{X}_x$) and how each *y*-value varies from the mean for the *y*-variable ($\overline{X}_y$). The convenience comes from not having to compute the individual *z*-values for each data point. Normally a table is set up for the terms required in the equation (Table 13.1).

Using this method, the researcher must first calculate the sample mean for both the *x*- and *y*-variable. As seen in Table 13.1, values for the observed data are represented in the first two columns, where *x* is the value for each measurement associated with the *x*-axis and *y* is the corresponding measure on the *y*-axis for that same data point. The third and fourth columns reflect the deviations of the *x*- and *y*-scores about their respective sample means. The fifth column is the product of these deviations, the sum of which becomes the numerator in the Pearson product-moment equation. The last two columns are the deviations squared for both the *x*- and *y*-variables and are used in the denominator.

As an example, consider the data collected on six volunteer subjects during a Phase I clinical trial (Table 13.2). For whatever reason, the investigator is interested in determining if there is a correlation between the subjects' weights and heights. First, both the volunteers' mean weights and mean heights are calculated:

$$\overline{X}_x = \frac{\sum x}{n} = \frac{511.1}{6} = 85.18$$

Table 13.2 Clinical Trial Data for Six Volunteers

Subject	Weight (kg)	Height (m)
1	96.0	1.88
2	77.7	1.80
3	100.9	1.85
4	79.0	1.77
5	73.0	1.73
6	84.5	1.83
$\sum =$	511.1	10.86

Table 13.3 Sample Data for Pearson's *r* Calculation – Definitional Formula

x	y	$X-\overline{X}_x$	$Y-\overline{X}_y$	$(x-\overline{X}_x)(y-\overline{X}_y)$	$(x-\overline{X}_x)^2$	$(y-\overline{X}_y)^2$
96.0	1.88	10.52	0.07	0.7574	117.07	0.0049
77.7	1.80	−7.48	−0.01	0.0748	55.96	0.0001
100.9	1.85	15.72	0.04	0.6288	247.12	0.0016
79.0	1.77	−6.18	−0.04	0.2472	38.19	0.0016
73.0	1.73	−12.18	−0.08	0.9744	148.35	0.0064
84.5	1.83	−0.68	0.02	−0.0136	0.46	0.0004
			$\sum=$	2.6690	607.15	0.0150

$$\overline{X}_y = \frac{\sum y}{n} = \frac{10.86}{6} = 1.81$$

Table 13.3 shows the required information for: 1) the deviations from the respective sample means; 2) the squares of those deviations; and 3) the products of the deviations. Finally, the last three columns are summed and entered into the equation:

$$r = \frac{\sum(x-\overline{X}_x)(y-\overline{X}_y)}{\sqrt{\sum(x-\overline{X}_x)^2(y-\overline{X}_y)^2}}$$

$$r = \frac{2.6690}{\sqrt{(607.15)(0.015)}} = \frac{2.6690}{3.0178} = +0.884$$

The resulting *r*-value is the **product-moment correlation coefficient**, or simply the correlation coefficient. It shows a positive relationship and can be noted as a strong

Table 13.4 Data Layout for Computation of the Pearson Product-Moment Correlation Coefficient – Computational Formula

x	y	x^2	y^2	xy
x_1	y_1	x_1^2	y_1^2	x_1y_1
x_2	y_2	x_2^2	y_2^2	x_2y_2
x_3	y_3	x_3^2	y_3^2	x_3y_3
...	...	...	...	...
x_n	y_n	X_n^2	y_n^2	x_ny_n
$\sum x$	$\sum y$	$\sum x^2$	$\sum y^2$	$\sum xy$

relationship considering a perfect correlation is +1.00.

A second formula is available that further simplifies the mathematical process and is easier to compute, especially for hand-held calculators or computers. This computational formula is:

$$r = \frac{n\sum xy - \sum x \sum y}{\sqrt{n\sum x^2 - (\sum x)^2}\sqrt{n\sum y^2 - (\sum y)^2}} \qquad \text{Eq. 13.5}$$

Once again a table is developed based on the sample data (Table 13.4). In this case there are only five columns and the calculations of the sample means ($\overline{X}_x$, $\overline{X}_y$) are not required. Similar to Table 13.3, the first two columns in Table 13.4 represent the observed data, paired for both the *x* and *y* measurement scales. The third and fourth columns represent the individual *x*- and *y*-values squared and the last column is the product of *x* and *y* for each data point. Using this method to compute the correlation coefficient for the previous example of height and weight would produce the results seen in Table 13.5. The calculation of the correlation coefficient would be:

$$r = \frac{n\sum xy - \sum x \sum y}{\sqrt{n\sum x^2 - (\sum x)^2}\sqrt{n\sum y^2 - (\sum y)^2}}$$

$$r = \frac{6(927.76) - (511.1)(10.86)}{\sqrt{6(44144.35) - (511.1)^2}\sqrt{6(19.6716) - (10.86)^2}}$$

$$r = \frac{5566.56 - 5550.546}{(60.356)(0.3)} = \frac{16.014}{18.107} = +0.884$$

The results from using either formula (Eq. 13.4 or 13.5) produce the identical answers since algebraically these formulas are equivalent.

Table 13.5 Sample Data for Pearson's *r* Calculation – Computational Formula

x	y	x^2	y^2	xy
96.0	1.88	9216.00	3.5344	180.480
77.7	1.80	6037.29	3.2400	139.860
100.9	1.85	10180.81	3.4225	186.665
79.0	1.77	6241.00	3.1329	139.830
73.0	1.73	5329.00	2.9929	126.290
84.5	1.83	7140.25	3.3489	154.635
511.1	10.86	44144.35	19.6716	927.760

Correlations can be measured on variables that have completely different scales with completely different units of measure (e.g., a correlation between weight in kilograms and height in meters). Thus, the value of the correlation coefficient is completely independent of the values for the means and standard deviations of the two variables being compared. Thus, even though the correlation coefficient is a parametric procedure, we do not need be concerned about the homogeneity of variance because each axis may involve a different measurement scale. However, it is critical that the underlying population distributions are assumed to be normally distributed.

Correlation Line

The correlation coefficient is an index that can be used to describe the linear relationship between two continuous variables and deals with paired relationships (each data point represents a value on the *x*-axis as well as a value on the *y*-axis). As will be seen in the next chapter, the best line to be fitted between the points on the bivariate scatter plot is very important for the linear regression model where prediction is required for *y* at any given value on the *x*-axis. However, it is also possible, and sometimes desirable to approximate a line that best fits between the data point in our correlation model. Note that the correlation coefficient does not require a line, nor do the calculations for this coefficient actually define a line. This is in contrast to defining the line of best fit that is required for regression models. As will be discussed in greater detail in Chapter 14, a straight line between our data points can be defined as follows:

$$y = a + bx \qquad \text{Eq. 13.6}$$

where *y* is a value on the vertical axis, *x* is a corresponding value on the horizontal axis, *a* indicates the point where the line crosses the vertical axis, and *b* represents the amount by which the line rises for each unit increase in *x* (the slope of the line). We can define the line that fits best between our data points using the following formulas and data from Table 13.3 for our computational method of determining the correlation coefficient.

$$b = \frac{n\sum xy - (\sum x)(\sum y)}{n\sum x^2 - (\sum x)^2} \qquad \text{Eq. 13.7}$$

$$a = \frac{\sum y - b\sum x}{n} \qquad \text{Eq. 13.8}$$

Such lines are illustrated in Figure 13.1. The correlation coefficient provides an indication of how close the data points are to this line. As mentioned previously, if we produce a correlation coefficient equal to +1.00 or –1.00, then all the data points will fall directly on the straight line. Any value other than a perfect correlation, positive or negative, indicates some deviation from the line. The closer the correlation coefficient is to zero, the greater the deviation from this line.

In our previous example of weight and height for our six subjects, the correlation line that fits based between these points is calculated as follows:

$$b = \frac{(6)(927.76) - (511.1)(10.86)}{(6)(44144.35) - (511.1)^2} = \frac{16.014}{3642.89} = +0.0044$$

$$a = \frac{(10.86) - (0.0044)(511.1)}{6} = 1.43$$

The data and resultant line with the slope of +0.044 and *y*-intercept of 1.43 are presented in Figure 13.2. As can be seen data are relatively close to the straight line, indicative of the high correlation value of $r = +0.884$.

Statistical Significance of a Correlation Coefficient

A positive or negative correlation between two variables shows that a relationship exists. Whether one considers it as a strong or weak correlation, important or unimportant, is a matter of interpretation. For example in the behavioral sciences a correlation of 0.80 would be considered a high correlation. However, individuals in the pharmaceutical industry doing a process validation may require a correlation >0.999.

Verbal descriptions of correlations are inconsistent. The simplest might be: less than 0.25 is a "doubtful" correlation; 0.26 to 0.50 represents a "fair" correlation; 0.51 to 0.75 is a "good" correlation, and greater than 0.75 can be considered a "superior" correlation (Kelly et al., 1992). Another rough guide (Guilford, 1956) is as follows:

<0.20	Slight; almost negligible relationship
0.20 - 0.40	Low correlation; definite but small relationship
0.40 - 0.70	Moderate correlation; substantial relationship
0.70 - 0.90	High correlation; marked relationship
>0.90	Very high correlation; very dependable relationship

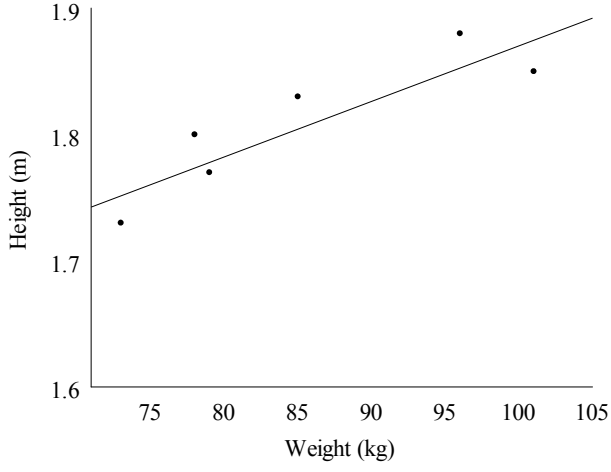


Figure 13.2 Correlation line representing data in Table 13.4.

Similar levels, but slightly different terminology can be seen with yet another guide (Roundtree, 1981):

<0.20	Very weak, negligible
0.20 - 0.40	Weak, low
0.40 - 0.70	Moderate
0.70 - 0.90	Strong, high, marked
>0.90	Very strong, very high

The sign (+ or −) would indicate a positive or negative correlation. In the previous example of weight versus height the result of +0.884 would represent a "high," "strong," or "marked" positive correlation.

The values for correlation coefficients do not represent equal distances along a linear scale. For example, a correlation of 0.50 is not twice as large as $r = 0.25$. Instead, the coefficient is always relative to the conditions under which it was calculated. The larger the r, either in the positive or negative direction, the greater the association between the two measures.

In addition to identifying the strength and direction of a correlation coefficient, there are statistical methods for testing the significance of a given correlation. Two will be discussed here: 1) use of a Pearson product-moment table and 2) the conversion to a Student t-statistic. In both cases, the symbol r_{yx} or ρ (rho) can be used to represent the correlation for the populations from which the samples were randomly selected. The hypotheses being tested are:

$$H_0: \; r_{yx} = 0 \qquad \text{or} \qquad H_0: \; \rho = 0$$
$$H_1: \; r_{yx} \neq 0 \qquad\qquad H_1: \; \rho \neq 0$$

The null hypothesis indicates that a correlation does not exist between the two continuous variables; the population correlation coefficient is zero, whereas the alternative hypothesis states that a significant relationship exists between variables *x* and *y*. Pearson's correlation coefficient, symbolized by the letter *r*, represents the sample value for the relationship; whereas ρ or r_{yx} represents true population correlation.

Using Table B14 in Appendix B, it is possible to identify a critical *r*-value and if the correlation coefficient exceeds the critical value, H_0 is rejected. The first column in the table represents the degrees of freedom and the remaining columns are the critical values at various allowable levels of Type I error (α). For correlation problems the number of degrees of freedom is the number of data points minus two ($n - 2$). The reason for $n - 2$ is that there is one fewer degree of freedom because the mean of the *y*-axis is an estimate of the true population mean, μ_y. The decision rule is to reject H_0 (no correlation) if the calculated *r*-value is greater than $r_{n-2}(\alpha)$

$$\text{with } \alpha = 0.05, \text{ reject } H_0 \text{ if } r > r_{n-2}(0.05)$$

In the previous example comparing weights and heights of volunteers in a clinical trial, the decision rule would be with $\alpha = 0.05$, reject H_0 if $r > r_4(0.05) = 0.8114$. The result of the calculations produces a correlation coefficient of 0.884, which is greater than the critical *r*-value of 0.8114; therefore, we would reject H_0 and conclude that there is a significant correlation with 95% confidence. One might question how well we can trust a correlation coefficient from a sample size of only six to predict the relationship in the population from which the sample is drawn. Two factors will influence this decision: 1) the strength of the correlation (the *r*-value itself); and 2) the sample size. Looking at the table of critical values for the correlation coefficient (Table B14, Appendix B) it is possible to find significance for a relatively small *r*-value, if the result comes from a large sample.

The second method for calculating the level of significance for the sample *r*-value is to enter the results into a special formula for a t-test and compare the results to a critical value from a Student *t*-distribution (Table B5, Appendix B). This converted *t*-value, from an *r*-value, is compared to the critical *t*-value with $n - 2$ degrees of freedom. The null hypothesis is that there is no correlation and the decision rule is

$$\text{with } \alpha = 0.05, \text{reject } H_0 \text{ if } t > +t_{n-2}(1 - \alpha/2) \text{ or } t < -t_{n-2}(1 - \alpha/2)$$

The statistical formula is:

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}} \qquad \text{Eq. 13.9}$$

The correlation coefficient (*r*) incorporates the concept of how scores vary within a given distribution. These potential deviations are considered as a standard error of the correlation coefficient and represent the standard deviation for the theoretical

Table 13.6 Comparison of Critical r-Values and t-Values

Table of Critical Values	Statistical Results	$\alpha = 0.05$		$\alpha = 0.01$	
		C.V.	Result	C.V.	Result
Table B11	$r = 0.884$	0.8114	Significant	0.9172	NS
Table B3	$t = 3.78$	2.776	Significant	4.604	NS

distribution of correlation coefficients for samples from the population with a given size. The closer the correlation coefficient to a perfect result (+1.00 or –1.00), the smaller the standard error (the denominator in Eq. 13.9). Approximately 95% of all possible correlation coefficients will be within two standard deviations of the population ρ. Therefore, we can use information from Chapter 9 to create a *t*-statistic to calculate significance of the correlation coefficient.

Using our previous example (weight versus height) to illustrate the correlation *t*-conversion, the decision rule is with $\alpha = 0.05$, reject H_0 if $t > t_4(0.975) = 2.776$. The computations are:

$$t = \frac{.884\sqrt{6-2}}{\sqrt{1-(.884)^2}} = \frac{1.768}{0.467} = 3.79$$

In this case the decision, with $t > 2.776$, is to reject H_0 and conclude that there is a significant correlation between the volunteers' weights and heights. Based on the *t*-conversion, a significant result would indicate that the results could not have occurred by chance alone from a population with a true zero correlation. Note in Table 13.6 that both methods produce identical outcomes.

The *r*-value can be considered a ratio of the actual amount of deviation divided by the total possible deviation, whereas the square of the *r*-value is the amount of actual deviation that the two distributions have in common. The interpretation of the correlation between two variables is concerned with the degree to which they **covary**. In other words, how much of the variation in one of the continuous variables can be attributed to variation in the other. This square of the correlation coefficient, r^2, indicates the proportion of variance in one of the variables accounted for by the variance of the second variable. The r^2 term is sometimes referred to as the "**common variance**." In the case of $r^2 = 0.49$ (for $r = 0.7$), 49% of the variance in scores for one variable is associated with the variance in scores for the second variable. The r^2 term will be discussed in greater detail in the following chapter.

Correlation and Causality

As a correlation approaches +1.0 or –1.0 there is a tendency for numbers to concentrate closer to a straight line. However, one should not assume that just

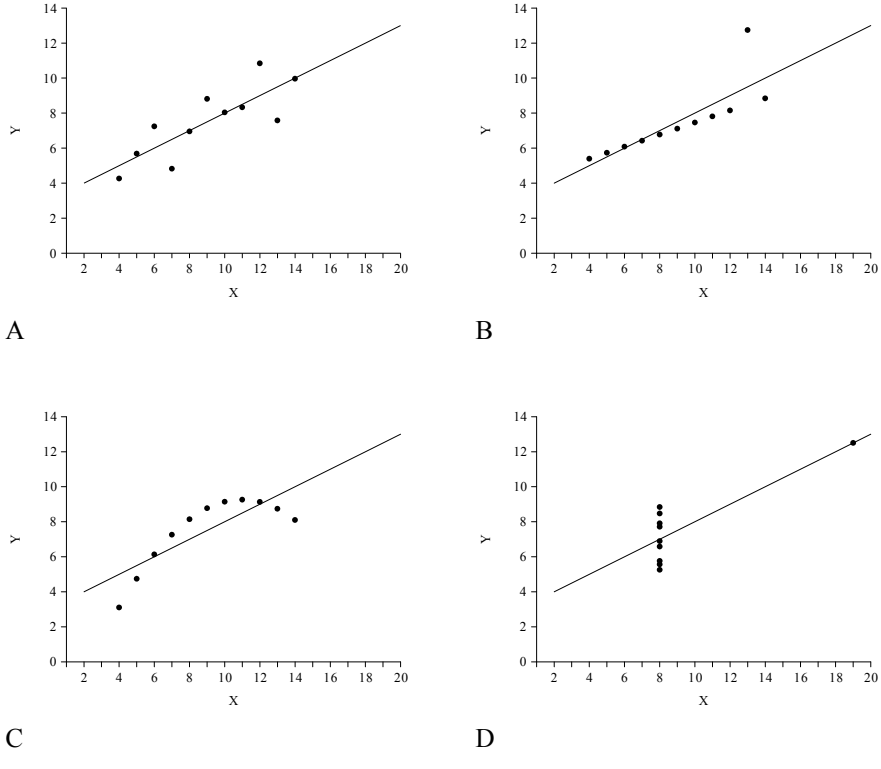


Figure 13.3 Graphs of four sets of data with identical correlations (r = 0.816). Recreated from: Anscombe, F.J. (1973). "Graphs in statistical analysis," *American Statistician* 27:17-27.

because correlations come closer to a perfect correlation they form a straight line. The correlation coefficient says nothing about the percentage of the relationship, only its relative strength. It represents a convenient ratio, not an actual measurement scale. It serves primarily as a data reduction technique and as a descriptive method. Figure 13.3 illustrates this point where four different data sets can produce the same "high" correlation ($r = 0.816$). As discussed in the next chapter, if lines were drawn that best fit between the points in each data set, they would be identical with a slope of 0.5 and a y-intercept of 3.0. This figure also shows the advantage of plotting the data on graph paper, or creating a computer-generated visual, to actually observe the distribution of the data points.

The correlation coefficient does not suggest nor prove the reason for this relationship; only that it exists, whether the two variables vary together either positively or negatively, and the degree of this relationship. It does not indicate anything about the **causality** of this relationship. Did the x-variable cause the result in y? Did y affect variable x? Could a third variable have affected both x and y? There could be many reasons for this relationship.

With correlation the relationship identified between two dependent variables is purely descriptive and no conclusions about causality can be made. By contrast, with experimental or regression studies in the next chapter, where the predictor or independent variable is controlled by the researcher, there is a better likelihood that interpretations about causality can be stated. However, with correlation, this relationship may be due to external variables not controlled for by the experiment. These are called **confounding variables** and represent other unidentified variables that are entwined or confused with the variables being tested. Two factors must be established before the researcher can say that *x*, assumed to be the independent variable, caused the result in *y*. First, *x* must have preceded *y* in time. Second, the research design was such that it controlled for other factors that might cause or influence *y*.

Even a significant result from a correlation coefficient does not necessarily represent simply a cause-and-effect relationship between the two variables. In the previous example, does the height of the person directly contribute to his or her weight? Does the weight of the person influence the person's height? The former assumption may be true, but probably not the latter. In this particular case, both variables were influenced by a third factor. The patients volunteering to take part in the study were screened using an inclusion criterion that they must fall within 10% of the ideal height and weight standards established by the Metropolitan Life Insurance Company. Thus, if we approximate ideal weight and height standards, taller volunteers will tend to weigh more and shorter volunteers will weigh less because of the ratio established between these variables based on the standardized tables used by Metropolitan Life.

In some cases causality may not be as important as the strength of the relationship. For example if the researchers were comparing two methods (e.g., analytical assays, cognitive scales, physiological measures), the individual is not interested in whether one method produced a higher mean value than the other, rather he or she is interested in whether there is a significant correlation between the two methods.

Various types of relationships can exist between two continuous variables and still produce a correlation coefficient. Many are illustrated in Figures 13.1 and 13.3. A **monotonic relationship** is illustrated by Figures 13.1-A, -B, and -D where the relationship is ever-increasing or ever-decreasing. The monotonic relationship could be linear (best represented by a straight line) or **nonlinear** or **curvilinear relationships** where a curved line best fits the data points. In contrast, Figure 13.3-C is an example of a **nonmonotonic relationship**. In this case the relationship is not ever-increasing or ever-decreasing, the points begin as a positive correlation but change near 10 on the *x*-axis and become a negative correlation. This figure represents a nonmonotonic, concave downward relationship. One last type is a **cyclical relationship** where waves are formed as the correlation continues to change from a positive to a negative to a positive relationship.

In Vivo and *In Vitro* Correlation

One example of the use of the correlation coefficient is to establish a relationship between an *in vitro* measure for a pharmaceutical product and an *in vivo* response in

living systems. This relationship is referred to as an ***in vivo–in vitro* correlation**, or an **IV/IV correlation**.

In 1977, the Food and Drug Administration issued regulations on bioequivalency and bioavailability, and included a list of drugs described as having "known or potential bioequivalency or bioavailability problems" (*Fed. Reg.*, 1977). In these regulations, it was pointed out that bioequivalence requirement for the majority of products could be the form of an *in vitro* test in which the product is compared to a reference standard. This point will be discussed in greater detail in Chapter 22. Preferably, these *in vitro* tests should be correlated with human *in vivo* data. In most cases the *in vitro* tests are dissolution tests.

Dissolution is a measure of the percent of drug entering a dissolved state over varying periods of time. Tests of dissolution are used to determine if drug products are in compliance with compendia standards in the *United States Pharmacopeia* (USP) or new drug application (NDA). Dissolution testing can be performed on a variety of dosage forms including immediate release and extended release solids, transdermal patches, and topical preparations. For immediate release solid dosage forms, one of the most commonly used comparisons for IV/IV correlation is between an *in vivo* parameter (e.g., AUC) and the mean *in vitro* dissolution time (Skelly and Shiu, 1993). If we can establish a strong relationship between this internal response and an equivalent external laboratory measurement we may be able to avoid the risks inherent with human clinical trials. In addition *in vivo* studies can be very expensive and equivalent laboratory results offer a considerable economic advantage.

In an ideal world we would see a correlation of +1.00 relationship between the two parameters. This represents a comparison between single point measures of outcome and rate (Figure 13.4). Using this model it is possible to perform *in vitro* laboratory exercises and predict the responses of *in vivo* systems. Unfortunately we do not live in an ideal world and both of these continuous variables will contain some error or variability resulting in an r less than 1.00. The larger the r-value, the more meaningful its predictive ability. As will be discussed in the next chapter, the strength of a correlation is commonly characterized by r^2, the square of the correlation coefficient. The r^2 is useful because it indicates the proportion of the variance explained by the line that best fits between the data points in the linear relationship.

In some cases *in vitro* dissolution testing can substitute for bioequivalency testing. This is particularly true for extended-release dosage forms. To use dissolution data as a substitute for bioequivalency testing, one is required to have a very strong correlation. In other words, the IV/IV correlation must be highly predictive of *in vivo* performance. In these cases, *in vitro* dissolution information may be meaningful for predicting an *in vivo* response. However, there is no complete assurance that *in vitro* dissolution equals *in vivo* dissolution. One needs to be confident for a given product tested that this IV/IV equality exists and that *in vivo* dissolution leads to absorption and absorption results in an *in vivo* response. The processes and potential problems associated with IV/IV correlation are beyond the scope of the book and readers interested in more information are referred to a series of papers in the book edited by Blume and Midha (1995) or the article by Amidon et. al. (1995).

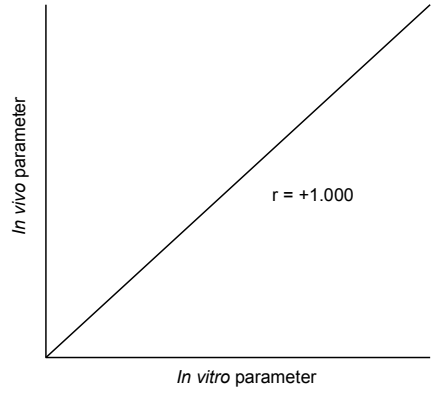


Figure 13.4 Hypothetical example of a perfect IV/IV correlation.

Other Types of Bivariate Correlations

Illustrated to this point in the chapter are correlations involving two continuous variables (interval or ratio scales). There are other special types of measures of relationships that handle various combinations of variables measured on nominal, ordinal, or interval/ratio scales. These are often described as measures of association and are presented in Chapter 17. Correlations involving ordinal scales for both the *x*- and *y*-axis are best evaluated with Spearman's rho, a nonparametric procedure (Chapter 21).

If at all possible, it is recommended to avoid grouping continuous data into categories that form dichotomous or nominal scale results. This attenuating of the data can lead to an underestimation of the measured effect. Tests for handling this type of edited data are discussed in Chapter 17 as measures of association and the terminology associated with such attenuated data sets is as follows. The **biserial correlation coefficient** is a special type of bivariate correlation coefficient for comparing a continuous normally distributed variable with a dichotomous variable that has an underlying normal distribution (e.g., a continuous variable that has been collapsed to create groups representing "above" and "below" the median result). Comparing two continuous variables that have both been dichotomized would involve a **tettachoric correlation coefficient**. This is in contrast to a **point biserial correlation coefficient** which involves the correlation between a continuous normally distributed variable with a truly dichotomous variable.

Pair-Wise Correlations Involving More Than Two Variables

When there are more than just two continuous variables affecting each data point, it is possible to calculate pair-wise correlations. For example if we are evaluating three continuous variables (*X*, *Y,* and *Z*) on the same subjects, we can calculate the correlations (r_{xy}, r_{xz}, and r_{yz}). The simplest way to evaluate the relationship between

Table 13.7 Example of an Intercorrelation Matrix

Variables	X	Y	Z
X	r_{xx}	r_{xy}	r_{xz}
Y	r_{xy}	r_{yy}	r_{yz}
Z	r_{xz}	r_{yz}	r_{zz}

Table 13.8 Abbreviated Intercorrelation Matrix

Variables	Y	Z
X	r_{xy}	r_{xz}
Y	...	r_{yz}

these variables is to create a table referred to as an **intercorrelation matrix**. Also called a **correlation matrix** it arranges the correlation coefficients in a systematic and orderly fashion represented by a square with an equal number of rows and columns (Table 13.7). The diagonal coefficients have a perfect relationship (r = 1.00) between each variable correlated with itself. The number of cells above or below the diagonal can be calculated using either of the following determinants:

$$C = \frac{k(k-1)}{2} \qquad \text{Eq. 13.10}$$

$$C = \binom{k}{2} = \frac{k!}{2!(k-2)!} \qquad \text{Eq. 13.11}$$

where k equals the number of variables and Eq. 13.11 is simply the combination formula (discussed in Chapter 2) for paired comparisons. The cells in the lower portion of the correlation matrix are a mirror image of the cells above the diagonal. We could simplify the matrix by discarding the diagonal cells and either the lower or upper portion of the cells and express the matrix as seen in Table 13.8.

To illustrate the use of an intercorrelation matrix, consider the data presented in Table 13.9. In this table more information is presented for the volunteer included in the earlier clinical trial. These additional data include: entry laboratory values for blood urea nitrogen (BUN) and serum sodium; and study pharmacokinetic results as represented by the area under the curve (AUC).

Using the data presented in Table 13.9, the intercorrelation matrix is shown in Table 13.10 and the actual pair-wise correlations in Table 13.11. Based on the correlation coefficients presented on this matrix and the descriptive terminology discussed earlier, the results of the multiple correlation would be: 1) a high correlation

Table 13.9 Leveled Variables from Six Subjects in a Clinical Trial

		Entry Lab Values		Results
Weight (kg)	Height (m)	BUN (mg/dl)	Sodium (mmol/l)	AUC (ng/ml)
96	1.88	22	144	806
77.7	1.80	11	141	794
100.9	1.85	17	139	815
79.0	1.77	14	143	775
73.0	1.73	15	137	782
84.5	1.83	21	140	786

Table 13.10 Layout of Correlation Matrix for Table 13.9

Variables	Weight	Height	BUN	Na	AUC
Weight(A)	1.00	r_{ab}	r_{ac}	r_{ad}	r_{ae}
Height (B)	r_{ab}	1.00	r_{bc}	r_{bd}	r_{be}
BUN (C)	r_{ac}	r_{bc}	1.00	r_{cd}	r_{ce}
Na (D)	r_{ad}	r_{bd}	r_{cd}	1.00	r_{de}
AUC (E)	r_{ae}	r_{be}	r_{ce}	r_{de}	1.00

Table 13.11 Correlation Matrix for Table 13.9

Variables	Height	BUN	Na	AUC
Weight(A)	.884	.598	.268	.873
Height(B)		.665	.495	.781
BUN (C)			.226	.334
Na (D)				.051

between weight and height, weight and AUC, and height and AUC; 2) a moderate correlation between weight and BUN, height and BUN, and height and sodium; 3) a low correlation between weight and sodium, BUN and sodium, and BUN and AUC; and 4) an almost negligible relationship between sodium and AUC.

This matrix can be extended to include the intercorrelations for any number of continuous variables. Using the correlation matrix it is possible to identify those variables that correlate most highly with each other. Unfortunately, just by inspection of the matrix it is not possible to determine any joint effects of two or more variables on another variable. As discussed later, most computer programs will generate such a correlation matrix and include an associated *p*-value for each correlation coefficient

Table 13.12 Correlation Matrix with Accompanying *p*-Values

Variables	Height	BUN	Na	AUC
Weight(A)	.884 0.019	.598 0.210	.268 0.608	.873 0.023
Height(B)		.665 0.149	.495 0.318	.781 0.067
BUN (C)			.226 0.667	.334 0.517
Na (D)				.051 0.923

(Table 13.12). In this case, statistical significant ($p < 0.05$) positive relationships exist between weight and height ($p = 0.019$) and weight and AUC ($p = 0.023$).

Multiple Correlations

Many times when there are multiple concurrent correlations in an experiment, we may be interested in one key variable that has special importance and we are interested in determining how other variables influence this factor. This variable is labeled as our **criterion variable**. Other variables assist in the evaluation of this variable. These additional variables are referred to as **predictor variables** because they may have some common variance with the criterion variable; thus information about these latter variables can be used to predict information about our criterion variable. The terms **criterion variable** and **predictor variable** may be used interchangeably with dependent and independent variables, respectively.

In the next chapter we will discuss regression, where the researchers are able to control at least one variable in controlled experimental studies and the criterion or dependent variable becomes synonymous with the **experimental variable** or **response variable**. In these experimental studies we will reserve the expression *independent variable* to variables independent of each other.

In **multiple correlation** we use techniques that allow us to evaluate how much of the variation in our criterion variable is associated with variances in a set of predictor variables. This procedure involves weighing the values associated with our respective predictor variables. The procedures are complex and tedious to compute. However, through the use of computer programs it is possible to derive these weights (usually the higher weights are associated with predictor variables with the higher common variance with our criterion variable).

In a multiple correlation we once again compute a line that fits best between our data points and compute the variability around that line. The formula for a straight line ($y = a + bx$) can be expanded to the following for multiple predictor variables.

$$y_i = \beta_o + \beta_1 x_{1i} + \beta_2 x_{2i} + \ldots + \beta_k x_{ki} + e_i \qquad \text{Eq. 13.12}$$

In this equation the e_i is a common variance associated with the *y*-variable and β_o is

the point where a plane created by the other variables will intercept the *y*-axis. The remaining β's in Eq. 13.12 are weights that are applied to each of the predictor variables, which result in composite scores that correlate most highly with the scores of our criterion variable. These are referred to as **beta coefficients** or **beta weights**. These weights are a function of the correlation between the specific predictor variables and the criterion variables, as well as the correlations that exist among all the predictor variables.

The result of the mathematical manipulation, which is beyond the scope of this book, is a **multiple correlation coefficient** (*R*). It is the correlation resulting from the weighted predictor scores. Multiple correlations are closely related to multiple regression models. An excellent source for additional information on multiple correlation is Kachigan (1991, pp. 147-153). Others sources would include Zar (2010, pp. 438-440) and Daniel (2005, pp. 508-512).

Partial Correlations

An alternative method for the evaluation of multiple correlations is to calculate a **partial correlation coefficient** that shows the correlation between two continuous variables, while removing the effects of any other continuous variables. The simplest type of partial correlation coefficient is to extract the common effects of one variable from the relationship between two other variables of interest:

$$r_{yx,z} = \frac{r_{yx} - (r_{xz})(r_{yz})}{\sqrt{(1 - r_{xz}^2)(1 - r_{yz}^2)}} \qquad \text{Eq. 13.13}$$

where $r_{yx,z}$ is the correlation between variables *x* and *y*, eliminating the effect of variable *z*. This formula can be slightly modified to evaluate the correlations for the other two combinations (*XZ* and *YZ*). In this formula all three paired correlations must be calculated first and then placed into Equation 13.13.

As an example of a partial correlation for three continuous variables, assume that only the first two columns and fifth column from Table 13.12 were of interest to the principal investigator involved in the clinical trial and that the researcher is interested in the correlation between the AUC and the weight, removing the effect that height might have on the results. The partial correlation coefficient would be as follows:

$$r_{ae,b} = \frac{r_{ae} - (r_{ab})(r_{be})}{\sqrt{(1 - r_{ab}^2)(1 - r_{be}^2)}}$$

$$r_{ac,b} = \frac{.873 - (.884)(.781)}{\sqrt{(1 - (.884)^2)(1 - (.781)^2)}} = \frac{.183}{.292} = +0.627$$

Therefore, we see a moderate correlation between AUC and weight when we control the influence of height. In other words, what we have accomplished is to determine the relationship (r = +0.63) between our two key variables (AUC and weight) while

holding a third variable (height) constant. Is this a significant relationship? We can test the relationship by modifying the *t*-statistic that was used to compare only two dependent variables.

$$t_{yx.z} = \frac{r_{yx.z}\sqrt{n-k-1}}{\sqrt{1-(r_{yx.z})^2}} \qquad \text{Eq. 13.14}$$

In this case, k represents the number of variables being evaluated that might influence the outcome against the y-variable. In our example, k equals 2 for variables x and z. The decision rule is to reject the null hypotheses of no correlation if t is greater than the critical t-value of the $n - k - 1$ degrees of freedom. In t-conversion to evaluate the significance of $r = 0.63$ the critical value would be $t_3(0.975) = 2.78$ and the calculations would be as follows:

$$t_{yx.z} = \frac{(.627)\sqrt{6-2-1}}{\sqrt{1-(.627)^2}} = \frac{1.086}{0.779} = 1.394$$

The researcher would fail to reject the null hypothesis and conclude that there is no significant correlation between the AUC and weight excluding the influence of height.

The partial correlation can be expanded to control for more than one additional continuous variable.

Nonlinear Correlations

For nonlinear correlations the best measure of a relationship is the **correlation ratio**. This **eta-statistic** (η) can be used when data tend to be curvilinear in their relationships. Based on visual inspection of the data, the sample outcomes are divided into categories, at least 7, but no more than 14. These categories represent clusters of data with observable breaking points in the data. If there are fewer than seven categories the eta-statistic may not be sensitive to the curvilinear relationship. The statistic is based on a comparison of the differences, on the y-axis, between observed data points and their category mean, as well as a comparison with the total mean for all of the observations:

$$\eta = \sqrt{1 - \frac{\Sigma(y_i - \overline{Y_c})^2}{\Sigma(y_i - \overline{Y_t})^2}} \qquad \text{Eq. 13.15}$$

where y_i represents the data point, $\overline{Y}_c$ is the mean for the category, and $\overline{Y}_t$ is the mean for all of the y-observations.

If there is a nonlinear relationship, the traditional correlation coefficient tends to underestimate the strength of this type of relationship. For example consider

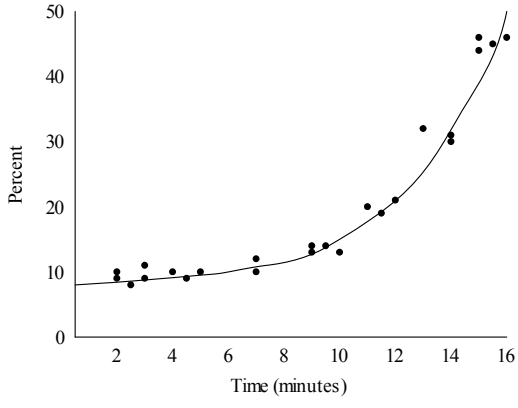


Figure 13.5 Graphic of a curvilinear relationship.

the relationship presented in Figure 13.5 where the data appears to curve. Calculation of the traditional correlation coefficient (Eq. 13.15) produces a correlation coefficient of $r = 0.883$. In this case the total mean for all the observations on the y-axis is $\overline{Y}_t = 20.25$. Calculation of the η is based on the data in Table 13.13.

$$\eta = \sqrt{1 - \frac{15.6167}{4140.50}} = \sqrt{0.9962} = +0.9981$$

Note that η is larger than r.

Assessing Independence and Randomness

Since this chapter introduced us to correlation and measures of the strength of relationships, two previously discussed topics will be revisited (independence and randomness) along with statistical procedures to evaluate these two critical assumptions associated with most inferential statistics.

As mentioned previously, the researcher should be concerned with obtaining an appropriate sample (preferably a random sample) and be comfortable that observations are independent of each other. Assessing independence can be visually determined by graphing how each observation differs from the mean. These differences are sometimes referred to as **residuals**. The residuals are plotted on the y-axis of a scatter plot against the x-axis, which represents the order in which the sample was collected or recorded. If there is **independence**, a pattern should not appear on the scatter plot. A more formal method for testing independence is to calculate the **Durbin-Watson coefficient**, which uses Studentized residuals:

Table 13.13 Sample Data Comparing Time and Percent Response

x (time)	y (%)	$\overline{Y}_c$	$(-\overline{Y}_c)$	$(y-\overline{Y}_c)^2$	$(y-\overline{Y}_t)$	$(y-\overline{Y}_t)^2$
2	9	9.40	−0.40	0.16	−11.25	126.5625
2	10		0.60	0.36	−10.25	105.0625
2.5	8		−1.40	1.96	−12.25	150.0625
3	9		−0.40	0.16	−11.25	126.5625
3	11		1.60	2.56	-9.25	85.5625
4	10	9.67	0.33	0.1089	−10.25	105.0625
4.5	9		−0.67	0.4489	−11.25	126.5625
5	10		0.33	0.1089	−10.25	105.0625
7	10	11.00	−1.00	1.00	−10.25	105.0625
7	12		1.00	1.00	−8.25	68.0625
9	13	13.50	−0.50	0.25	−7.25	52.5625
9	14		0.50	0.25	−6.25	39.0625
9.5	14		0.50	0.25	−6.25	39.0625
10	13		−0.50	0.25	−7.25	52.5625
11	20	20.00	0.00	0.00	−0.25	0.0625
11.5	19		−1.00	1.00	−1.25	1.5625
12	21		1.00	1.00	0.75	0.5625
13	32	31.00	1.00	1.00	11.75	138.0625
14	30		−1.00	1.00	9.75	95.0625
14	31		0.00	0.00	10.75	115.5625
15	44	45.25	−1.25	1.5625	23.75	564.0625
15	46		0.75	0.5625	25.75	663.0625
15.5	45		−0.25	0.0625	24.75	612.5625
16	46		0.75	0.5625	25.75	663.0625
		$\sum$ =	0	15.6167	0	4140.5000

$$d = \frac{\sum\left[(y_i - \overline{X}_y) - (y_{i-t} - \overline{X}_y)\right]}{\sum (y_i - \overline{X}_y)^2} \qquad \text{Eq. 13.16}$$

where $\overline{X}_y$ is the mean on the *y*-axis, y_i represents each data point and y_{i-t} is the *y*-value for the previous sequential value on the *x*-axis (**serial correlation**). This test is often used for time series correlations where the *x*-axis is time. For serial correlation a $d = 0$ would represent a perfect positive correlation, $d = 2$ no correlation, and $d = 4$ a perfect negative correlation. For testing independence, if the Durbin-Watson coefficient is between 1.5 and 2.5, independence can be assumed.

The second key consideration for inferential tests is randomness in the data. A **runs test** can be used for assessing randomness. A "run" is a series of similar responses. For example, 25 true and false questions give the following ordered

results:

TTFTTFTTTFFFFFTFFTTTFFFFF

This represents ten runs, TT, F, TT, F, TTT, FFFFF, T, FF, TTT, and FFFFF. The following symbols will be used: u = number of runs, n_1 = number with the first outcome (T in this case), and n_2 = number with the second outcome (F). Tables are available for small samples. For larger samples (n_1 or n_2 larger than 30) and as approximation for smaller samples, the following equations can be used. As the underlying distribution approaches normality, the mean is:

$$\mu_u = \frac{2n_1n_2}{N} + 1 \qquad \text{Eq. 13.17}$$

The standard deviation would be:

$$\sigma_u = \sqrt{\frac{2n_1n_2(2n_1n_2 - N)}{N^2(N-1)}} \qquad \text{Eq. 13.18}$$

The deviation from expect results would be evaluated using a z-statistic:

$$Z = \frac{|u - \mu_u| - 0.5}{\sigma_u} \qquad \text{Eq. 13.19}$$

If the result is less than $Z_{\alpha/2} = 1.96$, the sample can be assumed to be random. In our previous example the approximation would be:

$$n_1 = 11,\ n_2 = 14,\ N = 25\ u = 10$$

$$\mu_u = \frac{2(11)(14)}{25} + 1 = 13.32$$

$$\sigma_u = \sqrt{\frac{2(11)(14)[2(11)(14) - 25]}{25^2(24)}} = 2.41$$

$$Z = \frac{|10 - 13.32| - 0.5}{2.41} = 1.17$$

The runs test is a nonparametric procedure (Chapter 21) and thus assumes no specific distribution. In order to do a runs test the variable must have dichotomous results and categories should represent mutually exclusive and exhaustive outcomes. For ordinal or continuous data, the results must be dichotomized above or below the median.

An alternative to the runs test is **autocorrelation**, which also tests for non-

randomness in data. It is primarily used for time series tests. It is a correlation coefficient that involves evaluating *y*-values for their corresponding *x*-values arranged sequentially by time. The lag *k* autocorrelation is calculated using the following formula:

$$r_k = \frac{\sum (y_i - \overline{X}_y)(y_{i+k} - \overline{X}_y)}{\sum (y_i - \overline{X}_y)^2} \qquad \text{Eq. 13.20}$$

where $\overline{X}_y$ is the mean on the *y*-axis, y_i represents each data point and y_{i+k} is the *y*-value for the next sequential value on the *x*-axis. Additional information about the use of autocorrelation can be found in Box and Jenkins (1976).

Using Excel® or Minitab® for the Correlation

Excel 2010 has both function (*fx*) options and data analysis procedures for calculating single correlation coefficient or covariance, as well as the ability to create a correlation matrix. The function option for the covariance is **COVARIANCE.S** and for correlation coefficient it is **CORREL**. In both cases, Excel will request the range where the "array" is located for *x*-values and *y*-values (Array1 and Array2). The output is the simply covariance or correlation coefficient in the cell where the function option was initiated. One potential problem with Excel is that it also includes **COVARIANCE.P** for population data. Usually the population information is not known and one should use the ".S" options.

Both covariance and correlation are part of the Excel data analysis tools:

Data ➤ Data Analysis ➤ Covariance
Data ➤ Data Analysis ➤ Correlation

Either test will request the input range, whether the data is arranged by columns or rows and the location for printing the results (a new worksheet is the default setting – Figure 13.6). Single outcomes will be reported as a matrix table with each level as a row or column, with the one cell for the *x*-value and *y*-value showing the exact same results as the function option (similar to Table 13.8). More complex matrices for the correlation coefficients can be created when comparing multiple continuous variables by simply increasing the input range to accommodate all the dependent variables. Results for an evaluation for Table 13.9 are is presented in Figure 13.7 (each dependent variable is labeled because the "Labels in First Row" box was checked in Figure 13.6). Note that Excel does not provide the associated *p*-value for each correlation in the matrix.

It is recommended not to use the "Covariance" option under data analysis since it creates a single output or metric using the population calculation and results will be slightly lower (closer to zero) than for the sample calculation.

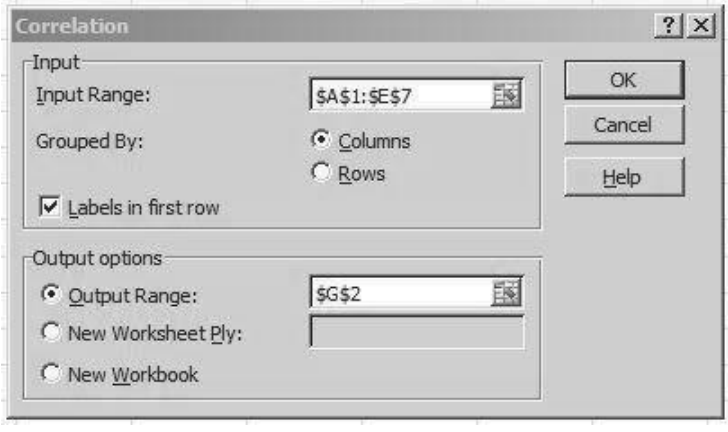


Figure 13.6 Options for covariance or correlation with Excel.

	Weight (kg)	*Height (m)*	*BUN (mg/dl)*	*Sodium (mmol/l)*	*AUC (ng/ml)*
Weight (kg)	1				
Height (m)	0.88441386	1			
BUN (mg/dl)	0.59755936	0.6651787	1		
Sodium (mmol/l)	0.26758273	0.4949747	0.22601347	1	
AUC (ng/ml)	0.87319086	0.7807796	0.33412276	0.051119863	1

Figure 13.7 Outcome matric for correlation with Excel for Table 13.9.

Minitab offers both covariance and correlation options under "Basic Statistics" on the title bar:

Stat ➤ Basic Statistics ➤ Covariance
Stat ➤ Basic Statistics ➤ Correlation

As with Excel, simple two variable or multiple variable matrices can be created. The advantage with Minitab is that the associated *p*-values are also displayed. Figure 13.8 illustrates the options panel for a correlation coefficient for the data from Table 13.2. The dependent variables on the left are selected by double clicking each column that then appears in the box to the left. Displaying the *p*-value is a default setting that can be turned off. The covariance looks similar except there are no associated *p*-values as an option. Figure 13.9 displays the output for both the covariance and correlation matrix for the data in Table 13.7 for just the laboratory values and AUC results. Note that the covariance is based on the sample statistic and not the population calculation.

References

"Bioequivalency requirements and *in vivo* bioavailability procedures," *Federal Register* 42:1621-1653 (1977).

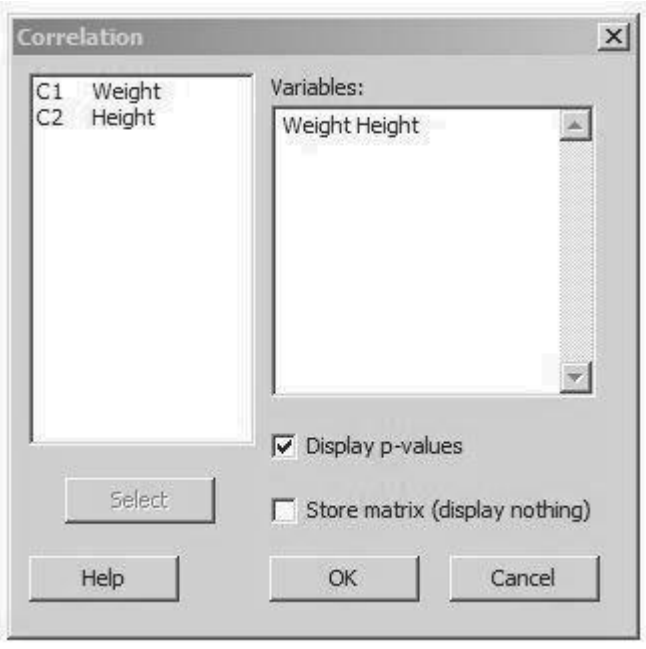


Figure 13.8 Options for correlation with Minitab.

Covariances: BUN (mg/dl), Sodium (mmol/l), AUC (ng/ml)

	BUN (mg/dl)	Sodium (mmol/l)	AUC (ng/ml)
BUN (mg/dl)	17.86667		
Sodium (mmol/l)	2.46667	6.66667	
AUC (ng/ml)	21.40000	2.00000	229.60000

Correlations: BUN (mg/dl), Sodium (mmol/l), AUC (ng/ml)

	BUN (mg/dl)	Sodium (mmol/l)	
Sodium (mmol/l)	0.226		
	0.667		
AUC (ng/ml)	0.334	0.051	
	0.517	0.923	

Cell Contents: Pearson correlation
P-Value

Figure 13.9 Outcome metric for covariance and correlation with Minitab for portions of Table 13.9.

Amidon, G.L. et al. (1995). "A theoretical basis for a biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability," *Pharmaceutical Research* 12:413-420.

Blume, H.H. and Midha, K.K., eds. (1995). *Bio-International 2: Bioavailability, Bioequivalence and Pharmacokinetic Studies*, Medpharm Scientific Publishers, Stuttgart, pp. 247-318.

Box, G.E.P. and Jenkins, G.M. (1976). *Time Series Analysis: Forecasting and Control*, revised edition, Holden-Day, San Francisco, pp. 23-45.

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Eighth edition, John Wiley and Sons, New York, pp. 508-512.

Guilford, J.P. (1956). *Fundamental Statistics in Psychology and Education*, McGraw-Hill, New York, p. 145

Kelly, W.D., Ratliff, T.A., and Nenadic, C. *Basic Statistics for Laboratories*, John Wiley and Sons, Hoboken, NJ, 1992, p. 93.

Rowntree, D. (1981). *Statistics Without Tears: A Primer for Non-Mathematicians*, Charles Scribner's Sons, New York, p. 170.

Kachigan, S.K. (1991). *Multivariate Statistical Analysis*, Second edition, Radius Press, New York, pp. 147-153.

Skelly, J.P. and Shiu, G.F. (1993). "*In vitro/in vivo* correlations in biopharmaceutics: scientific and regulatory implications," *European Journal of Drug Metabolism and Pharmacokinetics* 18:121-129.

Zar, J.H. (2010). *Biostatistical Analysis*, Fifth edition, Prentice-Hall, Upper Saddle River, NJ, pp. 438-440.

Suggested Supplemental Readings

Bolton, S. and Bon, C. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, New York, pp. 200-206.

Bradley, J. (1968). *Distribution-Free Statistical Tests*, Prentice-Hall, Englewood Cliffs, NJ, pp. 255-259.

Cutler, D.J. (1995). "*In Vitro/In Vivo* Correlation and Statistics," *Bio-International 2: Bioavailability, Bioequivalence and Pharmacokinetic Studies*, Blume, H.H. and Midha, K.K., eds., Medpharm Scientific Publishers, Stuttgart, pp. 281-289.

Havilcek, L.L. and Crain, R.D. (1988). *Practical Statistics for the Physical Sciences*, American Chemical Society, Washington, DC, pp. 83-93, 106-108.

Table 13.14 Data Comparing Two Methods

Method A	Method B
55	90
66	117
46	94
77	124
57	105
59	115
70	125
57	97
52	97
36	78
44	84
55	112
53	102
67	112
72	130

Example Problems (Answers are provided in Appendix D)

1. Two different scales are used to measure patient anxiety levels upon admission to a hospital. Method A is an established test instrument, while Method B (which has been developed by the researchers) is a quicker and easier instrument to administer. Is there a correlation between the two measures presented in Table 13.14?

2. Two drugs (A and B) are commonly used together to stabilize patients after strokes and the dosing for each is individualized. Listed in Table 13.15 are the dosages administered to eight patients randomly selected from admission records at a specific hospital over a 6-month period. Did the dosage of either drug result in a stronger correlation with shortened length of stay (LOS) in the institution?

Table 13.15 Data Comparing Two Drugs and Length of Stay

Patient	LOS (days)	Drug A (mg/kg)	Drug B (mcg/kg)
1	3	2.8	275
2	2	4.0	225
3	4	1.5	250
4	3	3.0	225
5	2	3.7	300
6	4	2.0	225
7	4	2.4	275
8	3	3.5	275

Table 13.16 Data Comparing Two Methods

Method GS	Method ALT
90.1	89.8
85.2	85.1
79.7	80.2
74.3	75.0
60.2	61.0
35.5	34.8
24.9	24.8
19.6	21.1

3. It is believed that two assay methods will produce identical results for analyzing a specific drug. Various dilutions are assayed using both the currently accepted method (GS) and the proposed alternative (ALT). Based on the results listed in Table 13.16, does a high correlation exist?

4. A random sample of twelve students graduating from a school of pharmacy was administered an examination to determine retention of information received during classes. The test contained four sections covering pharmacy law, pharmaceutical calculations (math), pharmacology (p'cology) and medicinal chemistry (medchem). Listed in Table 13.17 are the results of the tests. Create a correlation matrix to compare the results and relationships between the various sections and total test score. Which of the two sections most strongly correlated together? Which section has the greatest correlation with the total test score?

Table 13.17 Data for Problem 4

Student	Law	Math	P'cology	Medchem	Total
001	23	18	22	20	83
002	22	20	21	18	81
003	25	21	25	17	88
004	20	19	18	20	77
005	24	23	24	14	85
006	23	22	22	20	87
007	24	20	24	15	83
008	20	17	15	22	74
009	22	19	21	23	85
010	24	21	23	19	87
011	23	20	21	19	83
012	21	21	20	21	83

14

Regression Analysis

Unlike the correlation coefficient, regression analysis requires at least one independent variable. Where correlation describes pair-wise relationships between continuous variables, linear regression is a statistical method to evaluate how one or more independent (predictor) variables influence outcomes for one continuous dependent (response) variable. In linear regression a line is computed that best fits between the data points. If a linear relationship is established, the magnitude of the effect of the independent variable can be used to predict the corresponding magnitude of the effect on the dependent variable. For example a person's weight can be used to predict body surface area. The strength of the relationship between the two variables can be determined by calculating the amount of the total variability that can be accounted for by the regression line.

Both linear regression and correlation are similar, in that both describe the strength of the relationship between two or more continuous variables. However, with linear regression, also termed **regression analysis**, a relationship is established between the two variables and a response for the dependent variable can be estimated based on a given value for the independent variable. For correlation, two dependent variables can be compared to determine if a relationship exists between them. Similarly, correlation is concerned with the strength of the relationship between two continuous variables. In regression analysis, or **experimental associations**, researchers control the values of at least one of the variables and assign objects at random to different levels of these variables. Where correlation simply describes the strength and direction of the relationship, regression analysis provides a method for describing the nature of the relationship between two or more continuous variables.

The correlation coefficient can be very useful in exploratory research where the investigator is interested in the relationship between two or more continuous variables. One of the disadvantages of the correlation coefficient is that it is not very useful for predicting the value of y from a value of x, or vice versa. As seen in the previous chapter, the correlation coefficient (r) estimates the extent of the linear relationship between x and y. However, there may be a close correlation between the two variables that are based on a relationship other than a straight line (for example, Figure 13.3). The formulas for correlation and regression are closely related with similar calculations based upon the same sums and sums of squares. Therefore, if an independent variable is involved, calculating both is useful because the correlation

coefficient can support the interpretation associated with regression. This chapter will focus primarily on simple regression, where there is only one independent or predictor variable. The adjective *linear* is used to denote that the relationship between the two variables can be described by a straight line.

There are several assumptions associated with the linear regression model. First, values on the *x*-axis, which represent the independent variable are "fixed." These nonrandom variables are predetermined by the researcher so that responses on the *y*-axis are measured at only predetermined points on the *x*-axis. Because the researcher controls the *x*-axis it is assumed that these measures are without error. Second, for each value on the *x*-axis there is a subpopulation of values for the corresponding dependent variable on the *y*-axis. As will be discussed later, for any inferential statistics or tests of hypotheses, it is assumed that these subpopulations are normally distributed. For data that may not be normally distributed, for example, AUC or C_{max} measures in bioavailability studies, log transformations may be required to convert such positively skewed data to a more normally distributed subpopulation. Coupled with the assumption of normality is homogeneity of variance, in that it is assumed that the variances for all the subpopulations are approximately equal. Third, it is assumed that these subpopulations have a linear relationship and that a straight line can be drawn between them. The formula for this line is:

$$\mu_{y/x} = \alpha + \beta x \qquad \text{Eq. 14.1}$$

where $\mu_{y/x}$ is the mean for any given subpopulation for an *x*-value for the predictor independent variable. The terms α and β represent the true population *y*-intercept and slope for the regression line, respectively. Unfortunately, we do not know these population parameters and must estimate these by creating a line, which is our best estimate based on the sample data.

The Regression Line

As seen above, linear regression is involved with the characteristics of a straight line or **linear function**. This line can be estimated from sample data. Similar to correlation, a scatter plot offers an excellent method for visualizing the relationship between the continuous variables. In the simple regression design there are only two variables (*x* and *y*). The *x*-axis, or **abscissa**, represents the independent variable and the *y*-axis, the **ordinate**, is the dependent outcome. The scatter plot presented in Figure 14.1 shows a typical representation of these variables with *y* on the vertical axis and *x* on the horizontal axis. In this case *x* is a specific amount of drug (mcg) administered to mice, with *y* representing some measurable physiological response. The physiological response is obviously not controllable by the researcher and represents the dependent variable. However, prescribed (hopefully exact) doses of the drug are administered and represent the independent, researcher controlled variable on the *x*-axis.

The first step in a linear regression analysis is to draw a straight line that best fits between the data points. The slope of the line and its intercept of the *y*-axis are then used for the regression calculation. As introduced in the previous chapter, the general equation (Eq. 13.6) for a straight line is:

x	y
5	1.2
10	1.9
15	3.1
20	3.6
25	5.3
30	5.8
35	7.4
40	7.7

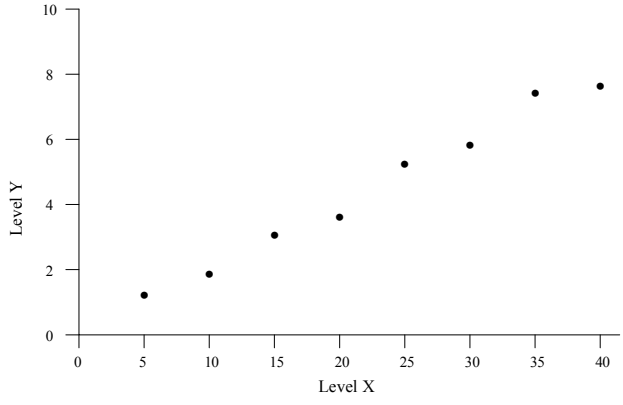


Figure 14.1 Simple examples of data points for two continuous variables.

$$y = a + bx$$

In this formula, y is a value on the vertical axis, x is a corresponding value on the horizontal axis, a is the point where the line crosses the vertical axis, and b represents the amount by which the line rises on the y-axis for each unit increase on the x-axis (the slope of the line). A second method for defining these values is that a is the value on the y-axis where $x = 0$ and b is the change in the y-value (the response value) for every unit increase in the x-value (the predictor variable).

Unfortunately, our estimate of the straight line is based on sample data and therefore subject to random error. Therefore, we need to modify our definition of the regression line to the following, where e is an error term associated with our sampling.

$$y = \alpha + \beta x + e \qquad \text{Eq. 14.2}$$

Once again, it is assumed that the e's associated with each subpopulation are normally distributed with all variances approximately equal.

Our best estimate of the true population regression line would be the straight line that we can draw through our sample data. However, if asked to draw this line using a straight edge, it is unlikely that any two people, using visual inspection, would draw exactly the same line to fit best among these points. Thus, a variety of slopes and intercepts could be approximated. There are in fact an infinite number of possible lines, $y = a + bx$, which could be drawn between our data points. How can we select the "best" line from all the possible lines that can pass through these data points?

The **least-squares line** is the line that best describes the linear relationship between the independent and dependent variables. The data points are usually scattered on either side of this straight line that fits best between the points on a scatter diagram. Also called the **regression line**, it represents a line from which the smallest sum of squared differences is observed between the observed (x,y_i)

coordinates and the line (x,y_c) coordinates along the y axis (sum of the squared vertical deviations). In other words, this **"best fit" line** shows where the sum of squares of the distances from the points in the scatter diagram to the regression line in the vertical direction of the y-variable is smallest. The calculation of the line that best fits between the sample data is presented below. The slope of this line (Eq. 13.7) is:

$$b = \frac{n\sum xy - (\sum x)(\sum y)}{n\sum x^2 - (\sum x)^2}$$

Data to solve this equation can be generated in a table similar to the one used for the correlation coefficient (Table 13.4). The sample slope (b) is our best estimate of the true **regression coefficient** (β) for the population, but as will be discussed later, it is only an estimate.

The greater the change in y, for a constant change in x, the steeper the slope of the line. With the calculated slope of the line that best fits the observed points in the scatter diagram, it is possible to calculate an "anchor point" on the y-axis (the y-intercept) using Eq.13.7 (that point where the x-value is zero):

$$a = \frac{\sum y - b\sum x}{n}$$

An alternative approach to the scatter diagram is to display the information in a table. The regression line can be calculated for the data points in Figure 14.1 by arranging the data in tabular format as presented in Table 14.1. Similar to the manipulation of data for the correlation coefficient, each x-value and y-value is squared, and the product is calculated for the x- and y-value at each data point. These five columns are then summed to produce Σx, Σy, Σx^2, Σy^2, and Σxy. Note that Σy^2 is not required for determining the regression line, but will be used later in additional calculations required for the linear regression model. Using the results in Table 14.1, the computations for the slope and y-intercept would be as follows:

Table 14.1 Data Manipulation of Regression Line for Figure 14.1

	x	y	x^2	y^2	xy
	5	1.2	25	1.44	6.00
	10	1.9	100	3.61	19.00
	15	3.1	225	9.61	46.50
n = 8	20	3.6	400	12.96	72.00
	25	5.3	625	28.09	132.50
	30	5.8	900	33.64	174.00
	35	7.4	1225	54.76	259.00
	40	7.7	1600	59.29	308.00
$\sum$ =	180	36.0	5100	203.40	1017.00

x	y
5	1.2
10	1.9
15	3.1
20	3.6
25	5.3
30	5.8
35	7.4
40	7.7

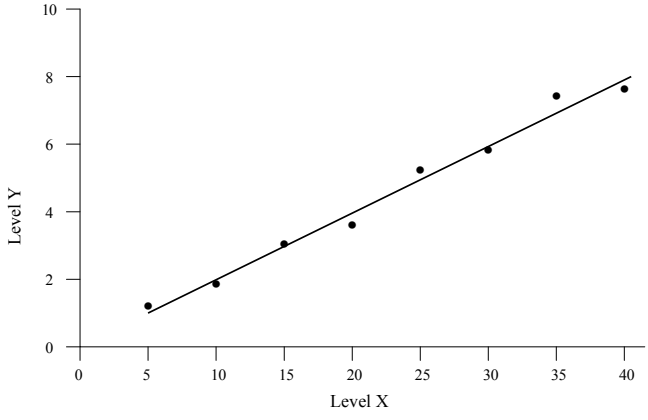


Figure 14.2 Regression line for two continuous variables.

$$b = \frac{8(1017) - (180)(36)}{8(5100) - (180)^2} = \frac{8136 - 6480}{40800 - 32400} = +0.1971$$

$$a = \frac{36 - 0.1971(180)}{8} = \frac{36 - 35.478}{8} = 0.06725$$

Based on these data, the regression line is presented in Figure 14.2, where the slope is in a positive direction +0.197 (as values of x increase, values of y will also increase) and the intercept is slightly above zero (0.067).

A quick check of the position of the regression line on the scatter diagram would be to calculate the means for both variables ($\overline{X}_x$, $\overline{X}_y$) and see if the line passes through this point. This can be checked by placing the slope, y-intercept, and $\overline{X}_x$ in the straight line equation and then determining if $\overline{X}_y$ equals the y-value for the mean on that axis. In this example, the mean for the abscissa is:

$$\overline{X}_x = \frac{\sum x}{n} = \frac{180}{8} = 22.5$$

The mean for the ordinate is:

$$\overline{X}_y = \frac{\sum y}{n} = \frac{36}{8} = 4.5$$

and the y-value for the mean of x is the same as the mean of y:

$$y = a + bx = 0.06725 + 0.1971(22.5) = 4.502 \approx 4.5$$

If there is a linear relationship (a statistical procedure will be presented later to prove that a straight line can fit the data), then it is possible to determine any point on the *y*-axis for a given point on the *x*-axis using the formula for a line (Eq. 13.6). Mechanically we could draw a vertical line up from any point on the *x*-axis; where it intercepts our regression line we draw a horizontal line to the *y*-axis and read the value at that point. Mathematically we can accomplish the same result using the formula for a straight line. For example, based on the regression line calculated above, if x = 32 mcg of the drug is administered, the corresponding physiological response for the *y*-value would be:

$$y = a + bx = 0.06725 + (0.1971)(32) = 0.06725 + 6.3072 = 6.3744$$

If instead the *x*-value is 8 mcg, the expected *y*-value physiological response would be:

$$y = a + bx = 0.06725 + (0.1971)(8) = 0.06725 + 1.5768 = 1.6441$$

Note that both of these results are approximations. As will be discussed later, if we can establish a straight line relationship between the *x*- and *y*-variables, the slope of the line of best fit will itself vary due to random error. Our estimate of the population slope (β) will be based on our best guess, b, plus or minus an amount of uncertainty. This will in fact create a confidence interval around any point on our regression line and provide a range of possible *y*-values. However, for the present time the use of the straight line equation provides us with a quick estimate of the corresponding *y*-value for any given *x*-value. Conversely, for any given value on the *y*-axis it is possible to estimate a corresponding *x*-value using an algebraic modification of the previous formula for a straight line:

$$x = \frac{y - a}{b} \qquad \text{Eq. 14.3}$$

If one wishes to determine the corresponding *x*-value for a physiological response of 5.0, the calculation for the approximate dose of drug would be:

$$x = \frac{y - a}{b} = \frac{5.0 - 0.06725}{0.1971} = 25.0266 \text{ mcg} \approx 25 \text{ mcg}$$

A method for calculating whether a relationship between two variables is in fact linear will be discussed subsequently. Many of the relationships that are encountered in research are linear, and those that are not can often be made linear with appropriate data transformation techniques. For example, if a scatter diagram shows that a nonlinear pattern may exist, it is possible to produce a linear pattern by doing a transformation on one of the variables.

Coefficient of Determination

As the spread of the scatter dots along the vertical axis (*y*-axis) decreases, the

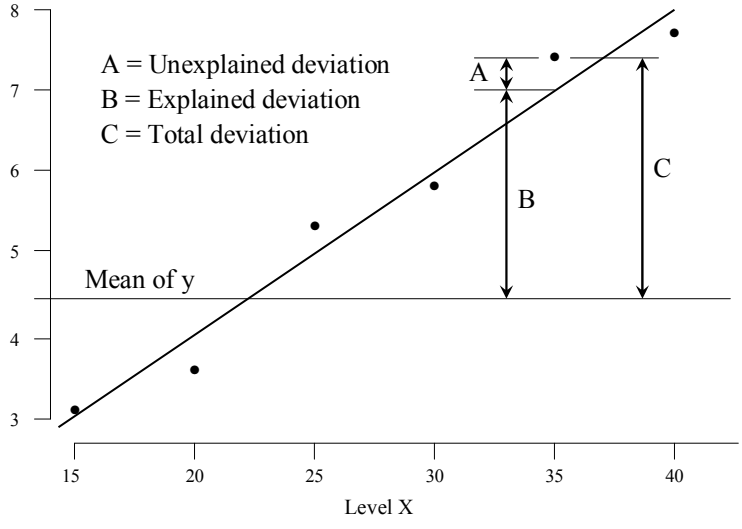


Figure 14.3 Variability of data points around the mean of the y-variable and the regression line.

precision of the estimated μ_y increases. A perfect (100%) estimate is possible only when all the dots (data points) lie on the straight regression line. The **coefficient of determination** offers one method to evaluate if the linear regression equation adequately describes the type of relationship. It compares the scatter of data points about the regression line with the scatter about the mean for the sample values of the dependent *y*-variable. Figure 14.3 shows a scattering of points about both the mean of the *y*-distribution ($\overline{X}_y$) and the regression line itself for part of the data presented in Figure 14.2. As discussed in Chapter 6, in normally distributed data we expect to see data vary around the mean, in this case $\overline{X}_y$. Also, it is possible to measure the deviation of each point (y_i) from the mean on the *y*-axis (labeled "*C*" in Figure 14.3). If there were no linear relationship between the *x*- and *y*-variables, we would expect a random distribution of points around the mean on the *y*-axis. However, if the data is truly represented by the straight regression line, then a certain amount of this total variation can be explained by the deviation from the mean to the line (*B*). The point on the straight line is labeled y_c. However, most data points will not fall exactly on the regression line and this deviation (*A*) must be caused by other source(s) (random error).

The coefficient of determination is calculated using the sum of the squared deviations that takes into consideration these deviations (*A*, *B*, and *C*). In this case the total deviation equals the explained deviations (defined by the line) plus the unexplained deviations:

$$\Sigma(y_i - \overline{X}_y)^2 = \Sigma(y_c - \overline{X}_y)^2 + \Sigma(y_i - y_c)^2 \qquad \text{Eq. 14.4}$$

Table 14.2 Residuals for Data Points from the Regression Line

X	y	y_c	Residual
5	1.2	1.0525	–0.1475
10	1.9	2.0375	+0.1375
15	3.1	3.0225	–0.0775
20	3.6	4.0075	+0.4075
25	5.3	4.9925	–0.3075
30	5.8	5.9925	+0.1775
35	7.4	6.9625	–0.4327
40	7.7	7.9475	+0.2475
		$\sum =$	0

where the total deviation is the vertical difference between the observed data points and the mean for the *y*-axis ($y_i - \overline{X}_y$). The explained deviation is the vertical difference between the points on the regression line and the mean for the *y*-axis ($y_c - \overline{X}_y$). The unexplained deviation is the vertical difference between the observed data points and their corresponding points on the regression line ($y_c - y_i$). These vertical distances between the data points and the regression line are called **residuals**. The residuals for this example are presented in Table 14.2. With the line of best fit between the data points, the sum of the residuals should equal zero, an equal amount of deviation above and below the line. Thus, the best fit line is the line that results in the smallest value for the sum of the squared deviations, $\Sigma(y_c - y_i)^2$. This term is referred to as the **residual sum of squares** or **error sum of squares**.

The computations presented in Eq. 14.4 can be long and cumbersome; involving the calculation of the mean of the *y*-values ($\overline{X}_y$), the *y*-value on the regression line (y_c) for each level of the independent *x*-value, various differences between those values, and then summation of the various differences. A more manageable set of formulas uses the sums computed in Table 14.1 to calculate the sum of squares due to linear regression:

$$SS_{Total} = SS_{Explained} + SS_{Unexplained} \qquad \text{Eq. 14.5}$$

These will produce the same results as the more time-consuming formula in Eq. 14.4. The sum of the total variation between the mean ($\overline{X}_y$) and each observed data point (y_i) would be the total sum of squares (SS_{total}) and can be more simply calculated using the tabular data in Table 14.1:

$$SS_T = \sum_{j=1}^{J} (y_i - \bar{y})^2 = \sum y^2 - \frac{(\sum y)^2}{n} \qquad \text{Eq. 14.6}$$

The variability explained by the regression line of the deviations between the mean

($\overline{X}_y$) and the line (y_c) taking into consideration the slope of the line is the explained sum of squares ($SS_{explained}$):

$$SS_E = \sum_{j=1}^{J} (y_c - \bar{y})^2 = b^2 \cdot \left[\sum x^2 - \frac{(\sum x)^2}{n} \right] \qquad \text{Eq. 14.7}$$

The remaining, unexplained deviation between the regression line (y_c) and the data points (y_i) is the unexplained sum of squares ($SS_{unexplained}$). This residual measure can be computed by subtracting the explained variability from the total dispersion:

$$SS_{unexplained} = SS_{total} - SS_{explained} \qquad \text{Eq. 14.8}$$

Calculation for these sums of squares for the previous example (Table 14.1) would be:

$$SS_{total} = \sum y^2 - \frac{(\sum y)^2}{n} = 203.4 - \frac{(36)^2}{8} = 41.4$$

$$SS_{explained} = b^2 \cdot \left[\sum x^2 - \frac{(\sum x)^2}{n} \right] = (0.1971)^2 \left[5100 - \frac{(180)^2}{8} \right] = 40.79$$

$$SS_{unexplained} = SS_{total} - SS_{explained} = 41.4 - 40.79 = 0.61$$

The sum of squares due to linear regression (**sum of squares regression**) is synonymous with the explained sum of squares and measures the total variability of the observed values that are associated with the linear relationship. The **coefficient of determination** (r^2) is the proportion of variability accounted for by the sum of squares due to linear regression.

$$r^2 = \frac{SS_{explained}}{SS_{total}} = \frac{b^2 \cdot \left[\sum x^2 - \frac{(\sum x)^2}{n} \right]}{\sum y^2 - \frac{(\sum y)^2}{n}} \qquad \text{Eq. 14.9}$$

In our previous example the coefficient of determination would be:

$$r^2 = \frac{(0.1971)^2 \cdot \left[5100 - \frac{(180)^2}{8} \right]}{203.4 - \frac{(36)^2}{8}} = \frac{40.79}{41.4} = 0.9853$$

The coefficient of determination measures the exactness of fit of the regression equation to the observed values for y. In other words, the coefficient of determination identifies how much variation in the dependent variable can be explained by variations in the independent variable. The rest of the variability ($1 - r^2$) is explained by other factors, most likely unidentifiable, random error unknown to the researcher (**coefficient of nondetermination**). In our example the computed r^2 is 0.9853; this indicates that approximately 98.5% of the total variation on the y-axis is explained by the linear regression model. As the r^2 becomes large, the regression equation accounts for a greater proportion of the total variability in the observed values. The coefficient of nondetermination ($1 - 0.9853$) represents a random error of approximately 1.15% in this example.

Similar to the correlation coefficient, the coefficient of determination is a measure of how closely the observations fall on a straight line. In fact, the square root of the coefficient of determination is the correlation coefficient:

$$r = \sqrt{r^2} \qquad \text{Eq. 14.10}$$

In this example the correlation coefficient is the square root of 0.9853 or 0.993. As proof of this relationship the correlation coefficient is calculated using Eq. 13.4 and the data in Table 14.1:

$$r = \frac{8(1017) - (180)(36)}{\sqrt{8(5100) - (180)^2}\sqrt{8(203.4) - (36)^2}} = \frac{1656}{1667.957} = 0.993$$

This linear correlation (correlation coefficient) can be strongly influenced by a few extreme values. One rule of thumb is to first plot the data points on graph paper and examine the points visually before reporting the linear correlation. An opposite approach would be to consider the correlation coefficient as a measure of the extent of linear correlation. If all the data points fall exactly on a straight line, the two variables would be considered to be perfectly correlated ($r = +1.00$ or -1.00). Remember that the correlation coefficient measures the strength of the relationship between two continuous variables and is not associated with the drawing of a straight line.

Sometimes termed the **common variance**, r^2 represents that proportion of variance in the response (dependent) variable that is accounted for by variance in the predictor (independent) variable. As the coefficient of determination approaches 1.0 we are able to account for more of the variation in the dependent variable with values predicted from the regression equation. Obviously, the amount of error associated with the prediction of the response variable from the predictor variable will decrease as the degree of correlation between the two variables increases. Therefore, the r^2 is a useful measure when predicting value for one variable from a second variable.

Some computer software packages (including Excel and Minitab) list an **adjusted r^2** along with the normal coefficient of determination when providing output for linear regression. The r^2 calculated previously is an estimate of the population coefficient of determination, R^2. Expressed as a percentage, the r^2 can be modified from Eq. 14.9 to be expressed as follows:

$$r^2 = \frac{SS_{explained}}{SS_{total}} \times 100\% \qquad \text{Eq. 14.11}$$

or it can be rewritten in terms of the unexplained sum of squares:

$$r^2 = 1 - \frac{SS_{unexplained}}{SS_{total}} \times 100\% \qquad \text{Eq. 14.12}$$

Both equations will give the same results. The adjusted coefficient of determination provides an approximate unbiased estimate of the population R^2. The formula is as follows:

$$Adj.R^2 = 1 - \frac{\frac{SS_{unexplained}}{n-p}}{\frac{SS_{total}}{n-1}} \qquad \text{Eq. 14.13}$$

where p is the number of variables involved in the evaluation (in simple linear regression $p = 2$). As will be seen in for multiple regression models, as the number of independent (predictor) variables increases, the p value will increase. For our previous example with the data from Table 14.1, the r^2 was 98.5%. The adjusted R^2 is:

$$Adj.R^2 = 1 - \frac{\frac{0.61}{8-2}}{\frac{41.4}{8-1}} = 1 - \frac{0.1016}{5.9143} = 0.983$$

This adjusted r^2 could also be multiplied and expressed as a percent.

ANOVA Table

Once we have established that there is a strong positive or negative relationship between the two continuous variables, we can establish the type of relationship (linear, curvilinear, etc.). This final decision on the acceptability of the linear regression model is based on an objective ANOVA test where a statistical test will determine whether the data is best represented by a straight line:

H_0: X and Y are not linearly related
H_1: X and Y are linearly related

In this case the ANOVA statistic is:

$$F = \frac{Mean\ Square\ Linear\ Regression}{Mean\ Square\ Residual} \qquad \text{Eq. 14.14}$$

Source of Variation	df	SS	MS	F
Linear Regression	1	Explained	$SS_{Explained}/1$	$MS_{Explained}/$
Residual	n – 2	Unexplained	$SS_{Unexplained}/n - 2$	$MS_{Unexplained}$
Total	n – 1	Total		

Figure 14.4 ANOVA table for linear regression.

where the amount of variability explained by the regression line is placed in the numerator and the unexplained residual (or error) variability is the denominator. Obviously as the amount of explained variability increases the *F*-value will increase and it becomes more likely that the result will be a rejection of the null hypothesis in favor of the alternative that a straight line relationship exists. The decision rule is

$$\text{with } \alpha = 0.05, \text{ reject } H_0 \text{ if } F > F_{1,n-2}(1 - \alpha)$$

The numerator degrees of freedom is one for the regression line, since the regression line is an estimate of two parameters (α and β) and degrees of freedom are the number of parameters minus one ($df = 2 - 1$). The denominator degrees of freedom is $n - 2$, where n equals the number of data points. The first page for Table B7 in Appendix B contains the critical values for one as the numerator degrees of freedom and a larger finite set of denominator degrees of freedom. Similar to the one-way ANOVA, the computed *F* is compared with the critical *F*-value in Table B7, and if it is greater than the critical value, the null hypothesis that no linear relationship exists between *x* and *y* is rejected. The ANOVA table is calculated as presented in Figure 14.4.

As an example of linear regression, assume that 12 healthy male volunteers received a single dose of various strengths of an experimental anticoagulant. As the primary investigators, we wish to determine if there is a significant relationship between the dosage and corresponding prothrombin times. In this case the independent variable is the dosage of the drug administered to the volunteers and the dependent variable, their responses are measured by their prothrombin times. Results

Table 14.3 Prothrombin Times for Volunteers Receiving Various Doses of an Anticoagulant

Subject	Dose (mg)	Prothrombin Time (seconds)	Subject	Dose (mg)	Prothrombin Time (seconds)
1	200	20	7	220	19
2	180	18	8	175	17
3	225	20	9	215	20
4	205	19	10	185	19
5	190	19	11	210	19
6	195	18	12	230	20

Table 14.4 Summations of Data Required for Linear Regression

Subject	Dose (mg)	Time (seconds)	x^2	y^2	xy
8	175	17	30625	289	2975
2	180	18	32400	324	3240
10	185	19	34225	361	3515
5	190	19	36100	361	3610
6	195	18	38025	324	3510
1	200	20	40000	400	4000
4	205	19	42025	361	3895
11	210	19	44100	361	3990
9	215	20	46225	400	4300
7	220	19	48400	361	4180
3	225	20	50625	400	4500
12	230	20	52900	400	4600
$\sum$ =	2430	228	495650	4342	46315

of the study are presented in Table 14.3. The hypotheses in this case are:

H_0: Dose (x) and prothrombin time (y) are not linearly related
H_1: Dose and prothrombin time are linearly related

and the decision rule with $\alpha = 0.05$, is to reject H_0 if $F > F_{1,10}(0.95)$, which is 4.96 (Table B7, Appendix B). The tabular arrangement of the data needed to calculate an ANOVA table is presented in Table 14.4. The slope and y-intercept for the regression line would be:

$$b = \frac{n\sum xy - (\sum x)(\sum y)}{n\sum x^2 - (\sum x)^2} = \frac{12(46315) - (2430)(228)}{12(495650) - (2430)^2} = 0.0406$$

$$a = \frac{\sum y - b\sum x}{n} = \frac{228 - (0.0406)(2430)}{12} = 10.7785$$

In this case there would a gradual positive slope to the line (as the dosage increases, the prothrombin time increases) and the predicted y-intercept would be 10.78 seconds. The total variability around the mean prothrombin time is:

$$SS_T = \sum y^2 - \frac{(\sum y)^2}{n} = 4342 - \frac{(228)^2}{12} = 10.0$$

of which the regression line explains a certain amount of variation:

$$SS_E = b^2 \cdot \left[\sum x^2 - \frac{(\sum x)^2}{n} \right] = (0.0406)^2 \left[495650 - \frac{(2430)^2}{12} \right] = 5.8811$$

However, an additional amount of variation remains unexplained:

$$SS_U = SS_T - SS_E = 10.0 - 5.8811 = 4.1189$$

For this particular example the coefficient of determination is:

$$r^2 = \frac{SS_{explained}}{SS_{total}} = \frac{5.8811}{10} = 0.5881$$

meaning that only approximately 59% of the total variability is explained by the straight line that we drew among the data points. The adjusted R^2 would be

$$Adj.R^2 = 1 - \frac{\frac{SS_{unexplained}}{n-p}}{\frac{SS_{total}}{n-1}} = 1 - \frac{\frac{4.1189}{12-2}}{\frac{10.00}{11}} = 1 - \frac{0.4119}{0.9091} = 0.5469$$

and the ANOVA table would be:

Source	SS	df	MS	F
Linear Regression	5.8811	1	5.8811	14.278
Residual	4.1189	10	0.4119	
Total	10.00	11		

The resultant *F*-value is greater than the critical value of 4.96; therefore we would reject H_0 and conclude that a linear relationship exists between the dosage of the new anticoagulant and the volunteers' prothrombin times.

Once the type of relationship is established, it is possible to predict values for the dependent variable (prothrombin time) based on the corresponding value for the independent variable (dose). Obviously, the accuracy of any prediction, based on a regression line, depends on the strength of the relationship between the two variables (the higher coefficient of determination the better our predictive abilities). Use of the regression analysis enables the researcher to determine the nature (e.g., linear) and strength of the relationship, and allows for predictions to be made.

It is important to realize that the linear regression line, which fits best between our data points, cannot be extrapolated beyond the largest or smallest point for our observations (to predict y_c values for x_i values outside the observed range of x_i). For example, in our previous example we identified a linear relationship between the dose of the experimental anticoagulant and volunteer prothrombin times. This linear relationship is illustrated by the solid line in Figure 14.5. What we do not know is what will happen beyond 230 mg, the highest dose. Could a linear relationship

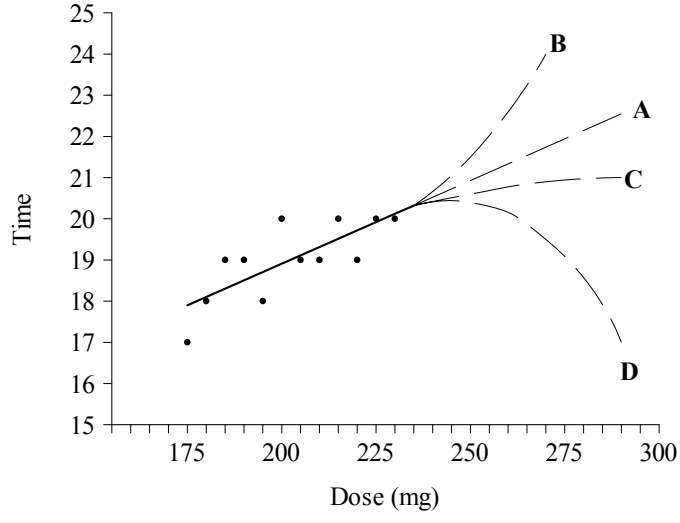


Figure 14.5 Example of the problems associated with extrapolation.

continue (*A*), might there be an acceleration in the anticoagulant effect (*B*), a leveling of response (*C*) or an actual decrease in prothromin time with increased doses (*D*)? Correspondingly, we do not know what the relationship is for responses at dosages less than 175 mg of the experimental anticoagulant. If more data were available beyond the last data point, it might be found that the regression line would level out or decrease sharply. Therefore, the regression line and the regression equation apply only within the range of the *x*-values actually observed in the sample data.

Confidence Intervals and Hypothesis Testing for the Population Slope (β)

With linear regression we are dealing with sample data and the only way to accurately determine the population parameters of slope (β) and intercept (α) would be to collect all the data for the entire population. Since in most cases this would be impossible, we have to estimate these parameters using our sample data and our best estimates, the sample slope (*b*) and sample intercept (*a*).

The correlation coefficient (*r*) and slope of the line (*b*) are descriptive statistics that define different aspects of the relationship between two continuous variables. When either *r* or *b* equals zero, there is no linear correlation and variables *x* and *y* can be considered independent of each other, and no mutual interdependence exists. An alternative test to our previously discussed ANOVA test for linearity is a null hypothesis that no linear relationship exists between the two variables. This is based on the population slope (β) of the regression line. In general, a positive β indicates that *y* increases as *x* increases, and represents a direct linear relationship between the two variables. Conversely, a negative β indicates that values of *y* tend to increase as values of *x* decrease, and an inverse linear relationship between *x* and y exists.

The hypothesis under test assumes that there is no slope; therefore, a relationship

between the variables does not exist:

$$H_0: \beta = 0$$
$$H_1: \beta \neq 0$$

In this case we can either: 1) calculate a *t*-value and compare it to a critical value or 2) compute a confidence interval for all possible slopes for the population (β) to determine if $\beta = 0$ is a possible outcome. The calculation of the *t*-value is similar to a paired t-test with an observed difference in the numerator and an error term in the denominator:

$$t = \frac{b - \beta_0}{S_b} \qquad \text{Eq. 14.15}$$

Based on the null hypothesis, β_0 is an expected outcome of zero or no slope and S_b is an error term that is defined below.

Calculation of the error term involves variability about the regression line. The variation in the individual y_i values about the regression line can be estimated by measuring their variation from the regression line for the sample data. The standard deviation for these observed y_i values is termed the **standard error of the estimate** ($S_{y/x}$) and is calculated as follows:

$$S_{y/x} = \sqrt{\frac{\sum (y_i - y_c)^2}{n-2}} = \sqrt{MS_{residual}} \qquad \text{Eq. 14.16}$$

where the numerator is $SS_{unexplained}$ and the denominator represents the degrees of freedom associated with the unexplained error. Thus, the standard error of estimate equals the square root of the mean square residual from the ANOVA table. If there is no relationship between the two continuous variables, the slope of the regression equation should be zero. The value $S_{y/x}$ is also referred to as the **residual standard deviation**. In this case the residual standard deviation would be

$$S_{y/x} = \sqrt{MS_{residual}} = \sqrt{0.4119} = 0.642$$

The standard error of the estimate could be used as a measure of precision for the regression line to predict a dependent variable (y_i) for a given independent value (x_i):

$$y = a + bx \pm S_{y/x} \qquad \text{Eq. 14.17}$$

The magnitude of $S_{y/x}$ is proportional to the magnitude of the *y*-variable and a poor method for comparing different regressions. To standardize this error term, it has been recommended that the standard error of the estimate be divided by the mean of the *y*-axis (Dapson, 1980, p. 545). This creates a relative standard deviation for the regression line:

$$RSD_{regression} = \frac{S_{y/x}}{\overline{X}_y} \times 100\% \qquad \text{Eq. 14.18}$$

For this particular example, the mean on the *y*-axis is 19 (Σy/n = 228/12). Therefore, the RSD for the regression line would be:

$$RSD_{regression} = \frac{\sqrt{0.4119}}{19} \times 100\% = 3.38\%$$

To test the null hypothesis H_0: $\beta = 0$, we need to calculate a standard error of the sample slope (*b*), which is our estimate of population slope (β):

$$S_b = \frac{S_{y/x}}{\sqrt{\Sigma(x_i - \overline{X})^2}} \qquad \text{Eq. 14.19}$$

where the sum of the deviations on the *x*-axis is:

$$\Sigma(x_i - \overline{X})^2 = \Sigma x^2 - \frac{(\Sigma x)^2}{n} \qquad \text{Eq. 14.20}$$

from data collected in tables such as Table 14.4 and the mean square residual from the ANOVA table. The formula can be simplified to:

$$S_b = \sqrt{\frac{MS_{residual}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}} \qquad \text{Eq. 14.21}$$

The decision rule is to reject H_0 (no slope) if *t* in Eq. 14.15 is greater than $t_{n-2}(1 - \alpha/2)$ or less than $-t_{n-2}(1 - \alpha/2)$ as previously used for a two-tailed test. With regression, we are dealing with sample data that provides the information for the calculation for an intercept (*a*) and slope (*b*), which are estimates of the true population α and β. Because they are samples, they are subject to random error similar to previously discussed sample statistics. The number of degrees of freedom is $n - 2$. The number two subtracted from the sample size represents the two approximations in our data: 1) the sample slope as an estimate of β and 2) the sample *y*-axis intercept as an estimate for α.

As noted, a second parallel approach would be to calculate a confidence interval for the possible slopes for the population:

$$\beta = b \pm t_{n-2}(1-\alpha/2) \cdot S_b \qquad \text{Eq. 14.22}$$

In this case the sample slope (*b*) is the best estimate of the population slope (β) defined in Eq. 14.1:

$$\mu_{y/x} = \alpha + \beta x$$

By creating a confidence interval we can estimate, with 95% confidence, the true population slope (β). As with previous confidence intervals, if zero falls within the confidence interval the result of no slope is a possible outcome; therefore, one fails to reject the null hypothesis and must assume there is no slope in the true population and thus no relationship between the two continuous variables.

Using our example of the 12 healthy male volunteers who received a single dose of various strengths of an experimental anticoagulant (Tables 14.3 and 14.4), one could determine a significant relationship between the dosage and the corresponding prothrombin time exist by determining a slope to the regression line for the population based on sample data. Once again, the null hypothesis states that there is no slope in the population:

$$H_0: \ \beta = 0$$
$$H_1: \ \beta \neq 0$$

The decision rule is, with $\alpha = 0.05$, reject H_0 if $|t| > t_{10}(1 - \alpha/2)$ which equals 2.228 (Table B5, Appendix B). The calculation of S_b is:

$$S_b = \sqrt{\frac{MS_{residual}}{\sum x^2 - \frac{(\sum x)^2}{n}}} = \sqrt{\frac{0.4119}{4956550 - \frac{(2430)^2}{12}}} = \sqrt{\frac{0.4119}{3575}} = 0.0107$$

and the calculation of the *t*-statistics is:

$$t = \frac{b - 0}{S_b} = \frac{0.0406 - 0}{0.0107} = 3.794$$

The decision in this case is, with $t > 2.228$, to reject H_0 and conclude that there is a slope and thus a relationship exists between dosage and prothrombin times. Note that the results are identical to those seen in the ANOVA test. In fact, the square of the *t*-statistic equals our previous *F*-value ($3.79^2 \approx 14.27$, with rounding errors).

A possibly more valuable piece of information is obtained by calculating the 95% confidence interval that estimates the true population slope (Eq. 14.22):

$$\beta = b \pm t_{n-2}(1 - \alpha/2) \cdot S_b$$

For our example the estimate on β would be:

$$\beta = 0.0406 \pm 2.228(0.0107) = 0.0406 \pm 0.0238$$

$$0.0168 < \beta < 0.0644$$

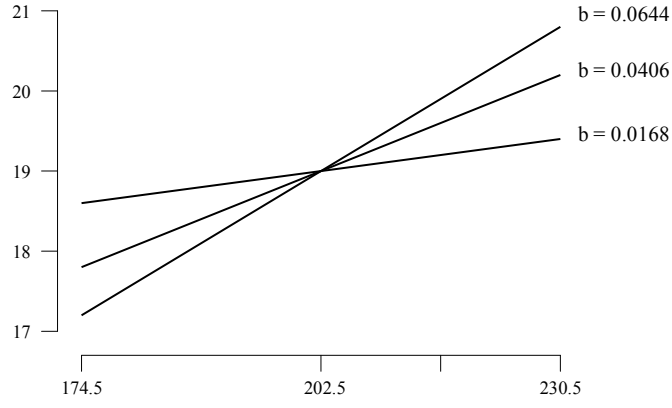


Figure 14.6 Range of possible population slopes (β).

Since zero does not fall within the confidence interval, $\beta = 0$ is not a possible outcome; therefore H_0 is rejected once again and the researcher concludes that a relationship exists between the two variables. It is possible to predict, with 95% confidence, that the true population slope is somewhere between +0.0168 and +0.0644. Figure 14.6 illustrates that even though our sample data provides us with an estimate of the slope (b = +0.04), the true slope for the population could range from +0.0168 to +0.0644 around the mean on the x-axis.

$$\overline{X} = \frac{\sum x}{n} = \frac{2430}{12} = 202.5$$

If the null hypothesis is rejected in favor of the alternate hypothesis that $\beta \neq 0$, then higher values of x would correspond with higher predicted values of y. In this case, there would be a positive correlation.

As mentioned previously, the population slope (β) is sometimes referred to as the population regression coefficient. An alternative formula for calculating the slope is:

$$b = r \cdot \frac{S_y}{S_x} \qquad \text{Eq. 14.23}$$

where r is our correlation coefficient and the standard deviations for each variable are represented by standard deviations for each axis (S_x and S_y). In the above example of prothrombin times, the standard deviation of the x-variable (dosage) is 18.0278, the standard deviation for the y-variable (prothrobin time) is 0.9535 and the correlation coefficient is 0.7676 (square root of r^2 = 0.5893).

$$b = (0.7676)\frac{0.9535}{18.0278} = 0.0406$$

This result is identical to our previous calculation for the slope of the line.

By testing the significance associated with the slope of the regression line we can be certain that the observed linear equation did not represent simply a chance departure from a horizontal line when there was no relationship between the two continuous variables. However, using a t-test to determine the significance of the relationship, we make additional assumptions that the *y*-values at different levels of *x* have equal variances and that their distributions are normal in shape.

Confidence Intervals and Hypothesis Testing for the Population Intercept (α)

Because the intercept (a) represents only a sample, it is possible to estimate the population intercept and test if it is significantly different form zero. The calculations involve the residual standard deviation ($S_{y/x}$) and follow similar procedures to those discussed in the previous section. The **standard error for the intercept** is calculated as follows:

$$S_a = S_{y/x}\sqrt{\frac{\sum x^2}{n\left(\sum x^2 - \frac{\left(\sum x\right)^2}{n}\right)}} \qquad \text{Eq. 14.24}$$

This value can be used to calculate either a *t*-statistic confidence interval:

$$t = \frac{a}{S_a} \qquad \text{Eq. 14.25}$$

$$\alpha = a \pm t_{n-2}(1-\alpha/2)\cdot S_a \qquad \text{Eq. 14.26}$$

In the previous example the *Sy/x* was 0.642 and therefore the standard error for the intercept would be

$$S_a = 0.642\sqrt{\frac{495650}{12\left(495650 - \frac{(2430)^2}{12}\right)}} = 0.642\cdot\sqrt{11.55} = 2.181$$

The calculation for the *t*-statistic and the hypothesis H_0: α = 0 would be:

$$t = \frac{a}{S_a} = \frac{10.779}{2.181} = 4.942$$

The result would be a significant difference ($p < 0.001$) and rejection of the hypothesis that α = 0. The confidence interval would be:

$$\alpha = 10.779 \pm 2.228(2.181) = 10.779 \pm 4.859$$

$$5.920 < \alpha < 15.648$$

With 95% confidence the true value for the prothrombin time at $x = 0$ is somewhere between 5.92 and 15.64 seconds.

Confidence Intervals for the Regression Line

The difference between the observed value and predicted value on our regression line ($y_i - \overline{X}_y$) is our best estimate of the variation of the y population around the true regression line. The variance term $S_{y/x}^2$, or the mean square residual, is an estimate of the variance of the $\overline{Y}$ population about the true population regression line. The **standard deviation about regression** is a third synonym for $S_{y/x}$ and is more meaningful than the variance term and signifies the standard deviation of y at a given x-value.

As discussed previously, for a given value on the x-axis it is possible to estimate a corresponding y-value using $y = a + bx$. Also, because we assume data is normally distributed on the y-axis for any point on the x-axis, a confidence interval for the expected y-value can be computed using a modification of the formula for the hypothesis test of the slope.

$$y = y_c \pm t_{n-2}(1-\alpha/2) \cdot \sqrt{MS_{residual}} \cdot \sqrt{\frac{1}{n} + \frac{(x_i - \overline{X})^2}{\sum x^2 - \frac{(\sum x)^2}{n}}} \qquad \text{Eq. 14.27}$$

where the $MS_{residual}$ is the mean square residual from the ANOVA table in the original regression analysis. Assuming that each point on the regression line (y_c) gives the best representation (mean) of the distribution of scores, it is possible to estimate the mean of y for any point on the x-axis.

In calculating the 95% confidence interval around the regression line, it is assumed that data are approximately normally distributed in the vertical direction along the y-axis (this may require transformation of the data before any of the previous calculations). If we have a large sample size, we would expect that approximately 95% of our prediction errors fall within ± 1.96 $S_{y/x}$. The errors in predicting y for a given value of x are due to several factors. Obviously, there is random variation of y about the true regression line that is expressed as $S_{y/x}$. In addition there is an error in estimating the y-axis intercept of the true regression line (α) and an error in estimating the slope of the true regression line (β). Because of the error due to estimating the slope of the line, the error in the estimate of the slope will become more pronounced for values of the independent variable (x_i) as those values deviate more from the center (the mean x-value, $\overline{X}$). This produces a bowing of the **confidence bands** as seen in Figure 14.7. The point at which the deviation is least, or where the confidence interval is the smallest, is at the mean for the observed x-values.

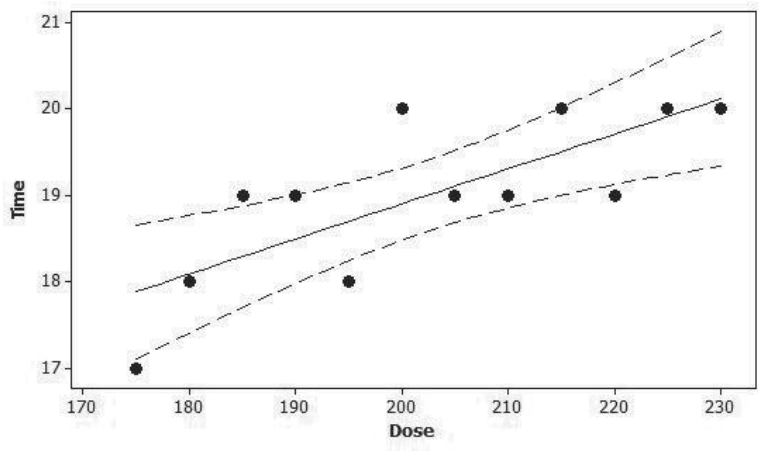


Figure 14.7 Graphic illustration of 95% confidence intervals.

This would seem logical since we expect less error as one moves to the middle of the distribution for *x*-values and we expect a larger error as one approaches the extreme areas of the data.

Once again, we will use the previous anticoagulant example to illustrate the determination of confidence intervals. With 95% confidence, what is the expected mean prothrombin time at a dosage of 210 mg of the anticoagulant? Based on the previous data in Table 14.4 we know the following: $\sum x$ = 2,430 and n = 12. The mean for the independent variable is:

$$\overline{X} = \frac{\sum x}{n} = \frac{2430}{12} = 202.5$$

Based on previous calculations the slope (b) is 0.04 and the y-intercept (a) is 10.9. Lastly, from the analysis of variance table, the mean square residual ($S_{x/y}^2$) is 0.4107. Using this data the first step is to calculate the y_c value for each point on the regression line for values of the independent variable (x_i). The y_c would be the best estimate of the center for the interval. For example, the expected value on the regression line at 210 mg would be:

$$y_c = a + bx_i = 10.9 + 0.04(210) = 19.3$$

The calculation of the confidence interval around the regression line at 210 mg of drug would be:

$$\overline{y} = 19.3 \pm 2.228\sqrt{0.4107}\sqrt{\frac{1}{12} + \frac{(210 - 202.5)^2}{495650 - \frac{(2430)^2}{12}}}$$

Table 14.5 95% Confidence Intervals at Selected Dosages

Dose (mg)	Time (seconds)	y_c	Lower Limit	Upper Limit	Range
175	17	17.9	17.10	18.70	1.60
180	18	18.1	17.40	18.80	1.40
190	19	18.5	17.98	19.02	1.04
200	20	18.9	18.47	19.33	0.86
210	19	19.3	18.85	19.75	0.90
220	19	19.7	19.10	20.30	1.20
230	20	20.1	19.31	20.89	1.58

$$\bar{y} = 19.3 \pm 2.228(0.6409)\sqrt{0.0833 + \frac{56.25}{3575}} = 19.3 \pm 0.449$$

$$18.851 < \bar{y} < 19.749$$

Thus, based on sample data and the regression line that fits best between the data points, the researcher could conclude with 95% confidence that the true population mean for a dosage of 210 mg would be between 18.85 and 19.75 seconds. Results from the calculation of the confidence intervals at the various levels of drug used are presented in Table 14.5 and graphically represented in Figure 14.7.

Inverse Prediction

As seen in the previous section, the original prediction of y for any given x using the straight line equation is:

$$y = a + bx$$

This offers a quick estimate, but a more exact estimate is the confidence interval (Eq. 14.27) at any point on the independent variable, the x-axis. Similarly, Eq. 14.3 is only a quick estimate of a possible value of the x-axis for any given value of the dependent (y-axis) variable. Following the same logic as the previous calculations (Eq. 14.27), a 95% confidence interval can be created on the x-axis around the value x_i determined by Eq. 14.3.

$$x = x_i + \frac{b(y_i - \bar{y})}{K} \pm \frac{t}{K}\sqrt{MS_{residual}}\sqrt{\frac{(y_i - \bar{y})^2}{\sum x^2 - \frac{(\sum x)^2}{n}} + K\left(1 + \frac{1}{n}\right)} \quad \text{Eq. 14.28}$$

where the intermediate K is based on the slope, the critical t-value and standard deviation about the line S_b:

$$K = b^2 - t^2 S_b^2 \qquad \text{Eq. 14.29}$$

For example, assume we want to predict a dosage that would be required to produce a prothrombin time of 20. In this case the y-value was 195, $\bar{y}$ was 19 (228/12) and the slope was calculated to be 0.04. The t-value remains the same for a 95% confidence interval (2.228) and the S_b was previously calculated. The best estimate of x, with 95% confidence, would be:

$$x = \frac{y - a}{b} = \frac{20 - 10.7785}{0.0406} = 227.13 \text{ mg}$$

The 95% confidence interval around that best estimate would be:

$$K = (0.0406)^2 - (2.228)^2 (0.0107)^2 = 0.00108$$

$$x = 227.13 + \frac{0.0406(20-19)}{0.00108} \pm \frac{2.228}{0.00108}\sqrt{0.4119} \cdot \sqrt{\frac{(20-19)^2}{495650 - \frac{(2430)^2}{12}} + 0.00108\left(1 + \frac{1}{12}\right)}$$

$$x = 264.7226 \pm 1323.9980\sqrt{0.00145} = 264.7226 \pm 50.4163$$

$$214.31 < x < 315.14$$

Thus, with 95% confidence, the true dosage to obtain a prothrombin time of 20 is somewhere between 214.31 and 315.14 mg of drug. This computation is sometimes referred to as **inverse prediction.** Note in the previously worked out example that the confidence interval is asymmetric around the estimated x-value. The inverse prediction interval is symmetrical only at the mean for sample data on the y-axis. The interval becomes more asymmetrical as y-values become more distant from the mean.

Multiple Data at Various Points on the Independent Variable

What if the data represents multiple measures (e.g., duplicate or triplicate assays) at the same points for the independent variable? Obviously, more data at each point on the x-axis will provide a better estimate of the true population values and should create a smaller confidence interval for both the estimate of β and the intervals around the line of least squares. For illustrative purposes, consider Table 14.1 where the results were single data points for each level of the independent variable. Instead, let us assume that each result is instead the mean of a duplicate assay (Scenario A) or

triplicate measure (Scenario B). The calculations would be the same as those originally used for the data in Table 14.1, but now the number of data points will increase from 16 or 24 for duplicate and triplicate measures. There would be a slight modification of Eqs. 14.6 and 14.7 where there are *j*-points on the *x*-axis, but now there are also *i*-possible points at each of these *j*-points.

$$SS_T = \sum_{j=1}^{J}\sum_{i=1}^{I}(y_i - \overline{X}_y)^2 = \sum y^2 - \frac{(\sum y)^2}{n} \qquad \text{Eq. 14.30}$$

$$SS_E = \sum_{j=1}^{J}\sum_{i=1}^{I}(y_c - \overline{X}_y)^2 = b^2 \cdot \left[\sum x^2 - \frac{(\sum x)^2}{n}\right] \qquad \text{Eq. 14.31}$$

The calculation would be basically the same, using ∑x, ∑y, etc., but the degrees of freedom for the residual error and total error would be adjusted for the new larger sample size. Results of the three outcomes are presented in Table 14.6. Due to the larger sample size and more information about the population, there are smaller ranges for the slopes and decreased widths in the confidence bands.

Lack-of-Fit Test

When there is more than one observation at different levels of the independent variable it is possible to evaluate how well the data fit a straight line. The test actually evaluates whether there is a lack-of-fit on the regression line.

H_0: There is no lack of linear fit
H_1: There is lack of linear fit

In the ideal situation each point on the independent (predictor) variable would have an equal number of observations, but the lack-of-fit analysis can be performed even if there is only one point with multiple observations. At the same time, this is one of the limitations of the lack-of-fit test, in that more than one observation is required for at least one level of the independent variable. With multiple observations at different levels of the independent variable, there two types of errors: 1) pure error and 2) error due to a lack of fit. The sum of squares due to error (unexplained variability) is a combination of a sum of squares due to "pure" error and a sum of squares due to lack of fit.

$$SS_U = SS_{PE} + SS_{LOF}$$

The "pure" error is the sum of squared of the differences between each data point and their corresponding average for all values at specific points on the independent variable. This is the variation of each data point around the mean of each given point on the x-axis.

Table 14.6 Results with Duplicate and Triplicate Measures

	Original Data	Scenario A	Scenario B
n	8	16	24
Slope	0.197	0.197	0.197
Intercept	0.064	0.064	0.064
Coefficient of Determination	0.986	0.986	0.986
F (p)	414.00 (9.2×10^{-7})	850.90 (6.1×10^{-14})	1392.43 (2.2×10^{-21})
β	0.1548<<0.2395	0.1711<<0.2232	0.1770<<0.2173
β range	0.085	0.052	0.040
CI at 5	0.554<<1.545	4.228<<4.772	7.454<<8.445
Range at 5	0.991	0.544	0.991
CI at 22.5	0.747<<1.353	4.334<<4.666	7.647<<8.253
Range at 2.5	0.606	0.326	0.606
CI at 40	0.804<<1.296	4.372<<4.628	7.704<<8.196
Range at 40	0.492	0.256	0.492

$$SS_{PE} = \sum_{j=1}^{J}\sum_{i=1}^{I}(y_{ij} - \bar{y}_j)^2 \qquad \text{Eq. 14.32}$$

If the $SS_U = SS_{PE} + SS_{LF}$, the determination of the sum of squares for the lack-of-fit would be

$$SS_{LOF} = SS_U - SS_{PE} \qquad \text{Eq. 14.33}$$

The ANOVA table is expanded to evaluate the lack of fit in the data (Figure 14.8). The degrees of freedom for the lack-of-fit error is expressed as $J - 2$ (the number of levels of the independent variable minus two) and pure error is $N - 2 - (J - 2)$ or $N - J$ (the total number of observations minus the number of levels of the independent variable). The number of degrees of freedom for the pure error and the lack–of–fit will sum up to the residual (unexplained) degrees of freedom. The mean squares are calculated as in the past by dividing the sum of squares by their respective degrees of freedom

Source of Variation	df	SS	MS	F
Linear Regression	1	Explained	$SS_{Explained}/1$	$MS_{Explained}/$
Residual	N – 2	Unexplained	$SS_{Unexplained}/n - 2$	$MS_{Unexplained}$
Lack of Fit	J – 2	LOF	$SS_{LOF}/$	MS_{LOF}/MS_{PE}
Pure Error	N – J	PE	$SS_{PE}/$	
Total	N – 1	Total		

Figure 14.8 ANOVA table for linear regression.

$$MS_{PE} = \frac{SS_{PE}}{N - J} \qquad \text{Eq. 14.34}$$

$$MS_{LOF} = \frac{SS_{LOF}}{J - 2} \qquad \text{Eq. 14.35}$$

To determine if there is a significant lack of fit from the linear model, the mean square for the lack–of–fit is divided by the mean square for the pure error

$$F = \frac{MS_{LOF}}{MS_{PE}} \qquad \text{Eq. 14.36}$$

The critical *F*-value is determined with $J - 2$ numerator degrees of freedom and $N - J$ denominator degrees of freedom and predetermined Type I error. If the resultant *F*-statistic exceeds the critical value or the associated *p*-value less than the Type I error, then the null hypothesis of no lack of linear fit is rejected.

As an example, assume the study in Table 14.1 was repeated with triplicate measures and by changing the mean for each point on the independent variable. The results are seen in Table 14.7. The same calculations are used to determine the initial ANOVA table:

$$SS_{total} = \sum y^2 - \frac{(\sum y)^2}{n} = 610.26 - \frac{(108)^2}{24} = 124.26$$

$$SS_{explained} = b^2 \cdot \left[\sum x^2 - \frac{(\sum x)^2}{n} \right] = (0.1971)^2 \left[15300 - \frac{(540)^2}{24} \right] = 122.42$$

$$SS_{unexplained} = SS_{total} - SS_{explained} = 124.26 - 122.42 = 1.84$$

Table 14.7 Repeat of Study in Table 14.1 with Triplicate Measures

	x	y	x^2	y^2	xy
	5	1.22	25	1.49	6.10
	5	1.24	25	1.54	6.20
	5	1.14	25	1.30	5.70
	10	1.98	100	3.92	19.80
	10	1.87	100	3.50	18.70
	10	1.85	100	3.42	18.50
	15	3.13	225	9.80	46.95
	15	3.10	225	9.61	46.50
	15	3.07	225	9.42	46.05
N = 24	20	3.63	400	13.18	72.60
J = 8	20	3.59	400	12.89	71.80
	20	3.58	400	12.82	71.60
	25	5.36	625	28.73	134.00
	25	5.28	625	27.88	132.00
	25	5.26	625	27.67	131.50
	30	5.88	900	34.57	176.40
	30	5.73	900	32.83	171.90
	30	5.79	900	33.52	173.70
	35	7.50	1225	56.25	262.50
	35	7.38	1225	54.46	258.30
	35	7.32	1225	53.58	256.20
	40	7.62	1600	58.06	304.80
	40	7.73	1600	59.75	309.20
	40	7.75	1600	60.06	310.00
$\sum$ =	540	108.00	15300	610.26	3051.00

The sum of squares for the pure error would be

$$SS_{PE} = \sum(y_{ij} - \bar{y})^2 = (1.22 - 1.2)^2 + (1.24 - 1.2)^2 \ldots + (7.75 - 7.7)^2 = 0.06$$

The sum of squares for the lack of fit would be

$$SS_{LOF} = SS_U - SS_{PE} = 1.84 - 0.06 = 1.77$$

An analysis of the data using Minitab shows identical results (Figure 14.9). The *F*-statistic for the lack of fit would be:

$$F = \frac{MS_{LOF}}{MS_{PE}} = \frac{1.77/6}{0.06/16} = 78.6$$

```
S = 0.288923   R-Sq = 98.5%   R-Sq(adj) = 98.5%

Analysis of Variance

Source          DF      SS      MS        F      P
Regression       1  122.43  122.43  1466.59  0.000
Residual Error  22    1.84    0.08
  Lack of Fit    6    1.77    0.30    76.07  0.000
  Pure Error    16    0.06    0.00
Total           23  124.26

Lack of fit test
Possible curvature in variable Concentr  (P-Value = 0.029)

Overall lack of fit test is significant at P = 0.029
```

Figure 14.9 Minitab results for the data in Table 14.7.

Even though the coefficient of determination (r^2) indicates that the line accounts for 98.5% of the variability on the *y*-axis, the resultant *p*-value is much less than the acceptable Type I error of 0.05. The null hypothesis would be rejected in favor of the alternative that there is a lack of fit with the line best fit.

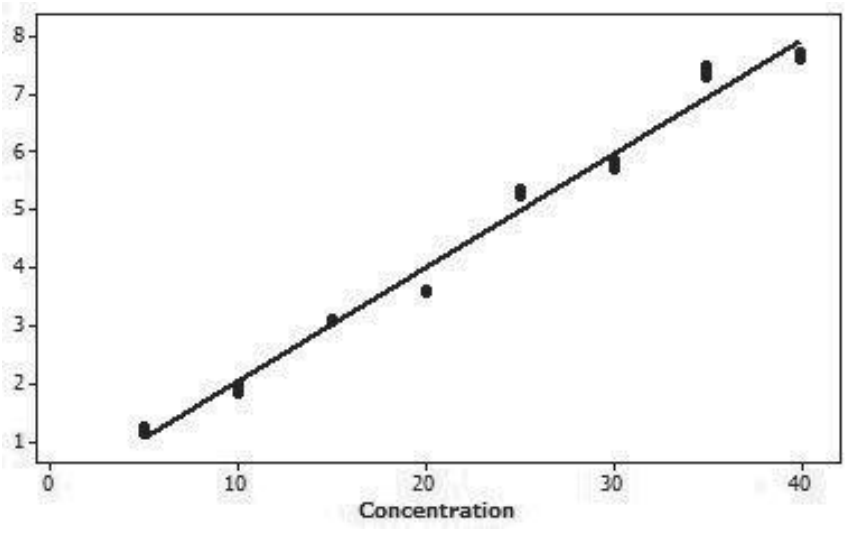
Lack of fit considers both the linear variation of the data as well as the variability (or precision of the data) at each point on the *x*-axis. Tighter numbers (greater precision) can result in a failure to meet the criteria for fit. Figure 14.10 shows the comparison between the data in Table 10.7 (on the left) and more dispersed data on the right. The tighter data fails the lack-of-fit test, but the less precise information successfully fits the linear model.

Assessing Parallelism of the Slopes of Two Samples

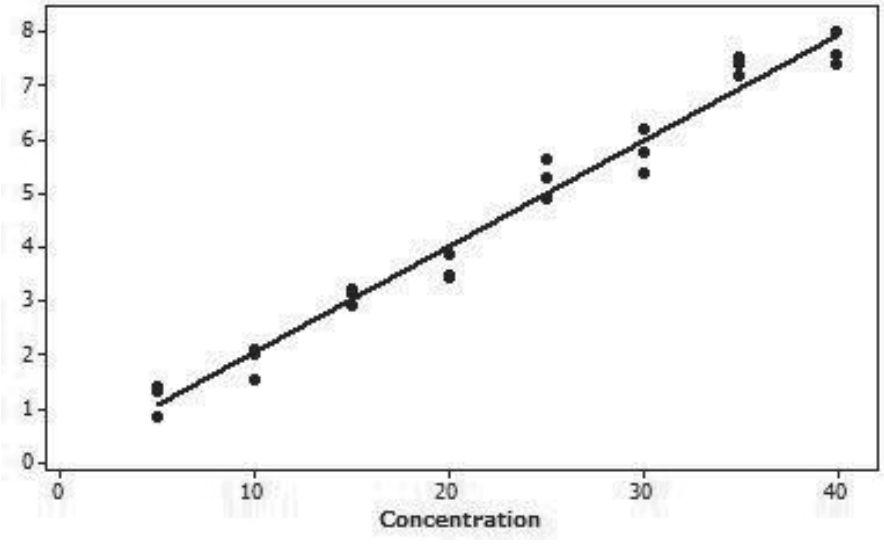
At times the researcher may wish to compare the linear regression lines from two different samples to determine if there are any statistical differences between the two slopes or distance between the lines (e.g., *y*-intercepts are different). When comparing the slopes for two samples to determine if the slopes for their respective populations are the same, the hypotheses tested are as follows:

$$H_0: \beta_1 = \beta_2$$
$$H_1: \beta_1 \neq \beta_2$$

If the null hypothesis is rejected, the two population slopes are different. The best estimate for the population slopes would be the sample slopes, b_1 and b_2. The evaluation of parallelism is handled similarly to the ratio method for dealing with a two-sample t-test (Chapter 9); where the numerator is the best guess of the difference ($b_1 - b_2$) and the denominator contains an error term (as will be seen in Eq. 14.31). The first step is to create a weighted average deviation term, analogous to the pooled



Tight dispersion - Coefficient of determination = 0.985
Lack of fit test - Possible curvature in dependent variable (p-value = 0.029)



Wide dispersion - Coefficient of determination = 0.975
Lack of fit test - No evidence of lack–of–fit ($p \geq 0.1$).

Figure 14.10 Lack-of-fit with different dispersions.

variance in the two-sample t-test (Eq. 9.3). In this case we create what is called a **pooled residual mean square,** a term that represents the addition of the two sum-of-squares residuals (SS_R) for each slope (this involves calculating an ANOVA table for each regression line) divided by their representative degrees of freedom ($n - 2$ for each line):

$$(S^2_{x/y})_p = \frac{SS_{R1} + SS_{R2}}{n_1 + n_2 - 4} \qquad \text{Eq. 14.37}$$

This pooled residual mean square then becomes part of the denominator in the calculation of the *t*-statistic. Notice the similarities between this equation and the two-sample t-test (Eq. 9.6):

$$t = \frac{b_1 - b_2}{\sqrt{\frac{(S^2_{x/y})_p}{\sum x_1^2 - \frac{(\sum x_1)^2}{n_1}} + \frac{(S^2_{x/y})_p}{\sum x_2^2 - \frac{(\sum x_2)^2}{n_2}}}} \qquad \text{Eq. 14.38}$$

Assessment of significance is determined by comparing the calculated *t*-value with the critical value off a t-table (Table B5 in Appendix B) for $1 - \alpha/2$ level of significance and the appropriate degrees of freedom ($n_1 + n_2 - 4$).

A confidence interval can be used also to assess parallelism, by creating the interval and determining if zero falls within the interval. Once again, this interval is analogous to the interval created for the two-sample t-test (Eq. 9.4):

$$\beta_1 - \beta_2 = (b_1 - b_2) \pm t_{n_1 - n_2 - 4}(1 - \alpha/2) \sqrt{\frac{(S^2_{x/y})_p}{\sum x_1^2 - \frac{(\sum x_1)^2}{n_1}} + \frac{(S^2_{x/y})_p}{\sum x_2^2 - \frac{(\sum x_2)^2}{n_2}}} \qquad \text{Eq. 14.39}$$

If the null hypothesis is rejected ($t > t_{critical}$ or $t < -t_{critical}$) we can assume that the two lines do not have the same slopes and are not parallel to each other.

As an example, assume the following data have been collected at a pharmacy department in a large hospital for two different suspensions of a carbonic anhydrase inhibitor. Table 14.8 lists the results for the two formulations (Suspension B involving a sugar-free vehicle). Results of the various calculations are presented in Table 14.9. The null hypothesis would be that both suspensions degrade at the same rate, illustrated by the fact that the slopes of the two regression lines are equal:

$$H_0: \beta_A = \beta_B$$
$$H_1: \beta_A \neq \beta_B$$

Table 14.8 Stability Data for Suspensions

	% Labeled Amount	
Time (months)	Suspension A	Suspension B
0	99.2	99.50
1	98.7	98.80
2	96.9	97.10
2.5	-	96.50
3	96.1	95.90
3.5	-	95.40
4	95.5	95.10

Table 14.9 Summary Results for Suspensions

	Suspension A	Suspension B
n	5	7
Σx	10	16
Σy	486.4	678.3
Σx^2	30	48.50
Σy^2	47327.40	65744.33
Σxy	962.8	1536.25
b	−1.000	−1.186
a	99.28	99.61
SS_T	10.408	17.060
SS_E	10.0	16.785
SS_R	0.408	0.275
F (p)	73.5 (p = 0.003)	305.3 (p < 0.001)

Additional information needed to evaluate the results of the study is presented in Table 14.10. The calculation to assess parallelism (using the *t*-ratio approach) would be as follows:

$$(S^2_{x/y})_p = \frac{0.408 + 0.275}{8} = 0.085$$

$$t = \frac{-1.000 - (-1.186)}{\sqrt{\dfrac{0.085}{30 - \dfrac{(10)^2}{5}} + \dfrac{0.085}{48.5 - \dfrac{(16)^2}{7}}}} = \frac{0.186}{0.125} = 1.488$$

Table 14.10 Additional Summary Results for Suspensions

	Suspension A	Suspension B
n	5	7
df	3	5
Σx	10	16
Σx^2	30	48.50
b	−1.000	−1.186
SS_R	0.408	0.275

The decision rule would be, with $\alpha = 0.05$, reject H_0 if $t > t_8(0.975)$ or $t < t_8(0.975)$, which is 2.306 from Table B5 for $n_1 + n_2 - 4$ degrees of freedom. With 1.488 < 2.306, we fail to reject the null hypothesis and cannot identify a significant difference between the slopes for the two carbonic anhydrase inhibitor suspensions. Creation of a confidence interval confirms that the difference of

$$H_0: \ \beta_1 - \beta_2 = 0$$

is a possible outcome that falls within the interval:

$$\beta_1 - \beta_2 = (-1-(-1.186)) \pm 2.306 \sqrt{\frac{0.085}{30-\frac{(10)^2}{5}} + \frac{0.085}{48.5-\frac{(16)^2}{7}}}$$

$$\beta_1 - \beta_2 = (+0.186) \pm (2.306)(0.125) = +0.186 \pm 0.288$$

$$-0.102 < \beta_1 - \beta_2 < 0.474$$

Because zero falls within the interval we fail to find a significant difference between the two slopes.

As seen in Figure 14.11, the two slopes are not identical, but relatively close, especially considering the results are based on only a total of 12 data points. Unfortunately, if we fail to reject the null hypothesis we do not prove that the two lines are parallel to each other; we simply fail to identify a difference. Problems with this approach to assessing parallelism have been discussed by Hauck (Hauck, 2005).

Curvilinear and Non-Linear Regression

Simple linear regression (SLR) has been discussed in the previous sections of this chapter. It assesses the relationship between data and a straight line that fits between these points. Such information can be assessed visually, using an ANOVA table or the strength of the coefficient of determination (r^2). Other graphic assessments of linearity including the plotting of the residuals or the if the residuals follow a normal

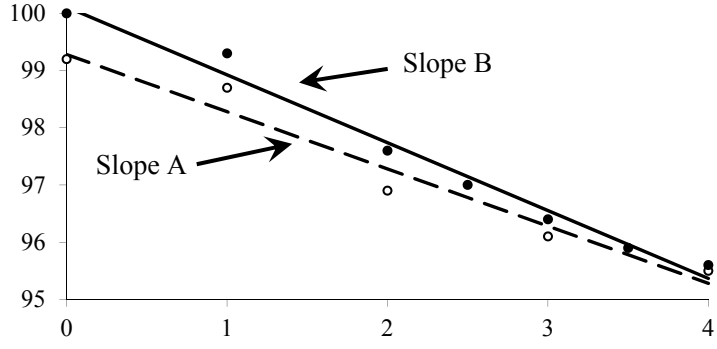


Figure 14.11 Graphic illustration of two similar slopes.

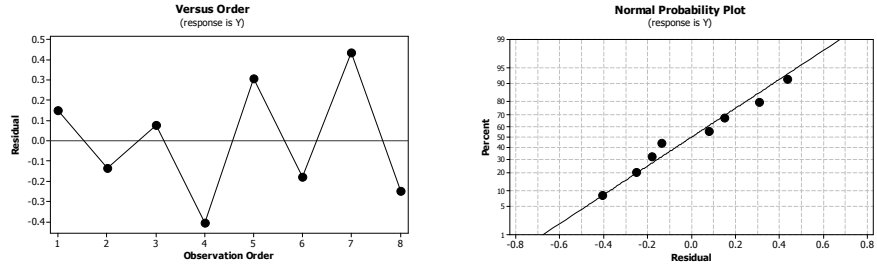


Figure 14.12 Plot of residuals for data in Table 14.1

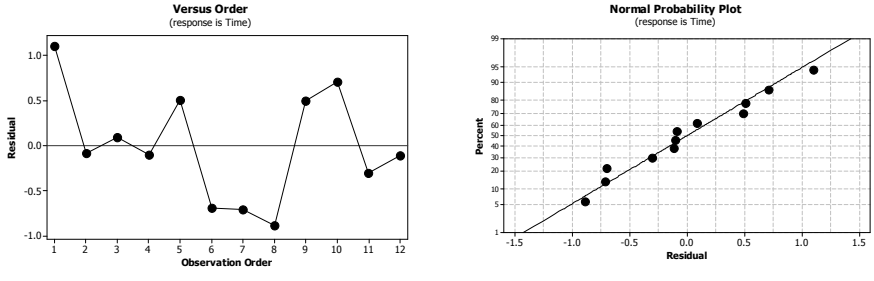


Figure 14.13 Plot of residuals for data in Table 14.3.

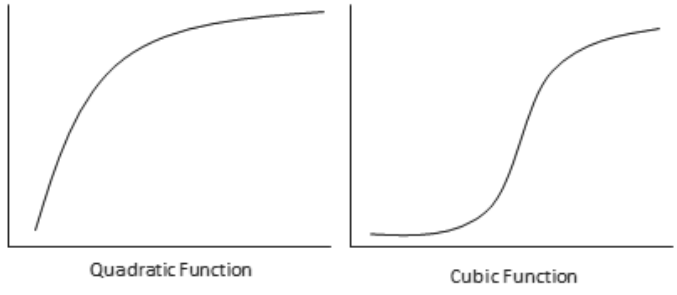


Figure 14.14 Examples of curvilinear distributions.

distribution. If a linear relationship exists we would expect the residuals to be randomly distributed between positive and negative values. For example, a plot of the residuals for data in Table 14.1 is presented in Figure 14.12. However, in a similar plot of the data in Table 14.3, even though statistically a linear relationship ($p < 0.004$), the residuals appear to be less random (Figure 14.13). Residual plots can be helpful
in identifying possible nonlinear relationships. Often in research there are curvilinear relationships instead of simple linear relationships. Fortunately, many such relationships can be expressed and evaluated as linear relationships.

The goal of a curvilinear regression is to describe the shape of the relationship between two continuous variables. Is the best fit linear or could it be something else? Curvilinear regression is sometime called **trend analysis**. For curvilinear regression we use models to fit a curve instead of a straight line through our sample data points. Also referred to as **polynomial regression**, various polynomial equations are used to fit the curve. The most common are quadratic or cubic models, as illustrated in Figure 14.14: quadratic (one bend or parabolic curve in the line) and cubic (two bends in the line). The linear equation for a polynomial equation is referred to as the first degree, the quadratic is of the second degree, and the cubic is of the third degree. More complex and rarely used trends (higher order trends) would include quartic (three bends) and quantic (four bends) trends. Sometimes a visual inspection of a scatter plot for sample data can reveal a degree of curvilinearity. These nonlinear relationships can also be applied to a correlation model where the independent variable is sampled at random and not specifically spaced or timed like regression.

With polynomial regression, polynomial equations are employed for the calculation of possible relationships. Curvilinear regression uses a linear model to fit a curved line to data points. It involves a hierarchical format by adding powered vectors to the analysis (e.g., quadratic where x is squared, cubic where x is raised to the third power). Modifying the equation for simple linear regression (Eq. 14.2) these are:

$$Linear \quad y = \alpha + \beta_1 x + e$$

$$Quadratic \quad y = \alpha + \beta_1 x_1 + \beta_2 x_1^2 + e \qquad \text{Eq. 14.40}$$

Table 14.11 Responses for Dietary Supplement Based on Days Treated

Days	% Response	Days	% Response	Days	% Response
1	11	6	36	11	87
1	12	6	41	11	89
2	16	7	53	12	89
2	19	7	59	12	93
3	28	8	67	13	93
3	24	8	63	13	96
4	27	9	75	14	95
4	33	9	83	14	98
5	31	10	81		
5	42	10	84		

$$Cubic \quad y = \alpha + \beta_1 x_1 + \beta_2 x_1^2 + \beta_3 x_1^3 + e \qquad \text{Eq. 14.41}$$

The introduction of the power terms is to account for the bends into the regression line. With simple linear regression, the regression line is straight (first order polynomial equation) and the formula is identical to Eq. 14.2. With the addition of the quadratic term, there is a single bend and the addition of x^2 (second order) and with the cubic term the addition of x^3 (third order). In all three cases the data represents a single independent variable and its effects on a single dependent variable. All of the assumptions for simple linear regression apply to the polynomial modifications.

Computer software can assist in determining if a curvilinear relationship is a better model than a simple linear relationship. To illustrate this, consider the data presented in Table 14.11. If we evaluate this data using a simple linear regression model we will find a significant result ($F = 691.28$, $p < 0.001$ and $r^2 = 0.964$). So, if we draw the straight line through this data we will find that the line accounts for 96.4% of the total variation of the y-axis. However, visually we are concerned that our data may be curvilinear, so we will let Minitab assess the possibility of a quadratic or cubic trend in the data. The results are as follows:

Relationship	Residual Standard Deviation	Coefficient of Determination
Linear	5.891	0.964
Quadratic	5.631	0.968
Cubic	4.171	0.983

In this example the regression line for this analysis is a curved line described by a third order polynomial equation. It is the best model with the smallest standard deviation for the residuals and the largest coefficient of determination. This is visually represented in Figure 14.15, where a cubic line is drawn to fit best between the data points in Table 14.11.

In contrast to curvilinear regression, **nonlinear regression** fits arbitrary nonlinear function associated with the dependent variable. One example of a nonlinear model

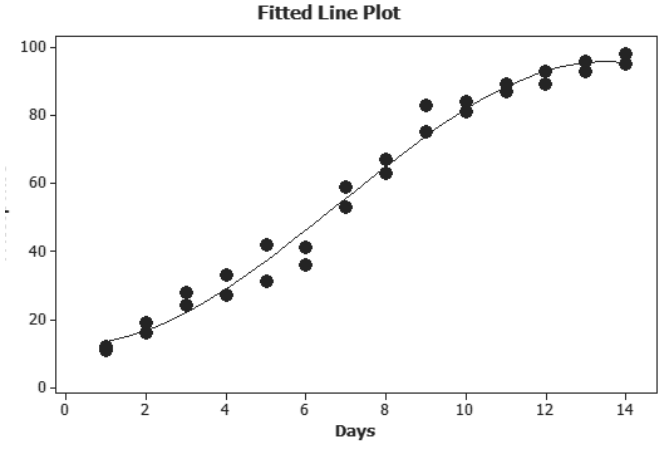


Figure 14.15 Cubic trend observed in data from Table 14.11.

might be something like $y = b(1 - e^{-bx})$. Nonlinear regression is beyond the scope of this book and additional material about the subject can be found in other references (Borowiak, 1989; Bates and Watts, 2006).

Multiple Regression Models

Multiple regression is a logical extension of the concepts illustrated for simple linear regression, where we were dealing with a single independent variable and were concerned with identifying the line which best fits between our data points (Eq. 14.2):

$$y = a + \beta x + e$$

Rather than using values for only one predictor or independent variable, (to estimate values on a dependent or **criterion variable**), with multiple regression we can control for several independent variables at the same time or look at the response by a dependent variable to these several predictor variables. By using many predictor variables (sometime referred to as **exploratory variables**), we will hopefully reduce our error of prediction even further, by accounting for more of the variance and at the same time we should be able to increase our predictive abilities. Multiple regression is designed to help the researcher learn more about the relationships among several predictor variables and a resultant dependent variable.

Any regression analysis allows us to make **predictions**, and could be referred to as **prediction analysis**. In the simple linear regression model, we can predict a value on the criterion variable, given its corresponding value on a predictor variable. With multiple regression we are interested in predicting a value for the criterion variable given a value for each of several corresponding predictor variables. The primary objectives for a multiple regression analysis are to: 1) determine whether a relationship exists between two continuous variables; 2) describe the nature of the

relationship, if one exists; and 3) assess the relative importance of each of the various predictor variables and their contribution to the criterion variable.

Although in most cases never confirmed, it is assumed that the relationship between variables is linear. Fortunately, multiple regression procedures are minimally affected by minor deviations from this assumption. However, if a curvilinear relationship is evident, one should consider transforming the data to make the situation more linear. Another assumption with multiple regression is that the residuals are normally distributed, but like other tests, these models are quite robust with regard to violations of this assumption. Some software packages offer the option of preparing a histogram of the residuals and visual inspection can identify severe deviations from a normal distribution. Also, similar to correlation, even with statistically significant results between certain predictor variables, and the dependent variable it does not prove causality.

Multiple linear regression is a powerful multivariate statistical technique for controlling any number of confounding variables:

$$y_j = a + \beta_1 x_1 + \beta_2 x_2 + \beta_2 x_2 + ... + \beta_j x_j + e_j \qquad \text{Eq. 14.42}$$

where y_j is the value of the dependent variable, α represents the point where the plane cuts the y-axis, and j is the number of independent variables. The values of β_1, β_2 ... β_k in the equation are referred to by several synonyms including **beta coefficients**, **beta weights**, and **regression coefficients**.

The beta coefficients indicate the relative importance of the various independent predictor variables and are based on their standardized z-scores. The prediction equation can be written as:

$$z_y = \beta_1 z_1 + \beta_2 z_2 + \beta_3 z_3 + ... \beta_k z_k \qquad \text{Eq. 14.43}$$

These beta weights are estimates of their corresponding coefficients for the population equation in standardized z-score form. They are also referred to as **partial regression coefficients**, because these regression coefficients are related to the partial correlation coefficients, which were discussed in Chapter 13.

As seen in the previous chapter, the multiple correlation coefficient (R) indicates the correlation for a weighted sum of the predictor variables and the criterion variable. The squared multiple correlation coefficient (R^2) will indicate the proportion of the variance for the dependent criterion variable, which is accounted for by combining the various predictor variables. In multiple regression the R^2 (also referred to as the *multiple* R^2) is analogous to the correlation coefficient also called the **coefficient of multiple determination**.

Unlike simple regression analysis, which was represented by a single line, multiple regression represents "planes in multidimensional space, a concept admittedly difficult to conceive and virtually impossible to portray graphically" (Kachigan, 1991). Two independent variables will create a "plane" in a three-dimensional space to best fit the data points. More than two independent variables will create what is called a "hyperplane" to account for the variability. Instead of thinking of a least-squares line to fit our data, we must think of a least-squares

solution based on weighted values for each of the various predictor variables. Using computer software it is possible to calculate the appropriate beta weights to create the least-squares solution, with those having the greatest correlation represented by the largest weightings. Computer programs utilize the ordinary least squares method to derive an equation by minimizing the sum of the squared residuals.

The calculations for multiple linear regression analysis are extensive, complex, and fall beyond the scope of this book. Excellent references for additional information on this topic include Zar (2010), Snedecor and Cochran (1989), and the Sage University series (Berry and Feldman, 1985; Achen, 1982; Schroeder, 1986). However, the following example will be helpful for interpreting computer reports and evaluating the relative importance of each predictor (independent) variable.

Table 14.12 contains data for 30 volunteers involved in a study of three different sleep aids. The first column is the result after 60 days of therapy as indicated by the change in Epworth sleep scores (the larger the negative result, the better the sleep score and lessening of the sleep problems). The next three columns are the predictor variables: the drug received, as well as each volunteer's age and gender. Note that the drug and gender are both numerical because software packages (including Excel and Minitab) require that the predictor variables have numeric values in order to perform the calculations. The coding of gender or other categorical data as numeric values instead of labels is referred to as **dummy coding**. In this example one represents male volunteers. This information is manipulated by computer software and the results from a Minitab analysis are reported in Figure 14.16.

Table 14.12 Change in Epworth Sleep Scores and Selected Predictor Variables

Change	Drug	Age	Gender[1]	Change	Drug	Age	Gender[1]
−5	1	40	1	+1	2	74	2
−3	1	53	1	−6	3	52	2
0	2	42	2	−4	3	65	1
−1	2	62	2	−4	1	45	1
−5	3	34	2	−3	1	55	1
−7	3	67	2	−2	2	28	1
−4	1	71	2	0	2	42	2
+1	1	69	2	−5	3	58	2
0	2	57	1	−7	3	29	1
−2	2	38	1	0	1	63	1
−4	3	48	1	−3	1	33	1
−6	3	32	1	+1	2	51	1
−1	1	25	2	−4	2	38	2
0	1	36	1	−6	3	47	1
+2	2	62	1	−3	3	76	2

[1] 1 = male; 2 = female

```
The regression equation is
Change in Sleep Score = - 1.54 - 1.65 Drug + 0.130 Gender + 0.0406 Age

Predictor      Coef  SE Coef      T      P
Constant     -1.538    1.898  -0.81  0.425
Drug         -1.6495   0.5196 -3.17  0.004
Gender        0.1300   0.8945  0.15  0.886
Age          0.04057  0.03010  1.35  0.189

S = 2.29196   R-Sq = 31.4%   R-Sq(adj) = 23.4%

Analysis of Variance

Source          DF       SS      MS     F      P
Regression       3   62.387  20.796  3.96  0.019
Residual Error  26  136.580   5.253
Total           29  198.967
```

Figure 14.16 Results of a multiple regression analysis on sleep scores with Minitab.

The computer will generate an equation that describes the statistical relationship between the multiple predictor variables and for predicting new observations. The results indicate the direction, size, and statistical significance of the relationship between each independent variable and the response by the dependent variable. The sign of each coefficient indicates the direction of the relationship. In this case note the top of Figure 14.16 that reports the regression equation which factors in all the beta coefficients in Eq.14.35 to create the plane that fits best through the 30 data points considering all three predictor variables:

$$y_j\,(Change) = -1.54 + (-1.65)(Drug) + (0.13)(Gender) + (0.0406)(Age)$$

This equation could be termed a **linear combination** and indicates the beta coefficient associated with each predictor variable. The lower portion of Figure 14.16 is the ANOVA table indicating a significant regression analysis ($p = 0.019$). Note that there are three degrees of freedom associated with the regression term, instead of one with simple linear regression. With multiple regression each independent variable parameter must be estimated and therefore counts as a loss of one degree of freedom for each estimate. An important portion of the printout in Figure 14.16 is the middle section that indicates the importance of each independent variable on the change in the sleep scores, where the beta coefficients appear in the second column of the table. Similar to slope in a simple linear relationship, each coefficient represents the change in the response for each unit of change in the particular independent variable if the other predictors are held constant. In this case for each year increase in age, there was only about a 0.04 change in the sleep score. The *t*-value and corresponding *p*-value represent a test of the null hypothesis that the beta coefficient is equal to zero while holding other predictors in the model constant. In this case the drug was most important *(*$t = -3.17$, $p = 0.004$) and this is reflected by the largest beta coefficient in

the regression equation. Age (t = 1.35, p = 0.189) and gender (t = 0.15, p = 0.886) were not significant contributing predictor variables to the overall response. In multiple regression the R^2 is the proportion of variation-dependent variable which can be accounted for by fitting the independent variables into a particular model. The greater the amount of variation that can be accounted for by the R^2 in the regression model the closer the data points will fit the regression plane. In Figure 14.16, even though the results were statistically significant the resultant regression equation could only account for 31.4% of the total variability on the y-axis.

Stepwise Regression

Another type of multiple regression model is called **stepwise regression**. In this situation there is a set of rules for deriving a multiple regression equation by adding or subtracting one independent variable at a time from the regression equation. When there is a set of independent variables it is not necessary to utilize every single one in the determination of a multiple R^2. The objective of stepwise regression is to identify a useful subset of predictors which contribute the most to the regression analysis.

With some computer software variable selection is automatic, removing all independent variables that have p-values greater than a designated Type I error rate. The standard stepwise procedure both adds and removes predictors as needed for each step. The results of this type of process automatically select the most significant models with the larger R^2 and adjusted R^2, and smaller standard errors.

Other models allow the researcher to add or subtract variables. In the **forward stepping** or **forward selection** model, the predictor variable with the highest correlation is entered first, followed by other variables (one at a time, in order of increasing correlations) that result in an increase the multiple R^2 until all statistically significant variables have been added to the equation. Forward stepping starts with no predictors in the model and then adds the most significant variable for each step. The opposite approach would be to begin with all the independent variables, then individual independent variables are subtracted according to a specified criterion (usually the lowest correlations first). They are eliminated if they do not contribute significantly or on some predetermined criterion. This latter approach is referred to as **backward stepping** or **backward elimination**. Both methods are designed for selecting the best set of variables and eliminating those that do not contribute. Backward stepping starts with all predictors in the model and then removes the least significant variable for each step.

The three stepwise processes stop when all variables not already included in the model have p-values that are greater than the specified Type I error rate.

However, there are potential problems. If two independent variables are highly correlated, only one may end up being in the model even though both may be equally important. The result may not always identify the model with the highest R^2 value. Computer programs that have automated procedures may not factor in special information about the predictor variables, resulting in a model that may not be the best fit.

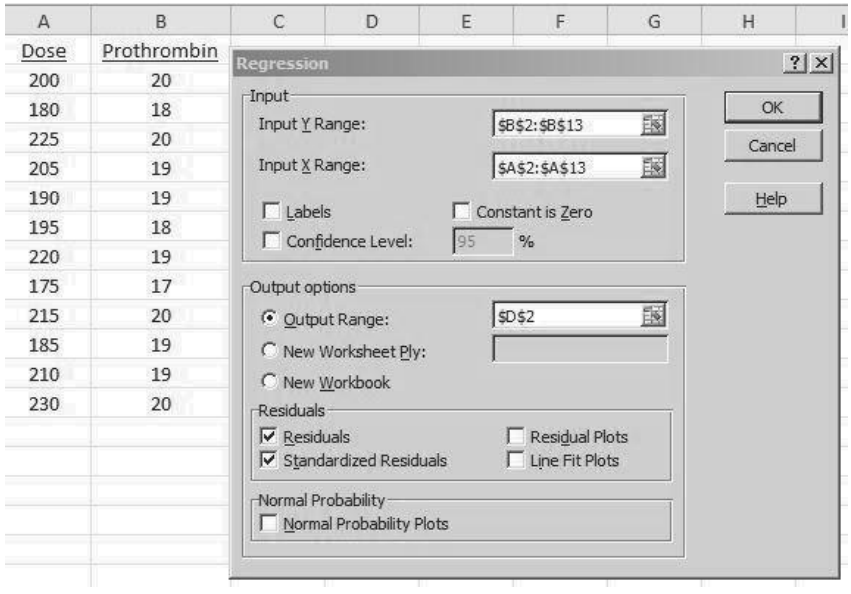


Figure 14.17 Options for regression with Excel.

Using Excel® or Minitab® for Regression Analysis

The regression analysis is available as part of the Excel data analysis tools:

Data ➤ Data Analysis ➤ Regression

Similar to the previous tests, each variable (independent or predictor variable and dependent or criterion variable) is represented by a different column. As seen in Figure 14.17 (for data in Table 14.3), for simple linear regression one needs to identify the column for the dependent variable ("Input Y Range:") and the independent variable ("Input X Range:"). A confidence level can be changed from the default value of 95% and the location for output ("Output range:") needs to be indicated, either starting at a cell on the current page (per this example, D2) or on a new worksheet (by default). There are several numeric summaries and visual graphics available under "Residuals" and "Normal Probability". The primary outcome of interest is the ANOVA table along with related information (Figure 14.18). The ANOVA table is presented in the center of Figure 14.18 and shows identical numbers (less some rounding errors) to those reported in the text for the data comparing prothrombin time and various doses of a drug. The coefficient of determination (r^2) appears in the top table, noted as "R Square" and the adjusted R^2 is also listed. The "Multiple R" represents the correlation coefficient (r) and the "Standard Error" term represents the residual standard deviation. The box immediately below the ANOVA table provides information about the slope and intercept. In the column "Coefficients"

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.76688453
R Square	0.58811189
Adjusted R Squ	0.54692308
Standard Error	0.6417851
Observations	12

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regression	1	5.881119	5.881118881	14.27844	0.0036098
Residual	10	4.118881	0.411888112		
Total	11	10			

	Coefficients	*andard Err*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	10.7867133	2.181466	4.944707398	0.000583	5.9261031	15.64732344
X Variable 1	0.04055944	0.010734	3.778682049	0.00361	0.0166431	0.064475733

RESIDUAL OUTPUT

Observation	*Predicted Y*	*Residuals*	*Standard Residuals*
1	18.8986014	1.101399	1.799911843
2	18.0874126	-0.08741	-0.142850146
3	19.9125874	0.087413	0.142850146
4	19.1013986	-0.1014	-0.16570617
5	18.493007	0.506993	0.828530848
6	18.6958042	-0.6958	-1.137087164
7	19.7097902	-0.70979	-1.159943188
8	17.8846154	-0.88462	-1.44564348
9	19.506993	0.493007	0.805674825
10	18.2902098	0.70979	1.159943188
11	19.3041958	-0.3042	-0.497118509
12	20.1153846	-0.11538	-0.188562193

Figure 14.18 Outcome report for regression with Excel.

is the sample "intercept" (a) and is the slope (b), labeled as "X Variable 1". If we chose "Labels" and included information in the first row (discussed below as part of multiple regression) on Figure 14.16, the "X Variable 1" descriptor would have to be replaced with "Dose". The "Standard Error", "t Stat", "P-value" and two 95% interval values in the columns represent calculations performed previously for the data in Table 14.3. The lower and upper 95% represent the 95% confidence limits around the estimates and evaluate the null hypothesis that $\beta = 0$. The last table of data represents

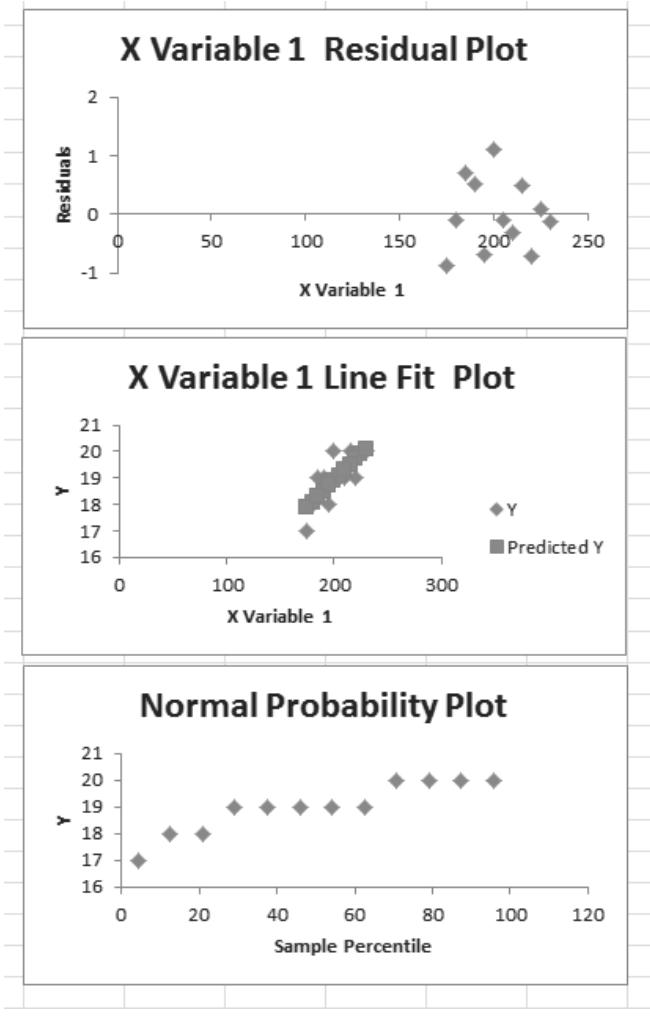
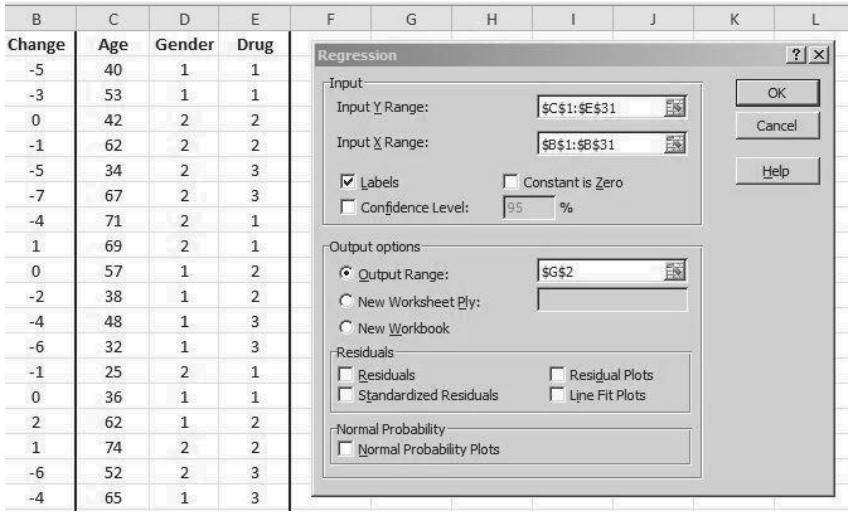


Figure 14.19 Graphics produced for regression with Excel.

the *y*-values on the regression line "Predicted Y" and the residual between the sample data point and the line, noted as "Residuals". The "Standardized Residuals" are listed in the last column, defined as

$$Standardized\ residual = \frac{Residual}{Standard\ deviation\ for\ all\ residuals} \qquad \text{Eq.14.37}$$



Change	Age	Gender	Drug
-5	40	1	1
-3	53	1	1
0	42	2	2
-1	62	2	2
-5	34	2	3
-7	67	2	3
-4	71	2	1
1	69	2	1
0	57	1	2
-2	38	1	2
-4	48	1	3
-6	32	1	3
-1	25	2	1
0	36	1	1
2	62	1	2
1	74	2	2
-6	52	2	3
-4	65	1	3

Figure 14.20 Options for multiple regression with Excel.

Figure 14.19 shows the graphic output available if the options are selected under "Residuals" and "Normal Probability" in Figure 14.17. Of primary interest would be the residuals plot to a random above and below the line distribution of data points. The software does not crop the data, starting with zero on the left margin of each plot.

For multiple regression with Excel, the same entry through data analysis is used:

Data ➤ Data Analysis ➤ Regression

However, in this case: 1) all the columns for independent variable are selected for the "Input X Range:"; 2) the first row should include the name of the variable and be included in the range; and 3) the "Label" option is selected. Figure 14.20 represents the data selection option of the study in Table 14.12. Here "Labels" are included so the resultant table will identify the appropriate coefficients for each variable. Note also that dummy variables are used for the independent variables of drug and gender. The results of the analysis are presented in Figure 14.21 and are identical to the results seen with Minitab in Figure 14.16. Note with "Labels" activated the lower table is easier to read. The one omission with Excel is the presentation of the actual linear combination that appears with Minitab. However this can be determined by selecting the beta coefficient for each variable from the lower table.

Minitab offers the regression options under "Stat" on the title bar. For simple linear regression and multiple regression the choices are found at:

Stat ➤ Regression ➤ Regression…

As with previous tests each column represents a variable and each row an observation. Figure 14.22 illustrates the decisions required for a simple linear

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.5599584
R Square	0.31355341
Adjusted R Squar	0.23434803
Standard Error	2.29195911
Observations	30

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	62.38667621	20.79556	3.958739	0.018899082
Residual	26	136.5799905	5.253077		
Total	29	198.9666667			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-1.5383253	1.898111108	-0.81045	0.425038	-5.43994855	2.363298
Age	0.04057078	0.030102563	1.347752	0.189365	-0.02130592	0.1024475
Gender	0.12997236	0.894471828	0.145306	0.88559	-1.70864081	1.9685855
Drug	-1.6495109	0.519600922	-3.17457	0.003837	-2.71756593	-0.5814559

Figure 14.21 Outcomes for multiple regression with Excel.

regression calculation for the data from Table 14.3. The dependent variable is labeled "Response" and the independent variable(s) as "Predictors". These are selected by double clicking on the variables in the box on the left. The confidence level can be changed from the default $1 - \alpha$ of 95% if desired under *Options*… selections. The results of the analysis are presented in Figure 14.23. The lower portion is the traditional ANOVA table with the *F*-statistic and associated *p*-value on the right side. The second line from the top defines the intercept and slope ($y = a + bx$); in this case "Time = 10.8 + 0.0406 Dose." The "Predictor" section in the middle provides similar information for the intercept "constant" and the slope (in this case "Dose" to the Excel printout with the coefficients (*a* and *b*), SE coefficients (how labeled before), and *t*-values and associated *p*-values for the intercept and slope. The following line includes *S* which is the residual standard deviation, the coefficient of determination (R-Sq) and the adjusted r^2 as "R-Sq(adj)". The lack-of fit test is available under the *Options*... choices.

For multiple regression, the process is the same, except more variables are added to the "Predictors:" area of the regression options (Figure 14.24). The *Graphic*... and *Options*... offer a variety of different output information. The simplest default results has already been displayed (Figure 14.16) and discussed previously. There is also a stepwise option for adding and subtracting independent variables:

Stat ➤ Regression ➤ Stepwise…

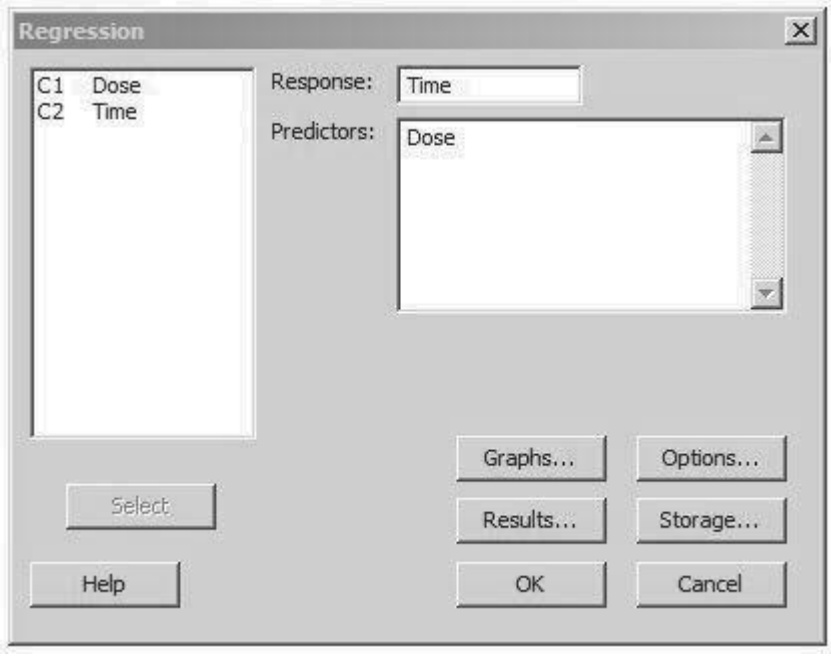


Figure 14.22 Options for simple linear regression with Minitab.

Regression Analysis: Time versus Dose

The regression equation is
Time = 10.8 + 0.0406 Dose

Predictor	Coef	SE Coef	T	P
Constant	10.787	2.181	4.94	0.001
Dose	0.04056	0.01073	3.78	0.004

S = 0.641785 R-Sq = 58.8% R-Sq(adj) = 54.7%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	5.8811	5.8811	14.28	0.004
Residual Error	10	4.1189	0.4119		
Total	11	10.0000			

Figure 14.23 Outcomes for simple linear regression with Minitab.

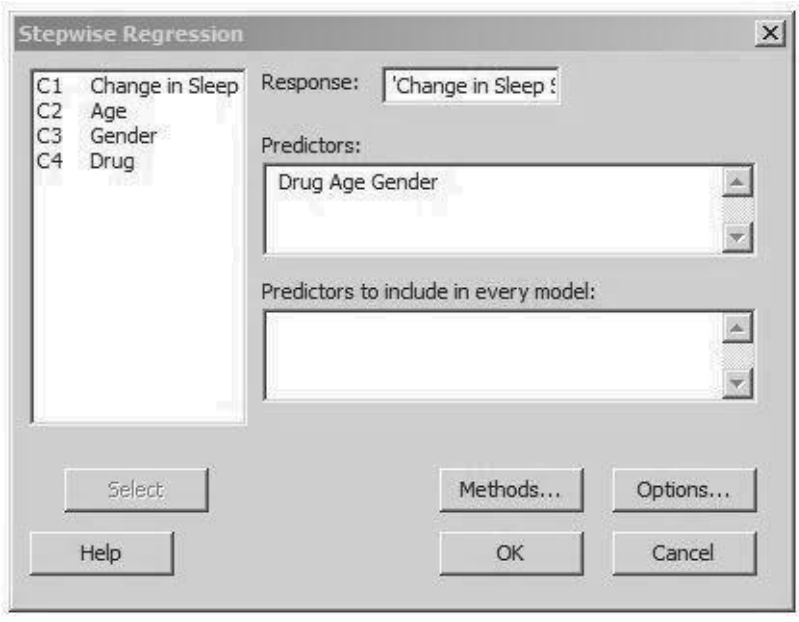


Figure 14.24 Options for multiple regression with Minitab.

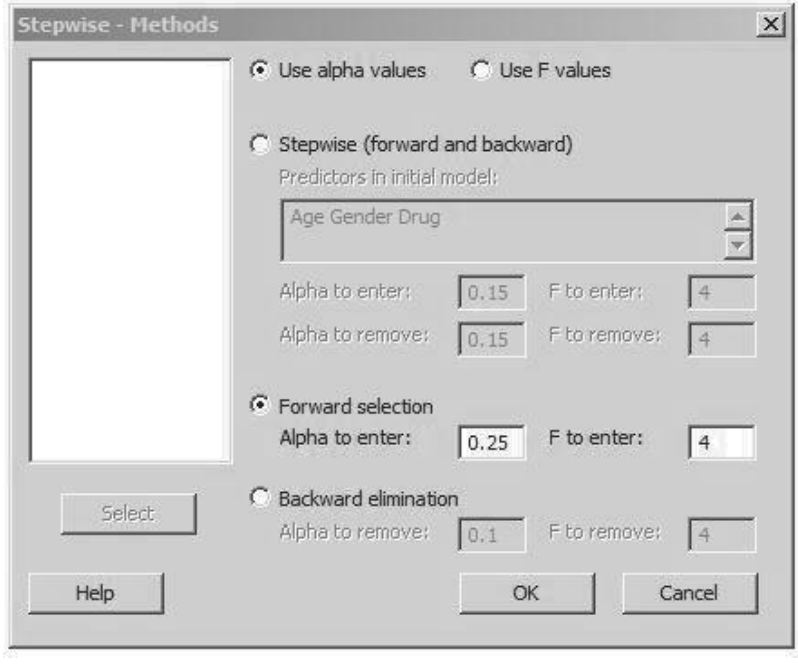


Figure 14.25 Options for stepwise regression using Minitab.

Like multiple regression, all the independent variables need to be selected and added to the "Predictors:" portion of the initial screen (Figure 14.24) which look similar to Figure 14.22. The *Methods*... option allows one to allow Minitab to do an automatic selection or either and forward stepping or backward stepping option. As seen in Figure 14.25, one can choose to use either Type I error (α) or *F*-values in selecting the predictor variables of interest. The *Stepwise* option will perform a standard stepwise procedure. For this example we have selected the "Forward selecting:" model and for illustrative purposes, set the α as large as 0.25 (the default value). The results are displayed in Figure 14.26. The first step selected only the "Drug" predictor variable with a resultant R^2 value of 25.73 and standard error of 2.30. In a second step the "Age" predictor variable is added (since *p*-value was less than 0.25) and the R^2 increases (31.30) and standard error slightly decreases (2.25). Gender was not considered in the stepwise regression model.

Many graphic choices are available through the *Graphs*... option including plots of residuals and standardized residuals. Curvilinear determinations can be made using

Stat ➤ Regression ➤ Fitted line plot…

```
Forward selection.  Alpha-to-Enter: 0.25

Response is Change in Sleep Score on 3 predictors, with N = 30

Step            1        2
Constant   0.5667  -1.4387

Drug        -1.60    -1.64
T-Value     -3.11    -3.25
P-Value     0.004    0.003

Age                  0.042
T-Value               1.48
P-Value              0.151

S            2.30     2.25
R-Sq        25.73    31.30
R-Sq(adj)   23.08    26.21
Mallows Cp    2.1      2.0
PRESS     166.979  165.877
R-Sq(pred)  16.08    16.63
```

Figure 14.26 Outcomes for a stepwise regression using Minitab.

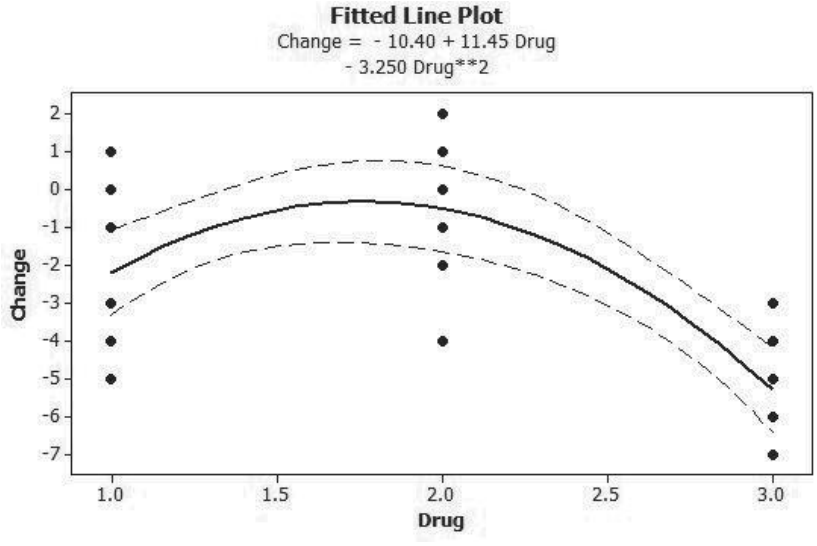


Figure 14.27 Quadratic distribution with confidence bands for data in Table 14.12 using Minitab.

Options include linear, quadratic and cubic, and confidence intervals can be created around the line of best fit. The "Options" alternative allows one to add confidence bands to the line of best fit and adjust the confidence limits ($1 - \alpha$). For example, using the linear fit, the significance for the data in Table 14.12 for sleep score versus drug is significant ($F = 9.70$, $p = 0.004$), but the quadratic fit displayed in Figure 14.27 is even better ($F = 23.18$, $p < 0.001$). Additional information about regression options and plotting appears in Lesik (2010).

References

Achen, C.H. (1982). *Interpreting and Using Regression* (Paper 29), Sage University Series on Quantitative Applications in the Social Sciences, Sage Publications, Newbury Park, CA.

Bates, D.M.. and Watts, D.G.. (2006). *Nonlinear Regression Analysis and Its Applications*, John Wiley and Sons, New York.

Berry, W.D. and Feldman, S. (1985). *Multiple Regression in Practice* (Paper 50), Sage University Series on Quantitative Applications in the Social Sciences, Sage Publications, Newbury Park, CA.

Borowiak, D.S. (1989). *Model Discrimination for Nonlinear Regression Models*, CRC Press, Boca Raton, Florida.

Dapson, R.W. (1980). "Guidelines for statistical usage in age-estimation technics," *Journal of Wildlife Management* 44:541-548.

Hauck, W.W. et al. (2005). "Assessing parallelism prior to determining relative potency," *PDA Journal of Pharmaceutical Science and Technology* 59:127-137.

Kachigan, S.K. (1991). *Multivariate Statistical Analysis*, Second edition, Radius Press, New York, p. 181.

Lesik, S.A. (2010). *Applied Statistical Inference with MINITAB®*, Chapman & Hall/ CRC Press, Boca Raton, FL, pp.239-306.

Schroeder, L.D. et al. (1986). *Understanding Regression Analysis: An Introductory Guide* (Paper 57), Sage University Series on Quantitative Applications in the Social Sciences, Sage Publications, Newbury Park, CA.

Snedecor, G.W. and Cochran, W.G. (1989). *Statistical Methods*, Eighth edition, Iowa State University Press, Ames, IA, pp. 333-365.

Zar, J.H. (2010). *Biostatistical Analysis*, Fifth edition, Prentice-Hall, Upper Saddle River, NJ, pp. 423-437.

Suggested Supplemental Readings

Bolton, S. and Bon, C. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, New York, pp. 200-206.

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Eighth edition, John Wiley and Sons, New York, pp. 410-440, 487-506.

Example Problems (Answers are provided in Appendix D)

1. Samples of a drug product are stored in their original containers under normal conditions and sampled periodically to analyze the content of the medication.

Time (months)	Assay (mg)
6	995
12	984
18	973
24	960
36	952
48	948

   Does a linear relationship exist between the two variables? If such a relation exists, what are the slope, *y*-intercept, and 95% confidence interval?

2. Acme Chemical is testing various concentrations of a test solution and the effect the concentration has on the optical density of each concentration.

Concentration (%)	Optical Density
1	0.24
2	0.66
4	1.15
8	2.34

Is there a significant linear relationship between the concentration and optical density? If there is a relationship, create a plot representing this relationship and 95% confidence intervals.

3. Acme Chemical reassesses the previous results by comparing the data against a reference standard solution at the same concentrations and found the results in the following table. Were the slopes of the test solution and reference standard parallel?

	Optical Density	
Concentration (%)	Test	Standard
1	0.24	0.22
2	0.66	0.74
4	1.15	1.41
8	2.34	2.76

4. During the formulation of a new product, various percent of a polymeric coating are added and the resultant release of the therapeutic agent is evaluated below. Is there a strong linear relationship between the percent and release rate and does the data fit that line?

Percent Coating	Rate of Release (mg/hr)		
5	3.70	3.28	3.56
10	3.00	2.91	3.17
15	2.74	2.61	2.53
20	2.34	2.47	2.21
30	1.41	1.60	1.76
40	0.56	0.91	0.69

5. Various dilutions are made of a homogeneous mixture and results analyzed. Is there a linear relationship between the dilution factor and the analytical outcome?

Dilution Factor	Analytical Results
2	2.187
4	2.167
8	2.149
16	2.097
32	1.987
64	1.763
128	1.578
256	1.132

15

z-Tests of Proportions

As an introduction to this new set of z-tests, consider the following two problems. First, we are presented with a coin and we wish to determine if the coin is "fair" (an equal likelihood of tossing a head or a tail). To test the assumption of fairness, we toss the coin 20 times and find that we have 13 heads and only 7 tails. Is the coin unfair, loaded in such a way that heads occur more often, or could the outcome be the result of chance error? In a second situation, 50 patients are randomly divided into two groups each receiving a different treatment. In one group 75% show improvement and in the second group only 52% improve. Do the results prove that the first therapy results in a significantly greater therapeutic response, or is this difference due to chance alone?

The z-tests of proportions can address each of these examples, when comparisons are made between proportions or percentages for one or two levels of a discrete independent variable.

z-Test of Proportions – One-Sample Case

The z-tests of proportions involve a dependent variable that has only two discrete possible outcomes (i.e., pass or fail, live or die). These outcomes should be mutually exclusive and exhaustive. Similar to the statistics used for t- and F-tests, this *z*-statistic involves the following ratio:

$$z = \frac{difference\,between\;\;proportions}{standard\;\;error\;\;of\;\;the\;\;difference\;\;of\;\;the\;\;proportions} \qquad \text{Eq. 15.1}$$

The simplest example would be the tossing of a fair coin. We would expect the proportion of heads to be equal to the proportion of tails. Therefore, we would expect a head to occur 50% of the time, or have a proportion of 0.50. Our null hypothesis is that we are presented with a fair coin:

$$H_0:\; P_{heads} = 0.50$$

The only alternative is that the likelihood of tossing a head is something other than 50%.

$$H_1: P_{heads} \neq 0.50$$

If we toss the coin 100 times and this results in 50 heads and 50 tails, the numerator of the above ratio (Eq. 15.1) would be zero, resulting in $z = 0$. As the discrepancy between what we observe and what we expect (50% heads) increases, the resultant z-value will increase until it eventually becomes large enough to be significant. Significance is determined using the critical z-values for a normalized distribution, previously discussed in Chapter 6. For example, from Table B2 in Appendix B, +1.96 or −1.96 are the critical values in the case of a 95% level of confidence. For a 99% level of confidence the critical z-values would be +2.58 or –2.58.

In the one-sample case the proportions found for a single sample are compared to a theoretical population to determine if the sample is selected from that same population.

$$H_0: \hat{p} = P_0$$
$$H_1: \hat{p} \neq P_0$$

The test statistic is as follows:

$$z = \frac{\hat{p} - P_0}{\sqrt{\frac{P_0(1-P_0)}{n}}} \qquad \text{Eq. 15.2}$$

where P_0 is the expected proportion for the outcome, $1 - P_0$ is the complement proportion for the "not" outcome, $\hat{p}$ is the observed proportion of outcomes in the sample, and n is the number of observations (sample size). The decision rule is

$$\text{with } \alpha = \_\_\_, \text{ reject } H_0 \text{ if } z > z_{(1-\alpha/2)} \text{ or } z < -z_{(1-\alpha/2)}$$

where $z_{(1-\alpha/2)} = 1.96$ for $\alpha = 0.05$ or 2.58 for $\alpha = 0.01$. Like the t-test, this is a two-tailed test and modifications can be made in the decision rule to test directional hypotheses with a one-tailed test.

The one-sample case can be used to test the previous question about fairness of a particular coin. If a coin is tossed 20 times and 13 heads are the result, is it a fair coin? As seen earlier the hypotheses are:

$$H_0: P_{heads} = 0.50$$
$$H_1: P_{heads} \neq 0.50$$

In this case the $\hat{p}$ is 13/20 or 0.65, P_0 equals 0.50 and n is 20. The calculation would be as follows:

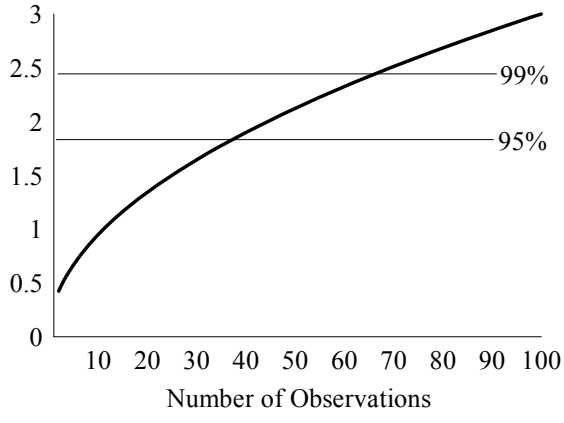


Figure 15.1 Effects of sample size on z-test results.

$$z = \frac{0.65 - 0.50}{\sqrt{\frac{(0.50)(0.50)}{20}}} = \frac{0.15}{0.11} = 1.36$$

Because the calculated *z*-value is less than the critical value of 1.96, we fail to reject the hypothesis, and we assume that the coin is fair and the difference between the observed 0.65 and expected 0.50 was due to random variability. What if we had more data and the results were still the same? The z-test is an excellent example of the importance of sample size. Figure 15.1 shows the same proportional differences with an increasing number of observations. Note that if these results appeared with more than 47 or 48 tosses the results would be significant at 95% confidence and the null hypothesis would be rejected. If the same proportional difference exists with over 75 tosses H_0 can be rejected with 99% confidence.

Similar to one-sample t-tests, confidence intervals can also be created for the z-test of proportions (best estimate plus and minus a reliability coefficient time - a standard error term):

$$P_0 = \hat{p} \pm Z_{(1-\alpha/2)} \sqrt{\frac{P_0(1-P_0)}{n}} \qquad \text{Eq. 15.3}$$

The interval indicates the range of possible results with 95% confidence. For the above example, the hypotheses would continue to be:

$$H_0: \ \hat{p} = 0.50$$
$$H_1: \ \hat{p} \neq 0.50$$

and the interval would be:

$$P_0 = 0.65 \pm 1.96\sqrt{\frac{(0.50)(0.50)}{20}}$$

$$P_0 = 0.65 \pm 0.22$$

$$0.43 < P_0 < 0.87$$

Therefore, based on a sample of only 20 tosses, with 95% confidence, the probability of tossing a head is somewhere between 0.43 and 0.87. The observed outcome of 0.65 is a possible outcome; therefore H_0 cannot be rejected.

z-Test of Proportions – Two-Sample Case

In the two-sample case, proportions from two levels of a discrete independent variable are compared and the hypothesis under test is that the two proportions for the population are equal.

$$H_0: \quad P_1 = P_2$$
$$H_1: \quad P_1 \neq P_2$$

If the two populations (P_1 and P_2) are equal, then the best estimation of that population proportion would be the weighted average of the two sample proportions:

$$\hat{p}_0 = \frac{n_1 \hat{p}_1 + n_2 \hat{p}_2}{n_1 + n_2} \qquad \text{Eq. 15.4}$$

This estimate of the population proportion is then used in the denominator of the *z*-ratio (Eq. 15.1) and the numerator is the difference between the two sample proportions:

$$z = \frac{\hat{p}_1 - \hat{p}_2}{\sqrt{\frac{\hat{p}_0(1-\hat{p}_0)}{n_1} + \frac{\hat{p}_0(1-\hat{p}_0)}{n_2}}} \qquad \text{Eq. 15.5}$$

In these two equations, $\hat{p}_1$, $\hat{p}_2$ are sample proportions and n_1, n_2 are the sample sizes. The decision rule for a two-tailed z-test would be

$$\text{with } \alpha = 0.05, \text{ reject } H_0, \text{ if } z > z_{(1-\alpha/2)} = 1.96 \text{ or } z < -1.96$$

To illustrate this test assume the following fictitious clinical trial. To possibly improve the survival rate for protozoal infections in AIDS patients, individuals with newly diagnosed infections were randomly assigned to treatment with either zidovudine alone or a combination of zidovudine and trimethoprim. Based on the following results, did either therapy show a significantly better survival rate?

Zidovudine alone, 23 out of 94 patients survived, $\hat{p}_Z = 0.245$

Zidovudine with trimethoprim, 42 out of 98 patients survived, $\hat{p}_{Z\&T} = 0.429$

Is there a significant difference between 0.245 and 0.429 based on fewer than 200 patients? The best estimate of the population proportion, if there is no difference between the two samples, is determined using weighted averages for the two sample proportions:

$$\hat{p}_0 = \frac{94(0.245) + 98(0.429)}{94 + 98} = 0.339$$

The null hypothesis would be that there was not a significant difference between the two groups of patients based on the proportions of patients surviving.

$$H_0: P_Z = P_{Z\&T}$$
$$H_1: P_Z \neq P_{Z\&T}$$

If the *z*-statistic is greater than +1.96 or less than −1.96, the researcher should reject the null hypothesis and conclude that there is a significant difference between the two groups. The computations would be:

$$z = \frac{0.245 - 0.429}{\sqrt{\frac{0.339(0.661)}{94} + \frac{0.339(0.661)}{98}}}$$

$$z = \frac{-0.184}{\sqrt{0.00467}} = \frac{-0.184}{0.068} = -2.71$$

With $z = -2.71$ (which is to the extreme of the critical value of −1.96) the decision would be to reject H_0 and conclude that there was a significant difference in the results for the two treatments. In this case the patients receiving both zidovudine and trimethoprim have a significantly better survival rate.

The formula can be slightly modified to create a confidence interval

$$P_1 - P_2 = (\hat{p}_1 - \hat{p}_2) \pm Z_{(1-\alpha/2)} \sqrt{\frac{P_0(1-P_0)}{n_1} + \frac{\hat{p}_0(1-\hat{p}_0)}{n_2}} \qquad \text{Eq. 15.6}$$

The results would be interpreted similar for the two-sample t-test; if zero is within the confidence interval, there is no significant difference between the two proportions.

Power and Sample Size for Two-Sample z-Test of Proportions

To calculate the power for a two-sample z-test of proportions we use the sum of

two probabilities associated with *z*-values:

$$power = p[z \le Z_1] + p[z \ge Z_2] \qquad \text{Eq. 15.7}$$

where:

$$Z_1 = \frac{-Z_{1-\alpha/2}(SE) - (\hat{p}_1 - \hat{p}_2)}{\sqrt{\frac{\hat{p}_1\hat{q}_1}{n_1} + \frac{\hat{p}_2\hat{q}_2}{n_2}}} \qquad \text{Eq. 15.8}$$

$$Z_2 = \frac{+Z_{1-\alpha/2}(SE) - (\hat{p}_1 - \hat{p}_2)}{\sqrt{\frac{\hat{p}_1\hat{q}_2}{n_1} + \frac{\hat{p}_1\hat{q}_2}{n_2}}} \qquad \text{Eq. 15.9}$$

with the *SE* representing the standard error in the denominator of the original *z*-ratio:

$$SE = \sqrt{\frac{p_O(1-p_O)}{n_1} + \frac{p_O(1-p_O)}{n_2}} \qquad \text{Eq. 15.10}$$

These are modified from Zar's equations (2010) to be consistent with the symbols used previously in this chapter. With a desired α of 0.05, the $Z_{1-\alpha/2}$ would be 1.96 (for 99% confidence the value would be 2.58). Using the previous example of the two HIV therapeutic approaches the power based on 192 patients would be:

$$SE = \sqrt{\frac{(0.323)(0.677)}{94} + \frac{(0.323)(0.677)}{98}} = 0.067$$

$$Z_1 = \frac{-1.96(0.068) - (0.245 - 0.429)}{\sqrt{\frac{(0.245)(.755)}{94} + \frac{(0.429)(0.571)}{98}}} = \frac{0.0501}{0.0668} = +0.75$$

$$Z_2 = \frac{+1.96(0.068) - (0.245 - 0.429)}{\sqrt{\frac{(0.245)(0.755)}{94} + \frac{(0.429)(0.571)}{98}}} = \frac{+0.3173}{0.0668} = +4.75$$

$$power = p[Z < +0.75] + p[Z > +4.75]$$

Using the area under the curve in Table B2, the probability of $z < +0.75$ is 1) 0.50 for the area below the mean; and 2) the probability of *z* at +0.75, which equals 0.2743, (sum = 0.7743). Similarly the probability of being >+4.75 is outside the table values, thus $p < 0.0001$. The combined probability for the power equation is:

$$power = 0.7743 + 0.0000 \approx 0.7743$$

In designing a study, sample sizes for each level of the discrete independent variable should be equal. The appropriate sample size per group for a given power ($1 - \beta$) can be estimated using the following formulas:

$$n = \frac{\left[\left(Z_{1-\alpha/2} \cdot \sqrt{2\hat{p}_0(1-\hat{p}_0)}\right) + \left(Z_\beta \cdot \sqrt{\hat{p}_1\hat{q}_1 + \hat{p}_2\hat{q}_2}\right)\right]^2}{\delta^2} \qquad \text{Eq. 15.11}$$

where $\hat{p}_0$ is the average of the two sample probabilities

$$p_0 = \frac{p_1 + p_2}{2} \qquad \text{Eq. 15.12}$$

and δ is the desired proportional difference we would like to be able to identify. Using the previous example, the estimated sample size to identify a 20% difference (δ = 0.20) with 80% power and a Type I error rate of 0.05, where $Z_{1-\alpha/2}$ is 1.96 and Z_β = 0.84, would be:

$$p_0 = \frac{0.245 + 0.429}{2} = 0.337$$

$$n = \frac{\left[\left(1.96 \cdot \sqrt{2(0.337)(0.663)}\right) + \left(0.84 \cdot \sqrt{(0.245)(0.755) + (0.429)(0.571)}\right)\right]^2}{(0.20)^2}$$

$$n = \frac{[1.3102 + 0.5508]^2}{(0.20)^2} = 86.6 \approx 87 \text{ volunteers}$$

Using these formulas for power and sample size it is possible to create different scenarios (Table 15.1). Notice that the results are similar to what would be expected based on the discussion in Chapter 8. As the differences increase the power increases. As the sample size increases the power increases.

z-Tests for Proportions – Yates' Correction for Continuity

In performing a z-test of proportions, the calculated *z*-value is based upon discrete or discontinuous data, but as discussed in Chapter 6 the normal standardized *z*-distribution is based on a continuous distribution. Therefore, the calculated *z*-values are only an approximation of the theoretical *z*-distribution. Therefore, Frank Yates (1934) argued that a more conservative approach was needed to estimate the *z*-statistic, which is more appropriate with the standardized normal distribution. In the

Table 15.1 Various Power and Sample Size Determinations*

For a Power of 80%		For a Difference of 20%	
δ	n	n	$1-\beta$
0.05	1,390	30	0.323
0.10	348	50	0.495
0.15	155	96	0.774
0.18	108	100	0.791
0.20	87	150	0.925

* Using the previous data comparing the two HIV therapies.

two-sample case, the Yates' correction for continuity is:

$$z = \frac{|\hat{p}_1 - \hat{p}_2| - \frac{1}{2}\left(\frac{1}{n_1} - \frac{1}{n_2}\right)}{\sqrt{\frac{\hat{p}_0(1-\hat{p}_0)}{n_1} + \frac{\hat{p}_0(1-\hat{p}_0)}{n_2}}} \qquad \text{Eq. 15.13}$$

Because of the smaller numerator, this will result in a slightly smaller calculated *z*-value, and a more conservative estimate. Obviously, as the sample sizes become smaller and we know less about the true population, there will be a decrease in the calculated *z*-value and an even more conservative answer.

Using this correction for continuity, we can recalculate the previous AIDS treatment example, where we were able to reject the null hypothesis and assumed that there was a better survival rate with the combination therapy. However, with Yates' correction:

$$z = \frac{|0.245 - 0.429| - \frac{1}{2}\left(\frac{1}{94} + \frac{1}{98}\right)}{\sqrt{\frac{0.339(0.661)}{94} + \frac{0.339(0.661)}{98}}}$$

$$z = \frac{0.184 - 0.010}{\sqrt{0.00467}} = \frac{0.174}{0.068} = 2.56$$

We again reject H_0 and with 95% confidence assume that there is a significant difference between the two therapeutic approaches. However, what we want is to be 99% confident in our decision ($|z| > 2.576$). In this case we would reject the null hypothesis with the first *z*-score, but fail to reject it with the Yates' correction.

Similarly, Yates' correction can be applied to the one-sample case (i.e., the previous example of tossing a fair coin):

Table 15.2 Comparison of Survival and Various Treatment Strategies

	Zidovudine alone	Zidovudine/ Trimethoprim	Zidovudine/ Drug A	Zidovudine/ Drug B
Lived	24	38	24	6
Died	70	60	86	51

$$z = \frac{|p - P_0| - \frac{1}{n}}{\sqrt{\frac{(P_0)(1-P_0)}{n}}} \qquad \text{Eq. 15.14}$$

Obviously the larger the amount of information (n), the smaller the correction factor. Recalculation of our previous example gives:

$$z = \frac{|0.65 - 0.50| - \frac{1}{20}}{\sqrt{\frac{(0.50)(0.50)}{20}}} = \frac{0.10}{0.11} = 0.91$$

We still fail to reject H_0, but the calculated z-value is much smaller (0.91 compared to 1.36).

Proportion Testing for More Than Two Levels of a Discrete Independent Variable

What if there are more than two levels of the discrete independent variable? Could the z-test of proportions be expanded beyond only two levels of the discrete independent variable? In these cases, it is best to establish a **contingency table** based on the frequency associated with each outcome. For example, assume in the previous zidovudine/trimethoprim study that there were actually four levels of treatment. The frequencies could be presented as a contingency table (Table 15.2). In this case a more appropriate test would be a chi square test of independence, where the interrelationship is measured between the survival rate and type of drug therapy received to determine if the two variables are independent of each other. This test will be discussed in the next chapter.

Using Minitab® for z-Tests for Proportions

Minitab offers applications for the one-sample and two-sample z-tests of proportions. These are accessed by choosing “Stat” on the title bar, then “Basic

Statistics" and the appropriate z-test:

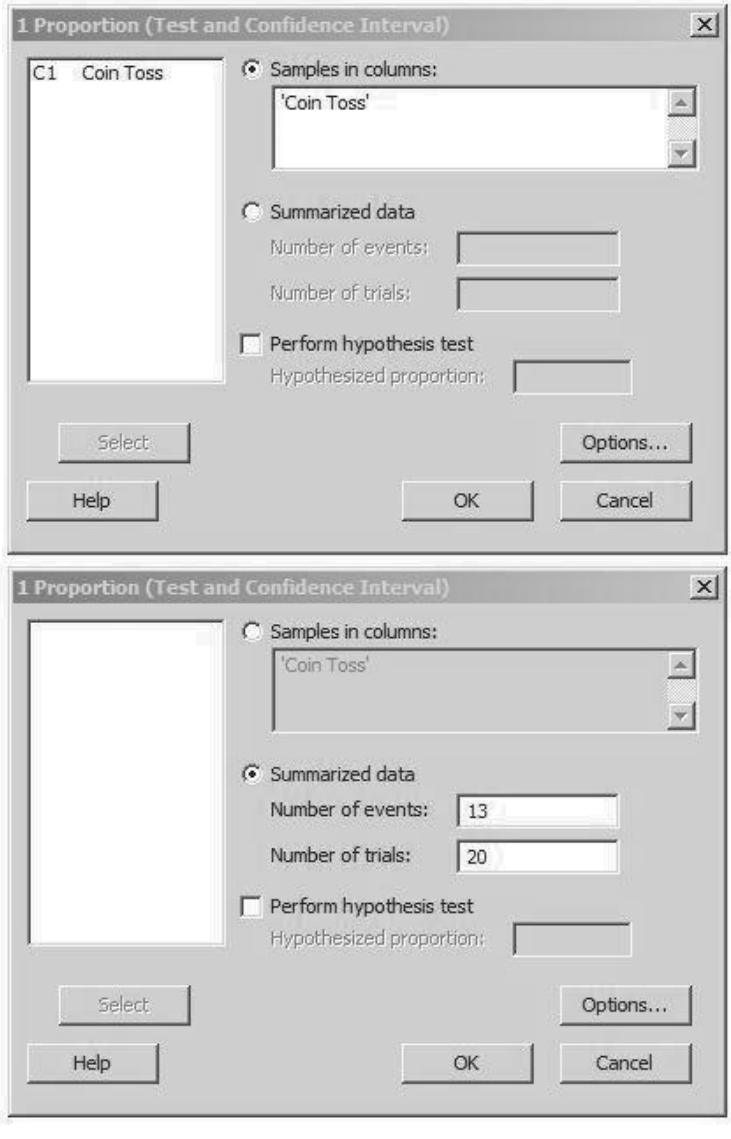


Figure 15.2 Options for a one-sample z-test of proportions with Minitab.

Stat ➤ Basic Statistics ➤ 1-proportion… one-sample CI
Stat ➤ Basic Statistics ➤ 2-proportions… two-sample t-test

As with previous examples, each column represents a variable and each row an observation. For the z-tests the data can be either nominal names or numbers; Minitab

will handle either as discrete data. Columns are chosen from the left box and placed in the right box for evaluation (top portion of Figure 15.2). *Options*... allows one to change the confidence interval from the default value of 95% or create a one-tailed interval. The result for the previous example of coin tossing is presented in the upper half of Figure 15.3. Minitab picks one of the two levels for evaluation and in this case chose "tails" but still created a confidence interval which included 0.50. In addition Minitab allows you to enter the frequency counts in the lower section "Summarized data" as illustrated in the lower portion of Figure 15.2. Here the "Number of trials:" would be the total number of possible events (e.g., 20 coin tosses) and the "Number of events:" is the number of outcomes of interest (e.g., 13 heads). The results appear in the lower half of Figure 15.3.

Minitab offers similar features for the two-sample z-test of proportions. As seen in Figure 15.4 information can be taken from specific columns on the left side or can be evaluated based on "Summary data." The results for the zidovudine and trimethoprim example are presented in Figure 15.5, with the "Summary data" approach in the second results. Also, as indicated in the middle of the decision options in Figure 15.4, data can be stacked in individual columns. Again, the *Options*... feature allows you to change the confidence interval from the default value of 95% or create a one-tailed interval.

References

Yates, F. (1934). "Contingency tables involving small numbers and the χ^2 test," *Royal Statistical Society Supplement* 1 (series B):217-235.

Zar, J.H. (2010). *Biostatistical Analysis*, Fifth edition, Prentice-Hall, Upper Saddle River, NJ, p. 549.

Test and CI for One Proportion: Coin Toss

```
Event = Tails

Variable   X   N  Sample p         95% CI
Coin Toss  7  20  0.350000  (0.153909, 0.592189)
```

Test and CI for One Proportion

```
Sample   X   N  Sample p         95% CI
1       13  20  0.650000  (0.407811, 0.846091)
```

Figure 5.3 Outcome report for a one-sample z-test of proportions with Minitab.

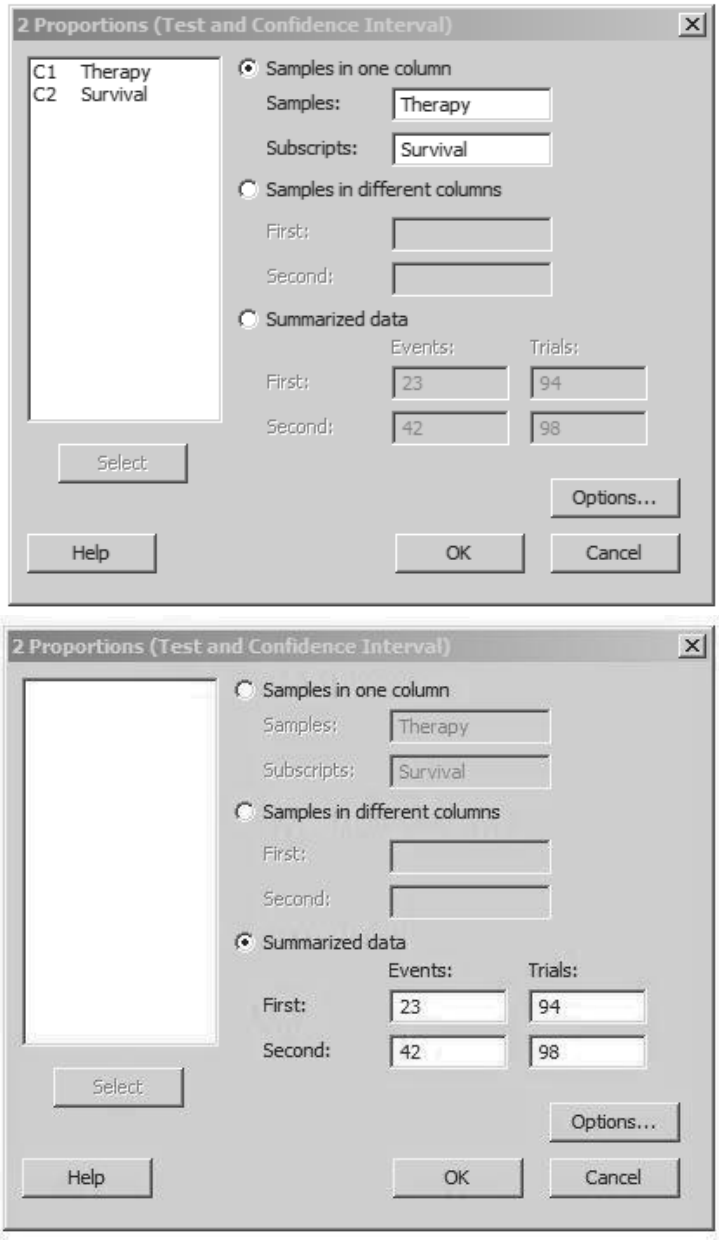


Figure 15.4 Options for a two-sample z-test of proportions with Minitab.

Test and CI for Two Proportions: Therapy, Survival

```
Event = Zido/Trim

Survival   X    N  Sample p
Died      56  127  0.440945
Lived     42   65  0.646154

Difference = p (Died) - p (Lived)
Estimate for difference:  -0.205209
95% CI for difference:  (-0.350015, -0.0604027)
Test for difference = 0 (vs not = 0):  Z = -2.78  P-Value = 0.005
```

Test and CI for Two Proportions

```
Sample   X   N  Sample p
1       23  94  0.244681
2       42  98  0.428571

Difference = p (1) - p (2)
Estimate for difference:  -0.183891
95% CI for difference:  (-0.314857, -0.0529238)
Test for difference = 0 (vs not = 0):  Z = -2.75  P-Value = 0.006
```

Figure 5.5 Outcome report for a two-sample z-test of proportions with Minitab.

Suggested Supplemental Readings

Bolton, S. and Bon, C. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, Inc., New York, pp. 131-134.

Glantz, S.A. (1987). *Primer of Biostatistics*, McGraw-Hill, New York, pp. 111-119.

Example Problems (Answers are provided in Appendix D)

1. During production runs, historically a specific dosage form is expected to have a defect rate of 1.5%. During one specific run, a sample of 100 tablets was found to have a defect rate of 5%. Does this differ significantly from what would normally be expected?

2. During initial Phase I and II studies, the incidence of nausea and vomiting of a new cancer chemotherapeutic agent was 36% for 190 patients, while 75 control patients receiving conventional therapy experienced nausea and vomiting at a rate of 55%.

    a. Is there a significant difference between the incidence of nausea and vomiting between these two drug therapies?

b. Did the new agent produce a significantly lower incidence of nausea and vomiting?

3. During the development of a final dosage form, the frequency of defects was analyzed to determine the effect of the speed of the tablet press. Samples were collected at 80,000 (lower) and 120,000 (higher) units per hour. Initially 500 tablets were to be collected at each speed; unfortunately due to an accident only 460 tablets were retrieved at the higher speed. Based on the following results were there any significant differences between the two tablet press speeds?

Speed	n	# of Defects
Low	500	11
High	460	17

4. During preapproval clinical trials with a specific agent, it was found that the incidence of blood dyscrasia was 2.5%. In a later Phase IV study involving 28 patients, two developed blood dyscrasia. Is this outcome possible or is there something unique about the population from which the sample was taken for this last clinical trial?

16

Chi Square Tests

The chi square tests are used when only discrete variables are involved. In the goodness-of-fit test there is one discrete variable. For the test of independence, two discrete variables are compared: one is usually independent (e.g., experimental versus control group) and the other variable is dependent upon the first (e.g., met goal versus did not meet goal). The chi square test, sometimes referred to as **Pearson's chi square**, evaluates the importance of the difference between what is expected (under given conditions) and what is actually observed. When criteria are not met for the chi square test of independence, the Fisher's exact test may be used. Pairing of dichotomous outcomes is possible using the McNemar test and the effects of a third possible confounding variable can be addressed using the Mantel-Haenszel test.

Chi Square Statistic

The chi square (χ^2) can best be thought of as a discrepancy statistic. It analyzes the difference between observed values and those values that one would normally expect to occur. It is calculated by determining the difference between the frequencies actually observed in a sample data set and the expected frequencies based on probability. Some textbooks classify χ^2 as a nonparametric procedure because it is not concerned with distributions about a central point and does not require assumptions of homogeneity or normality.

In the previous chapter, the z-tests of proportion evaluated the results of a coin toss. This one-sample case was a measure of discrepancy, with the numerator representing the difference between the observed frequency (p) and the expected population results for a fair coin (P_O) (Eq. 15.2). With the z-tests in Chapter 15, we were concerned with proportions, or percentages, and these were used with the appropriate formulas. With the chi square statistics, the frequencies are evaluated. The calculation involves squaring the differences between the observed and expected frequencies divided by the expected frequency. These results are summed for each cell in a contingency table or for each level of the discrete variable.

$$\chi^2 = \sum \frac{(f_O - f_E)^2}{f_E} \qquad \text{Eq. 16.1}$$

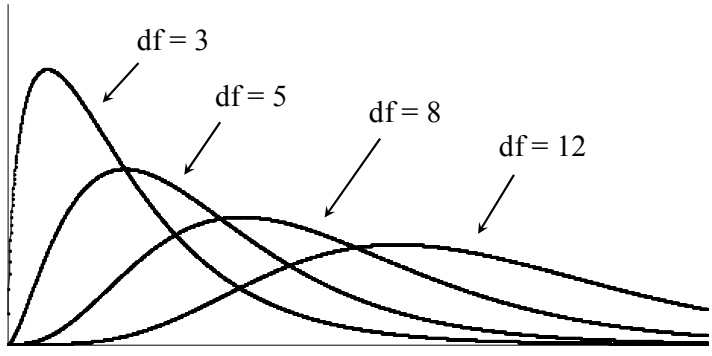


Figure 16.1 Various chi square distributions.

This formula can be slightly rewritten as follows:

$$\chi^2 = \Sigma\left[\frac{(Observed - Expected)^2}{Expected}\right]$$

or

$$\chi^2 = \Sigma\frac{(O-E)^2}{E} \qquad \text{Eq. 16.2}$$

Obviously, if all of the observed and expected values are equal for each level of the discrete variable the numerator is zero and the χ^2-statistic will be zero. Similar to the z-test, as the differences between the observed and expected frequencies increase, the numerator will increase and the resultant χ^2-value will increase. Because the numerator is squared, the resultant value must be equal to or greater than zero. Therefore at a certain point in the continuum from zero to positive infinity, the calculated χ^2-value will be large enough to indicate that the difference cannot be due to chance alone. Like the z-, t-, and F-tests, critical values for the chi square distribution are presented in tabular form (Table B15, Appendix B). Like the *t*- and *F*-distributions, there is not one single χ^2 distribution, but a set of distributions. The characteristics of each distribution are dependent on the number of degrees of freedom (Figure 16.1). As the number of degrees of freedom increases, the skew in the distribution decreases and the curve approaches a normal distribution. The first column on the left side of Table B15 indicates the number of degrees of freedom (determination of which will be discussed later) and the remaining columns are the critical chi square values at different acceptable levels of a Type I error (α).

The decision rule is written similarly to previous tests. For example, assume we are dealing with four degrees of freedom and wish to have a 95% level of confidence. The decision rule would be: with $\alpha = 0.05$, reject H_0 if $\chi^2 > \chi^2_4(0.05) = 9.448$. If the

calculated statistic derived from the formula (Eq. 16.2) is larger than the critical (9.448), the null hypothesis (the observed and expected values are the same) is rejected.

Chi Square Goodness-of-Fit for One Discrete Dependent Variable

As seen in the previous chapter, the z-tests of proportions were limited to only one or two levels of a discrete dependent variable. If the frequency counts are used (instead of proportions), the chi square test can be used and expanded to more than two levels. For example, assume a guidance counselor want to determine if the entry class of Pharm.D. students differs from previous classes based on where they obtained their pre-pharmacy course work. Traditionally incoming pharmacy students have represented approximately 60% from the parent campus, 25% from other in-state schools in the same university system, 5% from in-state non-system schools and 10% from out-of-state institutions. This year's entrance class is distributed as follows:

	Actual Students
Parent institution	85
In-state system school	35
In-state non-system school	5
Out-of-state school	25
Total	150

If the distribution was the same as previous years, based on 150 entry students, the expected number of students should be:

	Expected Students	
Parent institution	90	(150 × 0.60)
In-state system school	37.5	(150 × 0.25)
In-state non-system school	7.5	(150 × 0.05)
Out-of-state school	15	(150 × 0.10)
Total	150	

The null hypothesis would be that the incoming Pharm.D. students are distributed the same as previous classes based on the type on institution where they received their pre-pharmacy training. In selecting the appropriate critical χ^2-value, the number of degrees of freedom is one less than the number of levels of the discrete variable (k). Once again the $k - 1$ degrees of freedom is selected to correct for bias. In this example, since there are four types of institutions being tested, the number of degrees of freedom is three. The decision rule, assuming 95% confidence is: with $\alpha = 0.05$, reject H_0 if $\chi^2 > \chi^2_3(0.05) = 7.8147$. The value 7.8147 is found in Table B15 at the intercept of the third row (degrees of freedom equal to three) and the second column of critical values ($\alpha = 0.05$). If there were no differences between the observed institutions and the expected enrollment, we would expect to see similar proportions. The χ^2-statistic would be calculated as follows:

	Observed	Expected	O – E	$(O - E)^2/E$
Parent institution	85	90	–5.0	0.278
In-state system school	35	37.5	–2.5	0.167
In-state non-system school	5	7.5	–2.5	0.833
Out-of-state school	25	15	+10.0	6.667
			$\chi^2 =$	7.945

Based on the results of the chi square test, with the calculated χ^2 greater than 7.8147; we reject the null hypothesis and conclude that there is a significant difference between the characteristics of the new students compare to what is traditionally expected in an entry class based on institution where they received their previous education.

Unfortunately the chi square test does not indicate where the significant difference(s) exist between of the observed and expected results. The simplest way to estimate the major difference(s) is to observe which level(s) of the discrete variable contribute the most to a significant chi square statistic. This would be represented by the largest $(O-E)^2/E$. In this example it would be Out-of-State Schools which contributed 83.9% (6.667/7.945) of the total deviation from expected. Alternatively, one could perform two additional chi square tests to determine if the Out-of-State School proportion is significantly different previous experience by: 1) performing a second chi square test without the data for Out-of-State Schools; and 2) performing a third chi square on Out-of-State Schools to determine if it differs significantly form the average of the other three school sources.

The second chi square would be tested against a critical chi square value with two degrees of freedom (k - 1) which is 5.9915 (Table B15). The calculation would be as follows:

	Observed	Expected	O – E	$(O - E)^2/E$
Parent institution	85	90	–5.0	0.278
In-state system school	35	37.5	–2.5	0.167
In-state non-system school	5	7.5	–2.5	0.833
			$\chi^2 =$	1.278

The calculated χ^2 is less than 5.9915 and we fail to reject the null hypothesis that the proportions for these three types of institutions are the same as in previous years. Therefore, our best guess is that the proportions of students from the three remaining instructions are distributed similarly to previous years and only the out-of-state institutions are disproportionately represented.

The third chi square would be tested against a critical chi square value with one degree of freedom comparing out-of state schools to the composite for the three other types of institutions. In this case the critical value for one degree of freedom is 3.8415 (Table B15). The calculation would be as follows:

	Observed	Expected	O – E	$(O - E)^2/E$
In-state school	125	135	–10.0	0.741
Out-of-state school	25	15	+10.0	6.667
			$\chi^2 =$	7.408

Here calculated χ^2 is greater than the critical value of 3.8415, so we would reject the null hypothesis and conclude that there is a significantly greater proportion of out-of-state school students in the entering Pharm.D. class.

Chi Square for One Discrete Dependent Variable and Equal Expectations

If different batches of a particular product are compared, where production was similar, we could expect to see an equal result for some measure of the final product regardless of the batch tested. For example, assume that we wish to compare four lots of a particular drug for some minor undesirable trait (e.g., a blemish on the tablet coating). We randomly sample 1000 tablets from each batch and examine the tablets for that trait. The results of the experiment are as follows:

	Number of Tablets with Blemishes
Batch A	12
Batch B	15
Batch C	10
Batch D	9

A simple hypothesis to evaluate this data could be as follows:

H_0: The samples are selected from the same population
H_1: The samples are from different populations

Our best estimate of expected frequency is the average of the sample frequencies:

$$f_E = \frac{\sum frequencies\ per\ level}{number\ of\ levels} = \frac{\sum f_i}{k_i} \qquad \text{Eq. 16.3}$$

In this particular case:

$$f_E = \frac{12 + 15 + 10 + 9}{4} = 11.5$$

Therefore the χ^2-statistic would be calculated as follows:

	Observed	Expected	O – E	$(O - E)^2/E$
Batch A	12	11.5	+0.5	0.02
Batch B	15	11.5	+3.5	1.07
Batch C	10	11.5	–1.5	0.20
Batch D	9	11.5	–2.5	0.54
			χ^2 =	1.83

Based on the results of the chi square test, with the calculated χ^2 less than 7.8147, we fail to reject the null hypothesis. Therefore, our best guess is that they are from the same population; in other words, there is no difference among the four batches.

For a second example, refer to Table B1 in Appendix B in the back of this book. If numbers in this table are truly random we would expect an equal number of 0s, 1s, 2s, … and 9s. With 35 rows and 50 columns, there are 1,750 integers in the table; therefore we would expect 175 of each number (1,750/10). However, what we observe is some variation from these expected values:

Integer	0	1	2	3	4	5	6	7	8	9
Count	173	200	174	165	168	173	166	177	191	163

Are these differences significant or could we expect this variability by chance alone, since we are dealing with only 1,750 observations and not an infinite number of data points? To test the statistic, the critical value for chi square with nine degrees of freedom is 16.919 and the calculations are as follows:

	Observed	Expected	O – E	$(O - E)^2$
0	173	175	–2	4
1	200	175	+25	625
2	174	175	–1	1
3	165	175	–10	100
4	168	175	–7	49
5	173	175	–2	4
6	166	175	–9	81
7	177	175	+2	4
8	191	175	+16	256
9	163	175	–12	144
			Σ =	1.268

With the chi square well less than the critical value of 16.919, we cannot reject the hypothesis that there is an equal distribution of numbers in the random numbers table.

Chi Square Goodness-of-Fit Test for Distributions

All chi square tests can be thought of as goodness-of-fit procedures because they compare what is observed to what is expected in the hypothesized distribution. However, the term goodness-of-fit is reserved for comparisons of a sample distributions to determine if the observed set of data is distributed as expected by a preconceived distribution (the previous example could be considered as goodness-of-fit test for a uniform or rectangular distribution). It is assumed that the sample distribution is representative of the population from which it is sampled. Sample observations are placed into mutually exclusive and exhaustive categories, and the frequencies of each category are noted and compared to expected frequencies in the hypothetical distribution. The following are examples of the use of this method for both normal and binomial distributions.

Goodness-of-Fit for a Normal Distribution. The chi square goodness-of-fit test, can be used to determine if a sample is selected from a population that is normally distributed. The underlying assumption is that the sample distribution, because of

Table 16.1 Determination of Expected Values

Interval Range	Expected Values below the Largest Value in Each Class Interval	Expect Values within Range
705.5 to 716.5	1.74	1.74
716.5 to 727.5	7.57	5.83
727.5 to 738.5	23.01	15.44
738.5 to 749.5	52.59	29.58
759.5 to 760.5	84.20	31.61
760.5 to 771.5	109.36	25.16
771.5 to 782.5	120.51	11.15
782.5 to 793.5	125.00	4.49
	$\sum =$	125

random sampling, is reflective of the population from which it is sampled. Therefore, if the sample has characteristics similar to what is expected for a normal distribution, one cannot reject the hypothesis that the population is normally distributed.

H_0: Population is normally distributed
H_1: H_0 is false

Since many statistical procedures assume that sample data are drawn from normally distributed populations it is useful to have a method to evaluate this assumption. The chi square test provides an excellent method, but should be restricted to sample sets with 50 or more observations. For example, using Sturges' rule, the distribution presented in Table 16.1 is created from the data presented and discussed in Chapter 4. If this sample distribution is the best estimation of the population from which it was sampled, is the population in question normally distributed? Obviously, the greater the discrepancy between what is expected and what is actually observed, the less likely the difference is attributed to chance alone and the greater the likelihood that the sample is not from a normally distributed population. The test statistic would be Eq. 15.2:

$$\chi^2 = \sum \left[\frac{(O - E)^2}{E} \right]$$

Degrees of freedom are based on the number of categories or class intervals and a number of estimated values. In order to calculate areas within a normal distribution, and by extension the frequencies, one needs to know both the population mean and population standard deviation (Eq. 6.3):

$$z = \frac{x - \mu}{\sigma}$$

Calculation of z-values provides probabilities associated with the dividing points (boundaries) for our class intervals. The sample mean and standard deviation are the best available estimates of the population:

$$\overline{X} \approx \mu$$
$$S \approx \sigma$$

Therefore our best estimate of z-values would be an approximation based on our sample measurements:

$$z = \frac{x - \overline{X}}{S} \qquad \text{Eq. 16.4}$$

These estimates will affect the degrees of freedom associated with the critical value in Table B15. Because we are estimating two population parameters, each is subtracted from the number of levels of the dependent discrete variable. One additional degree is subtracted to control for bias. Thus, the degrees of freedom equal the number of levels minus three ($k - 3$); one for the estimate of the population mean; one for the estimate of the population standard deviation and one for bias. In the above example, degrees of freedom equal eight levels minus three, or five degrees of freedom. The decision rule is

$$\text{with } \alpha = 0.05, \text{ reject } H_0 \text{ if } \chi^2 > \chi^2_5(0.05) = 11.070$$

Based on the discussion in Chapter 6, we can use the information presented about areas under the curve of a normal distribution to estimate the expected frequencies in each interval of this sample distribution, if the population is normally distributed. For example, with a sample of 125 observations, if normally distributed, how many observations would be expected below 716.5 mg? The first step is to determine the *z*-value on a normal distribution representing 716.5 mg.

$$Z = \frac{x - \overline{X}}{S} = \frac{716.5 - 752.9}{16.5} = \frac{-36.4}{16.5} = -2.20$$

where 752.9 was the sample mean for the 125 data points and 16.5 was the sample standard deviation. Looking at the standardized normal distribution (Table B2, Appendix B) the area under the curve between the mean (0) and $z = -2.20$ is 0.4861. The proportion, or area under the curve, falling below the *z*-value is calculated by subtracting the area between the center and *z*-value from 0.5000, which represents all the area below the mean.

$$p(< 2.20) = 0.5000 - 0.4861 = 0.0139$$

The expected number of observations is the total number of observations multiplied by the proportion of the curve falling below $z = -2.20$:

$$E(<716.5) = 125(0.0139) = 1.74$$

Using this same method, it is possible to estimate the number of observations expected to be below 727.5 in a normal distribution (the greatest value in the second class interval).

$$Z = \frac{x - \overline{X}}{S} = \frac{727.5 - 752.9}{16.5} = \frac{-25.4}{16.5} = -1.54$$

$$p(<-1.54) = 0.5000 - 0.4394 = 0.0606$$

$$E(<727.5) = 125(0.0606) = 7.57$$

Continuing this procedure it is possible to calculate all areas below given points in the proposed normal distribution (Table 16.1, second column). By default, if all the observations are represented under the area of the curve, then the expected number of observations below the upper value of the highest interval must include all of the observations (in this case 125).

Unfortunately, we are interested in not only the areas below given points on the distribution, but also areas between the boundaries of the class intervals. Therefore, the number of observations expected between 716.5 and 727.5 is the difference between the areas below each point:

Expected (Range 716.5 to 727.5) = E(<727.5) – E(<716.5)
Expected (Range 716.5 to 727.5) = 7.57 – 1.74 = 5.83

Expected (Range 727.5 to 738.5) = E(<738.5) – E(<727.5)
Expected (Range 727.5 to738.5) = 23.01 – 7.57 = 15.44

Using this same procedure it is possible to determine the expected results for the remaining categories and create a table (Table 16.1, third column). The expected

Table 16.2 Comparison of Observed and Expected Data

Interval	Observed	Expected	(O – E)	$(O - E)^2/E$
705.5 to 716.5	2	1.74	0.26	0.039
716.5 to 727.5	6	5.83	0.17	0.005
727.5 to 738.5	18	15.44	2.56	0.424
738.5 to749.5	22	29.58	–7.58	1.942
759.5 to760.5	35	31.61	3.39	0.364
760.5 to771.5	28	25.16	2.84	0.321
771.5 to782.5	10	11.15	–1.15	0.119
782.5 to 793.5	4	4.49	–0.49	0.053
			$\chi^2 = \Sigma =$	3.267

amounts reflect a normal distribution. The chi square statistic is then computed comparing what is expected if the population distribution is normal to what was actually observed in the sample distribution. The greater the difference, the more likely one is to reject the hypothesis that the population represented by the sample is normally distributed. The chi square is a calculation using the data presented in Table 16.2. The decision is, with $\chi^2 < 11.070$, to not reject H_0 and conclude that we are unable to reject the hypothesis that the population is normally distributed. This process is laborious, but useful when evaluating data where it is important to determine if the population is normally distributed. Similar to previous tests, we cannot prove the null hypothesis and we simply cannot reject the possibility that the population from which our data were selected might be normally distributed.

Goodness-of-Fit for a Binomial Distribution. To illustrate the use of the chi square goodness-of-fit test for a binomial distribution, assume that four coins are tossed at the same time. This procedure is repeated 100 times. Based on the following results, are these "fair" coins?

0 heads	15 times
1 head	30 times
2 heads	32 times
3 heads	17 times
4 heads	6 times

From the discussion of probability in Chapter 2, using factorials, combinations, and the binomial equation (Eq. 2.12), it is possible to produce the theoretical binomial distribution given the coins are fair, p(head) = 0.50. For example, the probability of tossing only one head is:

$$p(x) = \binom{n}{x} p^x q^{n-x}$$

$$p(1) = \binom{4}{1} (0.5)^1 (0.5)^3 = 0.25$$

Table 16.3 Expected Outcomes from Tossing Four Coins

Outcome	p(x)	Frequency for 100 Times
0 heads	0.0625	6.25
1 head	0.2500	25.00
2 heads	0.3750	37.50
3 heads	0.2500	25.00
4 heads	0.0625	6.25

Table 16.4 Data for Comparing Observed and Expected Results for Four Tossed Coins

	Observed	Expected	(O-E)	$(O-E)^2/E$
0 heads	15	6.25	8.75	12.25
1 head	30	25.00	5.00	1.00
2 heads	32	37.50	–5.50	0.81
3 heads	17	25.00	–8.00	2.56
4 heads	6	6.25	–0.25	0.01
			$\chi^2 =$	16.63

A table can be produced for the probability of all possible outcomes. If the four coins are fair these would produce the expected outcomes displayed in Table 16.3. The comparison is made for the discrepancy between what was actually observed with 100 coin tosses and what was expected to occur if the p(head) was in fact 0.50. Is the discrepancy just due to change error or large enough to be significant? The hypotheses would be:

H_0: Population is a binomial distribution with $p = 0.50$
H_1: H_0 is false

The test statistic remains the same (Eq. 16.2):

$$\chi^2 = \Sigma\left[\frac{(O-E)^2}{E}\right]$$

The decision rule is: with $\alpha = 0.05$, reject H_0 if $\chi^2 > \chi^2_3(0.05) = 7.8147$. Here the degrees of freedom are based upon the number of discrete intervals minus two (k - 2); one degree of freedom is subtracted because we are estimating the population proportions (p) and one is subtracted to prevent bias. The data required for computing the χ^2-statistic is presented in Table 16.4. Based on 100 coin tosses, the decision is with $\chi^2 > 7.8147$, reject H_0, conclude that the sample does not come from a binomial distribution with p(head) = 0.50. The coins are not fair.

Chi Square Test of Independence

The most common use of the chi square test is to determine if two discrete variables are independent of each other. With this test we are concerned with conditional probability: what the probability is for some level of variable A given a certain level of variable B (Eq. 2.6)

$$p(A) \text{ given } B = p(A \mid B) = \frac{p(A \cap B)}{p(B)}$$

If the two discrete variables are independent of each other, then the probability of each level should be the same regardless of which level of the *B* characteristic it contains.

		Levels of the First Variable				
		A_1	A_2	A_3	...	A_K
Levels of the Second Variable	B_1				...	
	B_2				...	
	...	...	...	...	...	
	B_K				...	

Figure 16.2 Design of the contingency table, chi square test of independence.

$$p(A_1|B_1) = p(A_1|B_2) = ...p(A_1|B_k) = p(A_1) \qquad \text{Eq. 16.5}$$

A contingency table is created where frequency of occurrences is listed for the various levels of each variable. This **contingency table** is used to determine whether two discrete variables are contingent or dependent on each other. The table has a finite number of mutually exclusive and exhaustive categories in the rows and columns (Figure 16.2). Such a design is a "$K \times J$" contingency with K rows, J columns, and $K \times J$ cells. This bivariate table can be used to predict if two variables are independent of each other or if an association exists. The hypothesis under test implies that there is no relationship (complete independence) between the two variables and that each is independent of the other.

H_0: $P(B_1|A_1) = P(B_1|A_2) = P(B_1|A_3) \ldots = P(B_1|A_K) = P(B_1)$
$P(B_2|A_1) = P(B_2|A_2) = P(B_2|A_3) \ldots = P(B_2|A_K) = P(B_2)$
...
$P(B_K|A_1) = P(B_K|A_2) = P(B_K|A_3) \ldots = P(B_K|A_K) = P(B_K)$

H_1: H_0 is false

The chi square test of independence tests the hypothesis that two variables are related only by chance. A simpler terminology for the two previous hypotheses is:

H_0: Factor *B* is independent of Factor *A*
H_1: Factor *B* is not independent of Factor *A*

Thus, in the null hypothesis, the probability of B_1 (or B_2 ... or B_M) remains the same regardless of the level of the second variable, *A*. If we fail to reject H_0, the two variables have no systematic association and could also be referred to as **unrelated, uncorrelated**, or **orthogonal variables.** Once again the test statistic (Eq. 16.2) is:

$$\chi^2 = \Sigma\left[\frac{(O-E)^2}{E}\right]$$

Much like the goodness-of-fit model, if there is complete independence the difference between the observed and expected outcomes will be zero. As the difference in the numerator increases the calculated χ^2-value will increase and eventually exceed a critical value; past that point the difference cannot be attributed to chance or random variability. To determine the critical value, the degrees of freedom are based on the number of rows minus one ($K - 1$) times the number of columns minus one ($J - 1$). This is based on a contingency table such as the following:

	A_1	A_2	A_3	A_4	
B_1					100
B_2					200
B_3					100
	100	100	100	100	400

If we know the information for any six cells [$(J - 1)(K - 1)$] the remaining cells within the table would become automatically known and having no freedom to vary. With the following information for six cells (example bolded) the remaining cells could be easily determined and these last six cells have no freedom to change once the first six are identified.

	A_1	A_2	A_3	A_4	
B_1	**26**	**18**	10	46	100
B_2	43	**56**	**68**	33	200
B_3	**31**	26	22	**21**	100
	100	100	100	100	400

The decision rule is:

with $\alpha = 0.05$, reject H_0 if χ^2 is greater than $\chi^2_{(J-1)(K-1)}(\alpha)$.

In the case of four columns and three rows, the critical chi square value with $\alpha = 0.05$ from Table B15 is:

$$\chi^2_{(3)(2)}(\alpha) = \chi^2_6(\alpha) = 12.592$$

The expected value for any cell is calculated by multiplying its respective row sum by its respective column sum and dividing by the total number or the grand sum.

$$E = \frac{\Sigma C \cdot \Sigma R}{\Sigma Total} \qquad \text{Eq. 16.6}$$

To illustrate this, the calculations for the expected values for a three by two contingency table would be:

(C1 × R1)/T	(C2 × R1)/T	(C3 × R1)/T	∑ = R1
(C1 × R2)/T	(C2 × R2)/T	(C3 × R2)/T	∑ = R2
∑ = C1	∑ = C2	∑ = C3	∑∑ = T

For example, in a pharmacology study mice of various age groups are administered a chemical proposed to induce sleep. After 30 minutes the animals are assessed to determine if they are asleep or awake (based on some predetermined criteria). The purpose of the study is to determine if the particular agent is more likely to induce sleep in different age groups. The study results are as follows:

	Asleep (C_1)	Awake (C_2)
3 months (R_1)	7	13
10 months (R_2)	9	11
26 months (R_3)	15	5

Simply stated, the hypothesis under test is that age does not influence sleep induction by the proposed agent being tested.

H_0: $P(C_1|R_1) = P(C_1|R_2) = P(C_1|R_3) = P(C_1)$
$P(C_2|R_1) = P(C_2|R_2) = P(C_2|R_3) = P(C_2)$
H_1: H_0 is false

Or simply stated:

H_0: Sleep is independent of the age of the mice
H_1: H_0 is false, a relationship exists

The decision rule is, with $\alpha = 0.05$, reject H_0 if $\chi^2 > \chi^2_2(0.05) = 5.99$. A comparison of the observed and expected values are as follows:

Observed

	C_1	C_2	∑
R_1	7	13	20
R_2	9	11	20
R_3	15	5	20
∑	31	29	60

Expected

C_1	C_2
10.3	9.7
10.3	9.7
10.3	9.7

Calculation of the chi square statistic is:

Table 16.5 Original Data for Example Comparing Age Groups and Incidence of Side Effects

	Age in Years				
Side Effects	<18	18–45	46–65	>65	
None	80	473	231	112	896
Mild	9	68	43	27	147
Moderate	2	24	8	8	42
Severe	1	5	3	6	15
Total	92	570	285	153	1100

$$\chi^2 = \frac{(7-10.3)^2}{10.3} + \frac{(13-9.7)^2}{9.7} + \frac{(9-10.3)^2}{10.3} + \ldots \frac{(5-9.7)^2}{9.7} = 6.94$$

The decision based on a sample of 60 mice is that with $\chi^2 > 5.99$, reject H_0 and conclude that age does influence the induction of sleep by this particular chemical. It appears that the agent has the greatest effect on the older animals.

For the chi square test of independence there are two general rules: 1) there must be at least one observation in every cell, no empty cells and 2) the expected value for each cell must be equal to or greater than five. The chi square formula is theoretically valid only when the expected values are sufficiently large. If these criteria are not met, adjacent rows or columns should be combined so that cells with extremely small values or empty cells are combined to form cells large enough to meet the criteria. To illustrate this consider the following example of a multicenter study where patients were administered an experimental aminoglycoside for Gram negative infections. The incidences of side effects are reported in Table 16.5. Is there a significant difference in the incidence of side effects based upon the ages of the patients involved in the study?

Unfortunately, an examination of the expected values indicates that four cells fall below the required criteria of an expected value of at least five (Table 16.6). One

Table 16.6 Original Expected Values for Age Groups and Incidence of Side Effects

	Age in Years				
Side Effects	<18	18–45	46–65	>65	
None	74.94	464.29	232.15	124.62	896
Mild	12.30	76.17	38.08	20.45	147
Moderate	3.51	21.77	10.88	5.84	42
Severe	1.25	7.77	3.89	2.09	15
Total	92	570	285	153	1100

Table 16.7 Expected Values Resulting from Collapsing Side Effects

	Age in Years				
Side Effects	<18	18–45	46–65	>65	
None	74.94	464.29	232.15	124.62	896
Mild	12.30	76.17	38.08	20.45	147
Moderate/Severe	4.76	29.54	14.77	7.93	57
Total	92	570	285	153	1100

Table 16.8 Expected Values Resulting from Collapsing Both Age Groups and Side Effects

	Age in Years			
Side Effects	18–45	46–65	>65	
None	539.23	232.15	124.62	896
Mild	88.47	38.08	20.45	147
Moderate/Severe	34.30	14.77	7.93	57
Total	662	285	153	1100

Table 16.9 Observed Outcomes with Collapsing Both Age Groups and Side Effects

	Age in Years			
Side Effects	<46	46–65	>65	
None	553	231	112	896
Mild	77	43	27	147
Moderate/Severe	32	11	14	57
Total	662	285	153	1100

method for correcting this problem would be to combine the last two rows (moderate and severe side effects) and create a 3 × 4 contingency table (Table 16.7). This combination of adjacent cells is more logical than combining the severe side effects with either the mild side effects or the absence of side effects. However, one cell still has an expected value less than five. The next logical combination would be the first two columns (ages <18 and 18–45 years old) as presented in Table 16.8. This 3 × 3 design meets all of the criteria for performing a chi square analysis. Adjusting the initial observed data to the new 3 –3 design is presented in Table 16.9. It should be noted that the number of data points is the same as those in the original 4 × 4 design and we have not sacrificed any of our information by collapsing the cells. The new hypotheses would be:

H_0: $P(S_1 \mid A_1) = P(S_1 \mid A_2) = P(S_1 \mid A_3) = P(S_1)$
$P(S_2 \mid A_1) = P(S_2 \mid A_2) = P(S_2 \mid A_3) = P(S_2)$
$P(S_3 \mid A_1) = P(S_3 \mid A_2) = P(S_3 \mid A_3) = P(S_3)$
H_1: H_0 is false

or:

H_0: Severity of side effects is independent of age group
H_1: H_0 is false, a relationship exists

and the decision rule would be: with $\alpha = 0.05$, reject H_0 if $\chi^2 > \chi_4^2\ (0.05) = 9.4877$. Note the decrease from the original nine degrees of freedom (4 – 1 rows times 4 – 1 columns) to the new four degrees of freedom. The calculation for the chi square statistic would be:

$$\chi^2 = \frac{(553 - 539.23)^2}{539.23} + \frac{(231 - 232.15)^2}{232.15} + \ldots \frac{(14 - 7.93)^2}{7.93}$$

$$\chi^2 = 11.62$$

The decision is, with $\chi^2 > 9.4877$, reject H_0, conclude that there is a significant difference in side effects based on age.

If the chi square data for the test of independence is reduced to the smallest possible design, a 2 × 2 contingency table, and the expected values are still too small to meet the requirements (no empty cells and every expected value ≥5 per cell), then the **Fisher's exact test** should be considered (below).

The chi square test of independence provides little information about the strength or type of relationship between the two variables. Ways of assessing such associations are discussed in Chapter 17.

Chi Square Test for Trend for Ordinal Classifications

In the previous example of the incidence of side effects in the multicenter study of an experimental aminoglycoside, assume the study involved two different dosage forms of the same medication, tablets and a suspension. The types of side effects reported, based on the dosage form are as follows:

	None	Mild	Moderate	Severe	
Tablet	463	68	15	5	551
Suspension	433	79	27	10	549
	896	147	42	15	1100

If this data were to be evaluated using the chi square test of independence, with three degrees of freedom ($C - 1$ times $R - 1$), it would not be significant ($\chi^2 = 6.919$; with the critical value = 9.3484). However, there does appear to be a trend with the

suspension tending to cause more of the moderate and severe side effects. The severity of side effects is in an ordinal arrangement; therefore, it is possible to modify the data and test for a trend using a Student t-test.

To test for a trend, arbitrary values can be assigned to each of the ordinal levels. In this example assume a simple linear assignment of values of 0 for none, 1 for mild, 2 for moderate, and 3 for severe side effects. A table can be created to calculate the mean and standard deviation for the weighted scores for the tablet (T) and the suspension (S) dosage forms:

	Tablet			Suspension		
Weight (w)	fx_T	wfx_T	w^2fx_T	fx_S	wfx_S	w^2fx_S
0	463	0	0	433	0	0
1	68	68	68	79	79	79
2	15	30	60	42	84	168
3	5	15	45	15	45	135
Σ =	551	113	173	569	208	382

From the sums listed above Eqs. 5.2, 5.4 and 9.3 can be used to calculate the sample means and sample variances for the two dosage forms as well as a pooled variance:

	Tablet		Suspension
Mean =	0.205		0.365
Variance =	0.272		0.539
Pooled Variance =		0.408	

To determine if there is a trend in the data, a two-sample t-test (Eq. 9.6) can be used to evaluate significance. In this case the critical *t*-value (α = 0.05) is with 1098 degrees of freedom (551+569–2) is 1.96. The calculation would be:

$$t = \frac{\overline{X}_T - \overline{X}_S}{\sqrt{\frac{S_P^2}{n_1} + \frac{S_P^2}{n_2}}} = \frac{0.205 - 0.365}{\sqrt{\frac{0.408}{551} + \frac{0.408}{549}}} = -4.205$$

which is significant (even at a level of $p < 0.001$). Therefore, one could assume a significant trend with the suspension causing a significantly greater proportion of the more severe side effects.

One of the concerns with this test is the arbitrary selection of the weighted values and two investigators might assign different weights to the different levels of the discrete variables. In this example different ordinal weights do not substantially change the results:

Various Weights	*t*-statistic
0, 1, 2, 3 (original example)	–4.205
0, 1, 3, 5	–4.301
–1, 1, 5, 7	–4.371

a	b	a + b
c	d	c + d
a + c	b + d	n

Figure 16.3 Format for defining the contents of a 2 × 2 contingency table.

Yates' Correction for a Two-by-Two Contingency Table

A 2 row by 2 column (two-by-two or written 2 × 2) contingency table also could be set up by designating the four cells as *a*, *b*, *c*, and *d* (Figure 16.3). This particular format will be used for several tests in this and the following three chapters. Using these letters, another way to calculate χ^2 for a 2 × 2 design (which would produce the identical same results as Equation 15.2), is:

$$\chi^2 = \frac{n(ad - bc)^2}{(a+c)(b+d)(a+b)(c+d)} \qquad \text{Eq. 16.7}$$

As an example, consider the following data. A new design in shipping containers for ampules is compared to the existing one to determine if the number of broken units can be reduced. One hundred shipping containers of each design are subjected to identical rigorous abuse and failures are defined as broken ampules in excess of 1%. The results of the study are presented in Table 16.10. Notice the exact percent of breakage is ignored in favor of a success/failure criterion. Do the data suggest the new design is an improvement over the one currently used? In this case the expected values would be:

92.5	92.5
7.5	7.5

and the definitional calculation for the chi square statistic (Eq. 16.2) would be:

$$\chi^2 = \Sigma \frac{(O - E)^2}{E}$$

Table 16.10 Data Comparing Two Container Designs

Results	New Container	Old Container	Total
Success	97	88	185
Failure	3	12	15
Totals	100	100	200

$$\chi^2 = \frac{(97 - 92.5)^2}{92.5} + \frac{(88 - 92.5)^2}{92.5} + \frac{(3 - 7.5)}{7.5} + \frac{(12 - 7.5)^2}{7.5} = 5.84$$

Using the alternate formula (Eq. 16.7), the same results are obtained:

$$\chi^2 = \frac{n(ad - bc)^2}{(a+b)(c+d)(a+c)(b+d)}$$

$$\chi^2 = \frac{200(97(12) - 3(88))^2}{(100)(100)(185)(15)} = \frac{162{,}000{,}000}{27{,}750{,}000} = 5.84$$

In this particular example the hypotheses would be:

H_0: Success or failure is independent of container style
H_1: H_0 is false

or more accurately:

H_0: $P(S_1 \mid C_1) = P(S_1 \mid C_2) = P(S_1)$
$P(S_2 \mid C_1) = P(S_2 \mid C_2) = P(S_2)$
H_1: H_0 is false

and the decision rule is: with α = 0.05, reject H_0 if $\chi^2 > \chi_1^2$ (0.05) = 3.8415. Therefore, based on either formula, since $\chi^2 > 3.8415$, we would reject H_0 and conclude that the rate of damage is not independent of the type of container used.

Similar to the discussion of the z-test for proportions, the calculated chi square value is based upon discrete, discontinuous data, but the chi square critical value is based on a continuous distribution (Figure 16.1). Therefore, the calculated chi square value is only an approximation of the theoretical chi square distribution and these approximations are good for larger numbers of degrees of freedom, but not as accurate for only one degree. Also, since there is a decrease to the smallest possible degrees of freedom, the distribution no longer resembles a normal distribution (Figure 16.4). Therefore, we must once again use a correction to produce a more conservative estimate. Using the symbols in Figure 16.3, Yates' modification of Eq. 16.7 produces a smaller numerator and a more conservative estimate for the chi square statistic.

$$\chi^2_{corrected} = \frac{n(|ad - bc| - 0.5n)^2}{(a+c)(b+d)(a+b)(c+d)} \qquad \text{Eq. 16.8}$$

Recalculating the chi square statistic for data from the above example using Yates' correction for continuity, the results are:

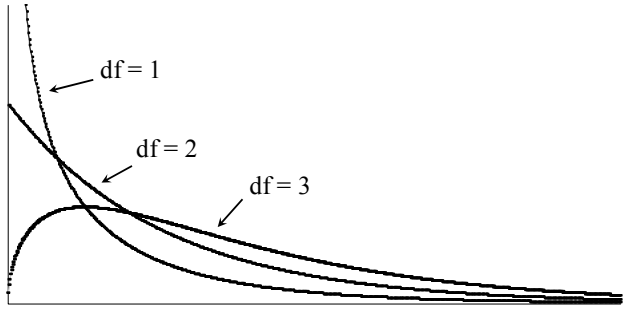


Figure 16.4 Chi square distributions for fewer than four degrees of freedom.

$$\chi^2_{corrected} = \frac{200[\,|(97)(12)-(3)(88)| - (0.5)(200)\,]^2}{(100)(100)(185)(15)}$$

$$\chi^2_{corrected} = \frac{128000000}{27750000} = 4.61$$

Yates' correction provides a more conservative, harder to reject, chi square value. If the above example were computed without Yates' correction the resulting χ^2 would have equaled 5.84. In this particular case either finding would have resulted in the rejection of H_0.

Likelihood-Ratio Chi Square Test

The likelihood-ratio chi square test compares the ratios between the observed and expected frequencies. It is computed using the log value for each ratio in each cell of the contingency table:

$$G^2 = 2 \cdot \sum \left[O \cdot ln\left(\frac{O}{E}\right)\right] \qquad \text{Eq. 16.9}$$

When there is independence between the row and column variables the likelihood ratio is represented by a chi square distribution with $(R - 1)(C - 1)$ degrees of freedom. This provides a measure of how good the results fit the alternative hypothesis. In the previous example (ampule breakage and packaging) the results would be as follows:

O/E

0.4000	1.6000
1.0486	0.9513

Ln O/E

–0.9163	+0.4700
+0.0475	–0.0499

O·Ln O/E

–2.7489	+5.6400
+4.6075	–4.3912

The sum of the last four cells is 3.1074. Therefore the likelihood ratio is

$$G^2 = 2(3.1074) = 6.215$$

This result is well in excess of the critical value of 3.84 and therefore a significant ratio showing a significant association between the row and column variables. Similar to the Yates' correction for the chi square statistic, the likelihood ratio will give a more conservative result, making rejection of the null hypothesis more difficult.

Comparison of Chi Square to the z-Test of Proportions

In the case of a 2 × 2 contingency table, one could either perform a chi square test of independence or a two-sample z-test of proportions on the same information and the results would be identical. For example, consider our previous example of the shipping containers and broken ampules. The exact *p*-value for a $\chi^2 = 5.84$ is 0.0157 (determined using the Excel® function **CHIDIST**). One could also present this same data in the format seen in Table 16.10. The proportion of failures, 0.03 (3/100) for the new design and 0.12 (12/100) for the old design is presented in the table. Using Eq. 15.5 to determine if there is a significant difference between the two proportions, the resulting *z*-value would 2.416, which represents a *p*-value of 0.0157 (determined using the Excel function [1-**NORMSDIST**(z)]*2). Similarly, using Yates' correction for either of the tests (Eq. 15.11 or Eq. 16.8) produces the same results, both *p*-values equal to 0.032. Thus, either test can be performed for data appearing in a 2 × 2 contingency table.

Fisher's Exact Test

If data for a chi square test of independence is reduced to a 2 × 2 contingency table and the expected values are still too small to meet the requirements (at least five per cell) or have a zero in one or more of the four cells, the Fisher's exact test can be employed (Fisher, 1936). The term "exact" is used because the result of the calculations produces the exact probabilities of obtaining the observed results if the two variables are independent. This test is sometimes referred to as **Fisher's four-fold test** because of the four cells of frequency data. The test used the previously described the *a-b-c-d* four-cell format (Figure 16.3). The formula involves the factorials for the cells and margins:

$$p = \frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!}{n!\,a!\,b!\,c!\,d!} \qquad \text{Eq. 16.10}$$

An alternative formula, using possible combinations (Chapter 2), produces the exact same results:

$$p = \frac{\binom{a+b}{a}\binom{c+d}{c}}{\binom{n}{a+c}} \qquad \text{Eq. 16.11}$$

The first formula is identical to the nonparametric median test that will be discussed in Chapter 21. However, in this test, cells are based on the evaluation of two independent variables and not on estimating a midpoint based on the sample data.

Multiple tests are performed to determine the probability of not only the research data, but also the probabilities for each possible combination to the extreme of the observed data. These probabilities are summed to determine the exact probability of the outcome observed given complete independence. For example, assume the following data is collected:

3	7	10
7	3	10
10	10	20

The *p*-value is calculated for this one particular outcome; however, *p*-values are also calculated for the possible outcomes that are even more extreme with the *same fixed margins*:

2	8
8	2

1	9
9	1

0	10
10	0

Then the probabilities of all four possibilities are summed and the result is a **one-tailed Fisher's exact test** using extremes in one direction. The decision rule compares this exact probability to a $p_{critical}$ (for example, 0.05). If it is smaller than the $p_{critical}$, reject H_0 and conclude that the rows and columns are not independent.

To illustrate the use of this test, assume the following example. Twelve laboratory rats are randomly assigned to two equal-sized groups. One group serves as a control, while the experimental group is administered a proposed carcinogenic agent. The rats are observed for the development of tumors. The following results are observed:

	Tumor	No Tumor	
Experimental	4	2	6
Control	1	5	6
	5	7	12

Is the likelihood of developing a tumor the same for both groups? The hypotheses are:

H_0: The group and appearance of a tumor are independent
H_1: The two variables are not independent

The decision rule is, with $\alpha = 0.05$, reject H_0 if $p < 0.05$. The computation for the probability of four tumors in the experimental group is:

$$p = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!a!b!c!d!}$$

$$p = \frac{6!6!5!7!}{12!4!1!2!5!} = 0.1136$$

There is only one more extreme result with fixed margins and that would be five tumors in the experimental group:

	Tumor	No Tumor	
Experimental	5	1	6
Control	0	6	6
	5	7	12

$$p = \frac{6!6!5!7!}{12!5!0!1!6!} = 0.0076$$

The probability of four or more experimental mice developing a tumor:

$$\begin{aligned} p(5) &= 0.0076 \\ p(4) &= \underline{0.1136} \\ &\quad 0.1212 \end{aligned}$$

Therefore the decision, with $p > 0.05$, is that H_0 cannot be rejected. It is assumed that the two variables are independent and that the incidence of tumor production is independent of the agent's administration.

To perform a **two-tailed Fisher's exact test** extremes in the opposite direction are considered when the probabilities of their outcomes are less than or equal to the probability associated with the observed results. For this example the extreme results in the opposite direction, with fixed margins would be:

0	6
5	1

1	5
4	2

2	4
3	3

The result for these three possible outcomes to the other extreme would be:

$$p(0) = \frac{6!\,6!\,5!\,7!}{12!\,0!\,5!\,6!\,1!} = 0.0076$$

$$p(1) = \frac{6!\,6!\,5!\,7!}{12!\,1!\,4!\,5!\,2!} = 0.1136$$

$$p(2) = \frac{6!\,6!\,5!\,7!}{12!\,2!\,3!\,4!\,3!} = 0.3788$$

However, the *p(2)* exceeds the original observed outcome of $p = 0.1136$, so it would not be included in the calculations. Therefore the result for a two-tailed Fisher's exact test would be:

$$\begin{aligned} p(5) &= 0.0076 \\ p(4) &= 0.1136 \\ p(0) &= 0.0076 \\ p(1) &= \underline{0.1136} \\ &\quad 0.2424 \end{aligned}$$

McNemar's Test

The McNemar test can be used to evaluate the relationship or independence of paired discrete variables. The test involves dichotomous measurements (e.g., pass/fail, yes/no, present/absent) that are paired. The paired responses are constructed into a four-fold, or 2 × 2 contingency table and outcomes are tallied into the appropriate cell. Measurements can be paired on the same individuals or samples over two different time periods (similar to our previous use of the paired t-test in Chapter 9) and the layout for the contingency table is presented in Figure 16.5. Alternatively subjects can be paired based on some predetermined and defined characteristic (replacing first measurement and second measurement with the two characteristics in Figure 16.5.

For example, if it were based on a yes/no response over two time periods, those individuals responding "yes" at both time periods would be counted in the upper left corner (cell *a*) and those answering "no" on both occasions are counted in the lower right corner (cell *d*). Mixed answers, indicating changes in responses, would be counted in the other two diagonal cells (*b* and c). If there was absolutely no change over the two time periods, we would expect that 100% of the results would appear in cells *a* and *d*. Those falling in cells *c* and *b* represent changes between the two measurement periods and are of primary interest to the researcher.

For the McNemar's test the statistic is as follows:

$$\chi^2_{McNemar} = \frac{(b-c)^2}{b+c} \qquad \text{Eq. 16.12}$$

		First Measurement	
		Outcome 1	Outcome 2
Second Measurement	Outcome 1	a	b
	Outcome 2	c	d

Figure 16.5 Design for a McNemar test for paired data.

As with the previous Yates' correction of continuity, a similar correction can be made to produce a more conservative approximation for the McNemar test:

$$\chi^2_{McNemar\ corrected} = \frac{(|b-c|-1)^2}{b+c} \qquad \text{Eq. 16.13}$$

In either case, the null hypothesis would be that there is no significant change between the two times or characteristics. Because we are dealing with a 2 × 2 contingency table, the number of degrees of freedom is one (rows – 1 × columns – 1). Thus we will compare our calculated statistic to a critical χ^2 with one degree of freedom or 3.8415 (Appendix B, Table B15). If the $\chi^2_{McNemar}$ exceeds 3.8415 we reject H_0 and assume a significant change between the two measurements (similar to our previous H_0: $\mu \neq 0$ in the paired t-test).

As an example, assume that 100 patients are randomly selected based on visits to a local clinic and assessed for specific behavior that is classified as a risk factor for colon cancer. The risk factor is classified as either present or absent. During the course of their visit and with a follow-up clinic newsletter, they are educated about the incidence and associated risks for a variety of cancers. Six months after the initial assessment patients are evaluated with respect to the presence or absence of the same risk factor. The following table represents the results of the study:

		Risk Factor Before Instruction		
		Present	Absent	
Risk Factor After Instruction	Present	40	5	45
	Absent	20	35	55
		60	40	100

The null hypothesis would be that the instructional efforts had no effect.

H_0: Instruction did not influence presence of the risk factor
H_1: H_0 is false

The decision rule would be to reject H_0, of independence, if $\chi^2_{McNemar}$ greater than $\chi^2_1(1-\alpha) = 3.8415$. The calculations would be:

$$\chi^2_{McNemar} = \frac{(b-c)^2}{b+c} = \frac{(5-20)^2}{5+20} = \frac{225}{25} = 9.0$$

Yates' correction of continuity would produce a more conservative estimation:

$$\chi^2_{McNemar\ corrected} = \frac{(|b-c|-1)^2}{b+c} = \frac{(|5-20|-1)^2}{5+20} = \frac{196}{25} = 7.84$$

Either method would result in the rejection of the H_0 and the decision that the instruction provided the patients resulted in a change in risk taking behavior.

Another way to think of McNemar's procedure is as a test of proportions, based on samples that are related or correlated in some way. The McNemar's test does not require the computation of the standard error for the correlation coefficient. The computation, using the previous notations for a 2 × 2 contingency table is:

$$z = \frac{a-d}{\sqrt{a+d}} \qquad \text{Eq. 16.14}$$

where in large samples $\chi^2 = z^2$.

Cochran's Q Test

Cochran's Q test can be thought of as a complement to the randomized complete block design, discussed in Chapter 10, when dealing with discrete data. It is an extension of the McNemar's test to three or more levels of the independent variable. Similar to the randomized complete block design, subjects or observations are assigned to blocks to reduce variability within each level of the independent variable. The design is used to create homogeneous blocks. Subjects within each block are more homogeneous than subjects within the different blocks. As seen in Table 16.11, the blocking effect is represented by the row and each block contains results for each level of the independent variable. There is only one observation per cell and this is reported as a pass (coded as 1) or fail (coded as 0) result. Each of the columns is summed (C) and the sum is squared (C^2). Also, each block is summed (R) and the sum squared (R^2). Lastly, both the R and R^2 are summed producing $\sum R$ and $\sum R^2$. The formula for Cochran's Q is:

$$Q = \frac{(k-1)[(k\sum C^2) - (\sum R)^2]}{k(\sum R) - \sum R^2} \qquad \text{Eq. 16.15}$$

where k is the number of levels of the discrete independent variable. The resultant Q-value is compared to the chi square critical value with $k - 1$ degrees of freedom. If the

Table 16.11 General Structure of a Randomized Block Design

	Levels of the Independent Variable					
	C_1	C_2		C_k	R	R^2
Block b_1	x_{11}	x_{12}	...	x_{1k}	$\sum x_{1k}$	$\sum x_{1k}^2$
Block b_2	x_{21}	x_{22}	...	x_{2k}	$\sum x_{2k}$	$\sum x_{2k}^2$
Block b_3	x_{31}	x_{32}	...	x_{3k}	$\sum x_{3k}$	$\sum x_{3k}^2$
...	...	...	...	...	...	...
Block b_j	x_{j1}	x_{j2}	...	x_{jk}	$\sum x_{jk}$	$\sum x_{ik}^2$
C	$\sum x_{j1}$	$\sum x_{j2}$		$\sum x_{jk}$		
C^2	$\sum x_{j1}^2$	$\sum x_{j2}^2$	...	$\sum x_{jk}^2$		
				$\sum R =$	$\sum\sum x_k$	
				$\sum R^2 =$		$\sum\sum x_k^2$

Q-value exceeds the critical value there is a significant difference among the various levels of the independent variable.

As an example, a pharmaceutical company is trying to decide among four different types of gas chromatographs produced by four different manufacturers. To evaluate the performances of these types of equipment, ten laboratory technicians are asked to run samples and evaluate the use of each piece of equipment. They are instructed to respond as either acceptable (coded 1) or unacceptable (coded 0) for the analysis performed by the equipment. The results of their evaluations appear in Table 16.12. Is there a significant relationship between the pieces of equipment and technicians' evaluations? The hypotheses being tested are:

Table 16.12 Evaluations for Various Types of Equipment

	Manufacturer			
Technician	A	B	C	D
1	0	1	0	1
2	0	0	0	1
3	1	0	0	1
4	0	1	0	1
5	0	0	1	0
6	0	0	1	1
7	0	0	0	1
8	0	1	1	1
9	0	0	0	1
10	1	0	0	1

Table 16.13 Example of Cochran's Q Test

	Manufacturer					
Technician	A	B	C	D	R	R^2
1	0	1	0	1	2	4
2	0	0	0	1	1	1
3	1	0	0	1	2	4
4	0	1	0	1	2	4
5	0	0	1	0	1	1
6	0	0	1	1	2	4
7	0	0	0	1	1	1
8	0	1	1	1	3	9
9	0	0	0	1	1	1
10	1	0	0	1	2	4
C =	2	3	3	9		
C^2 =	4	9	9	81		
				$\sum R$ =	17	
	$\sum C^2$ = 103			$\sum R^2$ =		33

H_0: Technician evaluations are independent of the equipment tested
H_1: H_0 is false

The decision rule is, with 95% confidence or α equal less than 0.05, reject H_0 if Q is greater than $\chi^2_{(k-1)}(1 - \alpha)$, which is 7.8147 ($k - 1 = 3$). The sum of columns and rows are presented in Table 16.13 and the calculation of Cochran's Q is as follows:

$$Q = \frac{(k-1)[(k\sum C^2) - (\sum R)^2]}{k(\sum R) - \sum R^2}$$

$$Q = \frac{(3)[(4)(103) - (17)^2]}{(4)(17) - 33} = \frac{369}{35} = 10.54$$

With Q greater than the critical value of 7.8147, the decision is to reject the hypothesis of independence and assume that the type of equipment tested did influence the technicians' responses. Based on the *C's* presented in Tables 16.12 and 16.13, manufacturer *D*'s product appears to be preferred.

Mantel-Haenszel Test

The Mantel-Haenszel test sometimes referred to as the **Cochran-Mantel-Haenszel test** or **Mantel-Haenszel-Cochran test**, can be thought of as a three-dimensional chi square test, where a 2 × 2 contingency table is associated with main

factors in the row and column dimensions. However a third, possibly confounding variable, is added as a depth dimension in our design. This third extraneous factor may have *k*-levels and the resultant design would be 2 × 2 × *k* levels of three discrete variables. In other words, we are comparing *k* different 2 × 2 contingency tables. Using the *a*,*b*,*c*,*d* labels as in the previous 2 × 2 designs, the Mantel-Haenszel compares each a_i (a_1 through a_k) with its corresponding expected value. The a_i is the observed value for any one level of the possible confounding variable. The statistic is:

$$\chi^2_{MH} = \frac{\left[\sum \frac{a_i d_i - b_i c_i}{n_i}\right]^2}{\sum \frac{(a+b)_i (c+d)_i (a+c)_i (b+d)_i}{(n_i - 1)(n_i^2)}} \qquad \text{Eq. 16.16}$$

This can be modified to create a numerator that compares the observed and expected values for one cell of the 2 × 2 matrix and sums this comparison for each level of the confounding variable.

$$\chi^2_{MH} = \frac{\left[\sum (a_i - \frac{(a_i + b_i)(a_i + c_i)}{n_i}\right]^2}{\sum \frac{(a+b)_i (c+d)_i (a+c)_i (b+d)_i}{n_i^2 (n_i - 1)}} \qquad \text{Eq. 16.17}$$

The null hypothesis reflects independence between the row and column variables, correcting for the third extraneous factor. The calculated χ^2_{MH} is compared to the critical value $\chi^2_1(1 - \alpha)$. If that value exceeds the critical value, the row and column factors are not independent and there is a significant relationship between the two factors.

For example, consider a study of smoking and the presence or absence of chronic lung disease. Assume that we are concerned that the subjects' environments might confound the finding. We decide to also evaluate the data based on home setting (e.g., urban, suburb, rural). The results of the data collection are presented in Table 16.14.

Equation 16.16 can be simplified by modifying certain parts of the equation. For example the e_i (the expected value) for each confounding level of a_i is:

$$e_i = \frac{(a_i + b_i)(a_i + c_i)}{n_i} \qquad \text{Eq. 16.18}$$

This is equivalent to stating that the sum of the margin for the row multiplied by the margin for the column divided by the total number of observations associated with the *i*th level is the expected value. This is the same way we calculated the expected value in the contingency table for a chi square test of independence. For example, for the suburban level the e_i is:

Table 16.14 Evaluation of Setting as a Possible Confounding Factor

Site	Chronic Lung Disease	Smoker	Nonsmoker	Totals
Urban	Yes	45	7	52
	No	16	80	96
		61	87	148
Suburban	Yes	29	10	39
	No	19	182	201
		48	192	240
Rural	Yes	27	18	45
	No	16	51	67
		43	69	112

$$e_2 = \frac{(39)(48)}{240} = 7.8$$

This will be compared to the observed result ($a_2 = 29$) to create part of the numerator for Eq. 16.18. In a similar manner, a v_i can be calculated for the denominator at each level of the confounding variable:

$$v_i = \frac{(a_i + b_i)(c_i + d_i)(a_i + c_i)(b_i + d_i)}{n_i^2 (n_i - 1)} \qquad \text{Eq. 16.19}$$

The v_i for the rural level is:

$$v_3 = \frac{(45)(67)(43)(69)}{(112)^2 (112 - 1)} = 6.43$$

These intermediate results can be expressed in a table format:

	Urban	Suburban	Rural
a_i	45	29	27
e_i	21.43	7.80	17.28
v_i	8.23	5.25	6.43

and entered into the following equation:

$$\chi^2_{MH} = \frac{[\Sigma(a_i - e_i)]^2}{\Sigma v_i} \qquad \text{Eq. 16.20}$$

The results are

$$\chi^2_{MH} = \frac{[(45-21.43)+(29-7.80)+(27-17.28)\,]^2}{(8.23+5.25+6.43\,)} = \frac{(54.49\,)^2}{19.91} = 149.13$$

With the χ^2_{MH} greater than $\chi^2_i(1-\alpha)$ we reject the null hypothesis of no association between the two main factors controlling for the potentially confounding environmental factor. If the value would have been less than the critical χ^2 value we would have failed to reject the null hypothesis and assumed that the confounding variable affected the initial χ^2 results for the 2 × 2 contingency table.

A correction for continuity can also be made with the Mantel-Haenszel procedure:

$$\chi^2_{MH\ corrected} = \frac{[\,\Sigma(a_i - e_i) - 0.5\,]^2}{\Sigma v_i} \qquad \text{Eq. 16.21}$$

In the previous example this correction would produce the expected, more conservative result:

$$\chi^2_{MH\ corrected} = \frac{(54.49-0.5\,)^2}{19.91} = 146.40$$

In this case, either the Mantel-Haenszel test or the corrected version would produce a statistically significant result and rejection of the null hypothesis.

Using Excel® or Minitab® for Chi Square Applications

Similar to *t*-distributions and *F*-distributions, Excel 2010 has several functions for calculating probabilities of critical values. Instead of referring to Table B15 to determine critical values for the test statistic it can be determined using the function **CHISQ.INV.RT**. For older versions of Excel this command was **CHIINV**. Either function will prompt for the probability (alpha as a decimal) and the degrees of freedom. Caution should be noted here. Excel 2010 has the command **CHISQ.INV** and this command will identify the location for a certain probability on the LEFT end of the curve. Other Excel functions allow one to determine the *p*-value for a calculated chi square statistic: **CHISQ.DIST.RT** (for Excel 2010) or **CHIDIST** (for older versions). Either function will prompt for the calculated chi square value and degrees of freedom. The result will be the *p*-value for the given chi square results. Once again caution is needed because **CHISQ.DIST** in Excel 2010 will do the calculation for the LEFT side of the distribution.

Excel does not provide a useful approach for handling chi square tests. There is a function application, **CHISQ.TEST** (Excel 2010) or **CHITEST** (older versions), that requires you enter your frequencies in cells similar to the method of the contingency table. However, it also requires another parallel contingency table with the expected

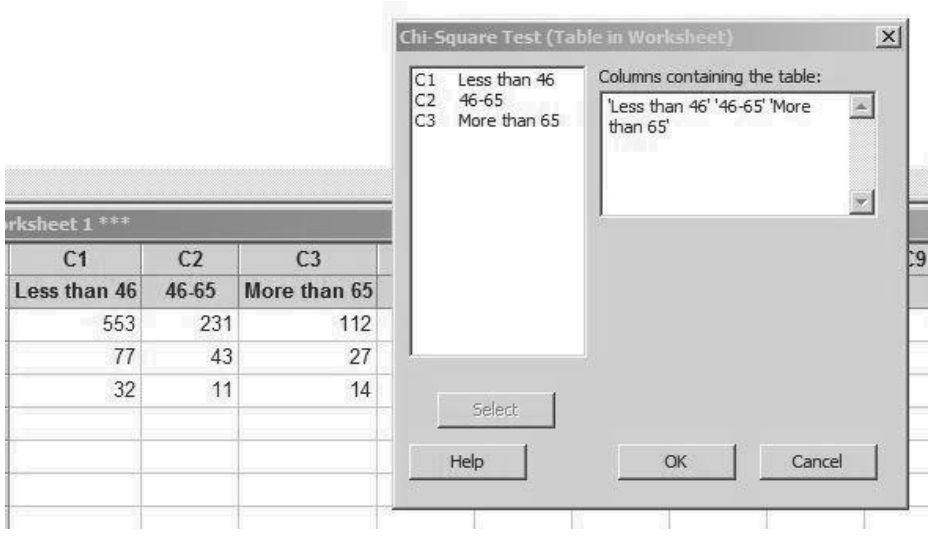


Figure 16.6 Chi square from worksheet layout with Minitab.

values (these would need to be calculated prior to using the software). Then the range of the observed data is identified for Excel as the "Actual_Range" and the expected results under independence identified as the "Expected_range". The resulting output is only the *p*-value (Pearson) and the chi square statistic is not reported.

Minitab offers better applications in the "Tables" option under "Stat" in the title bar:

Stat ➤ Tables ➤ Chi-Square Test (Table in Worksheet)
Stat ➤ Tables ➤ Cross Tabulation and Chi-Square
Stat ➤ Tables ➤ Chi-Square Goodness-of-Fit Test (one variable)

If data has already been summarized in a contingency table, the easiest approach is to enter the frequency counts directly into the Minitab worksheet, labeling each level for the columns. The "Chi-Square Test (Table in Worksheet)" option will request the columns which represent the data (Figure 16.6). These columns are selected from the left box by double clicking each one. An example output is presented in Figure 16.7 (from the previous example for Table 16.9). In addition to listing the chi square statistic and associated *p*-value at the bottom, the output will automatically list the expected values (second line) and the amount each cell contributes to the chi square statistic on the third line (the observed minus expected squared divided by expected value for each cell).

If data is arranged in columns as individual variables, the "Cross Tabulation and Chi-Square" option is the appropriate choice. Column and row variables are selected by clicking on choices in the left box (Figure 16.8). If the *Chi-Square…* option is selected, the smaller box in Figure 16.8 appears and "Chi-Square analysis" should be

Chi-Square Test: Less than 46, 46-65, More than 65

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

          Less                 More
       than 46   46-65   than 65  Total
1          553     231       112    896
        539.23  232.15    124.63
         0.352   0.006     1.279

2           77      43        27    147
         88.47   38.09     20.45
         1.486   0.634     2.101

3           32      11        14     57
         34.30   14.77      7.93
         0.155   0.961     4.650

Total      662     285       153   1100

Chi-Sq = 11.624, DF = 4, P-Value = 0.020
```

Figure 16.7 Output for chi square from worksheet layout with Minitab.

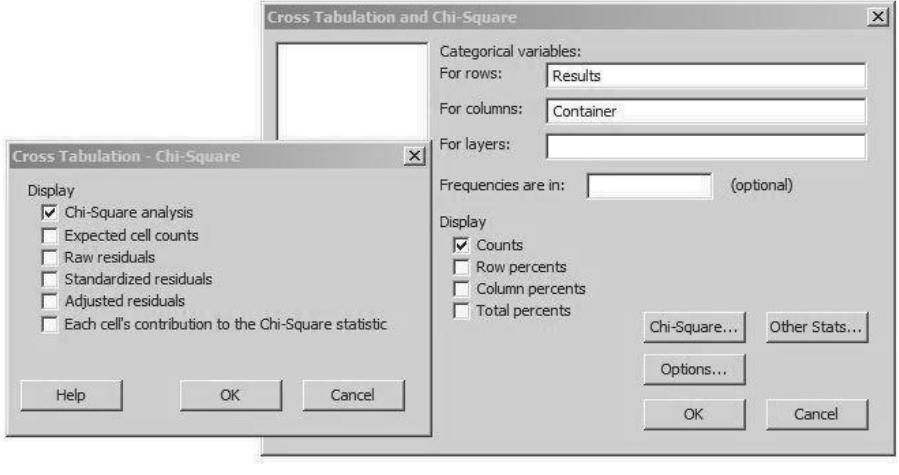


Figure 16.8 Chi square from column data with Minitab.

Tabulated statistics: Results, Container

Rows: Results Columns: Container

	New	Old	All
Fail	3	12	15
Pass	97	88	185
All	100	100	200

Cell Contents: Count

Pearson Chi-Square = 5.838, DF = 1, P-Value = 0.016
Likelihood Ratio Chi-Square = 6.220, DF = 1, P-Value = 0.013

Figure 16.9 Output for chi square from column data with Minitab.

selected. Multiple options are available in both boxes for what should appear in the output. Figure 16.9 represents the simplest report with just the frequency counts, chi square statistic, and *p*-value. Note that the likelihood ratio is provided for the 2 × 2 contingency table. Minitab does not provide an option to make the Yates' correction for a 2 × 2 chi square. However, there is warning message if cells have expected values less than five.

Additional tests described in this chapter are available under *Other Stats…* in Figure 16.8 including the Fisher's exact test and the Mantel-Haenszel test. The latter is labeled Mantel-Haenszel-Cochran and the confounding variable is selected for the "For layers:" variable in Figure 16.8. The results are reported with the correction for continuity formula (Eq. 16.21). Fisher's exact test uses the two-tailed approach.

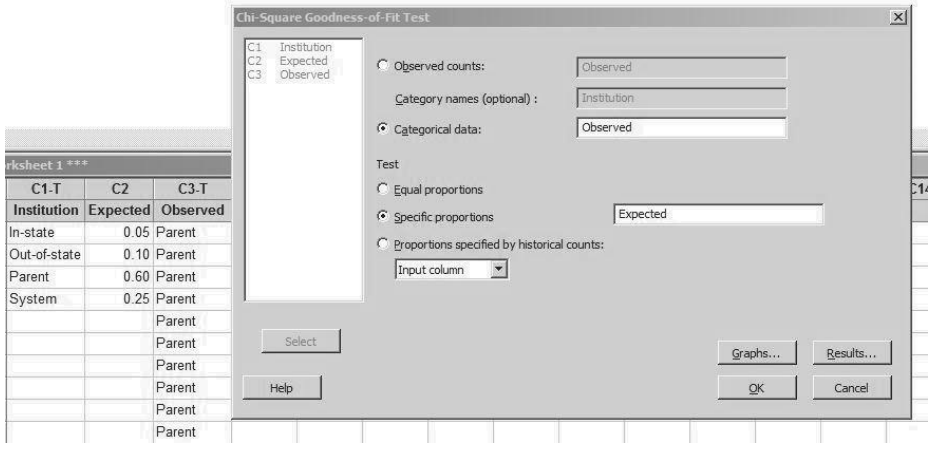


Figure 16.10 Chi square goodness-of-fit with Minitab for column data.

Chi-Square Goodness-of-Fit Test for Categorical Variable: Observed

Category	Observed	Test Proportion	Expected	Contribution to Chi-Sq
In-state	5	0.05	7.5	0.83333
Out-of state	25	0.10	15.0	6.66667
Parent	85	0.60	90.0	0.27778
System	35	0.25	37.5	0.16667

N	N*	DF	Chi-Sq	P-Value
150	0	3	7.94444	0.047

Figure 16.11 Output for chi square goodness-of-fit with Minitab.

Minitab also provides for a goodness-of-fit for one discrete independent variable. Data may be arranged by variables in columns or presented in tabular form on the worksheet. If data is presented in a single column of a worksheet, the first step is to create a column with the expected outcomes as proportions. These must be arranged alphabetically or with sequential numbers. For example, using the previous data for the students admitted into the Pharm.D. program the alphabetical order would be: Row 1 In-State, $p = 0.25$; Out-of-State, $p = 0.10$; Parent, $p = 0.60$; and System, $p = 0.25$). Then for the "Chi-Square Goodness-of-fit Test", the option would be the "Categorical data;" and enter the column with the appropriate data. The expected outcomes would be identified as the "Specific proportions" (Figure 16.10). The results of the tests are presented in Figure 16.11. Data can also be arranged as a table on a Minitab worksheet, with one column representing the levels of the independent variable, one column with the expected proportion for each level, and a third column with the actual observed results. Once the "Chi-Square Goodness-of-fit Tests" option is selected, Minitab will request the columns for "Observed counts:" (what was observed) and the "Categorical names (optional):" (the column with names for the levels of the dependent variable). Also requested is the "Specific proportions" (column with expected proportions) or "equal proportions" if each level of the dependent variable is expected to be equal (a uniform distribution). These options appear in Figure 16.12. The results will be identical to those presented in Figure 16.11, except the results would be in the same order as listed on the Minitab worksheet (in this example, "Parent" first and "Out-of-State" last).

Reference

Fisher, R.A. (1936). *Statistical Methods for Research Workers*, Oliver and Boyd, London, pp. 100-102.

Suggested Supplemental Readings

Agresti, A. (2002). *Categorical Data Analysis*, Second edition, John Wiley and Sons, Inc., New York, pp. 36-101.

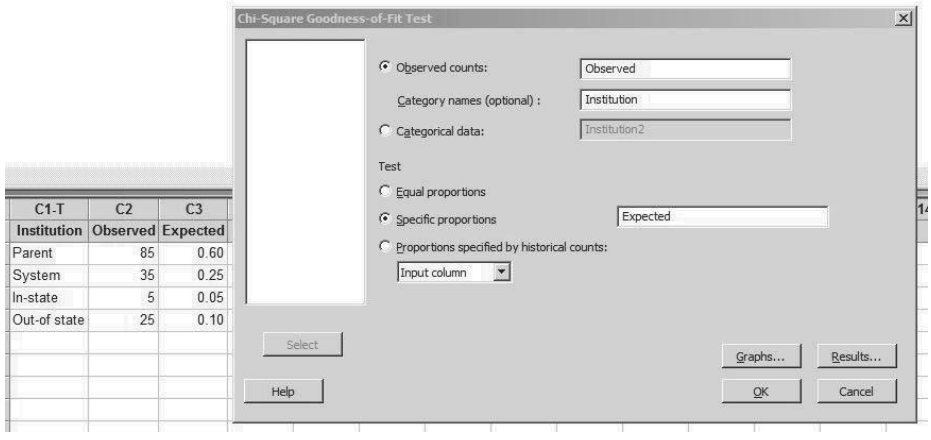


Figure 16.12 Chi square goodness-of-fit with Minitab from worksheet table.

Bolton, S. and Bon, C. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, Inc., New York, pp. 131-134.

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Eighth edition, John Wiley and Sons, New York, pp. 593-629.

Havilcek, L.L. and Crain, R.D. (1988). *Practical Statistics for the Physical Sciences*, American Chemical Society, Washington, DC, pp. 212-221.

Example Problems (Answers are provided in Appendix D)

1. A medication known to cause severe irritation to stomach mucosa is tested with a series of special tablet coatings to prevent release until after the tablet has passed through the stomach. Three variations of the coating formula are tested on 150 fasted volunteers, randomly assigned to each group. The presence or absence of irritation, through endoscopic examination, is noted for each subject.

	GI Irritation	
	Present(P_1)	Absent(P_2)
Formula A	10	40
Formula B	8	42
Formula C	7	43

   Was there a significant difference in the likelihood of irritation based on the coating formulas?

2. A pharmacist is evaluating the amount of time needed for nurse surveyors to observe drug delivery in 70 long-term care facilities. The median time required by the surveyors is 2.5 hours. The researcher wishes to know if the type of

delivery system (unit dose versus traditional) influences the amount of survey time required.

	Unit Dose	Traditional	Total
2.5 hours or less	26	10	36
More than 2.5 hours	14	20	34
Total	40	30	70

3. Immediately after training on a new analytical method, technicians were asked their preference between the new method and a previously used, "old" method. Six months later, after the technicians had experience with the new method, they were resurveyed with respect to their preference. The results of the two surveys are presented below. Did experience with the new method significantly change their preferences?

		Preferred Method before Experience		
		New	Old	
Preferred Method After Experience	New	12	8	20
	Old	3	7	10
		15	15	30

4. In preparing to market an approved tablet in a new package design, the manufacturer tests two different blister packs to determine the rates of failure (separation of the adhesive seal) when stored at various temperatures and humidities. One thousand tablets in each of two conditions were stored for three months and the number of failures were observed:

	40° 50% relative humidity	60° 50% relative humidity
Blister pack A	2	5
Blister pack B	6	6

Is there a significant relationship between the storage conditions and the frequency of failures based on the blister pack used?

5. A manufacturer is experimenting with a new 50-mm diameter screw-type container using various amounts of torque for closure. The tightness of the containers is tested based on moisture permeability. From the data reported below, is there any significant difference in moisture level based on the torque used to tighten the cap?

	Torque (inch-pounds)				
Moisture	21	24	27	30	
< 2000	26	31	36	45	138
≥ 2000	24	19	14	5	62
Total	50	50	50	50	200

6. Twenty volunteers were randomly assigned to a randomized three-way cross-over clinical trial involving the same topical medication presented in three different formulations (A, B, and C). During each phase of the study volunteers were assessed for the presence or absence of erythema (redness) at the site of application. Was there any significant difference among the formulations for the incidence of erythema?

	Formulation				Formulation		
Volunteer	A	B	C	Volunteer	A	B	C
001	0	1	0	011	0	0	1
002	1	0	1	012	0	0	0
003	0	0	0	013	1	0	1
004	0	0	0	014	0	0	0
005	0	1	1	015	0	0	0
006	0	0	0	016	0	0	0
007	0	0	0	017	1	1	0
008	0	0	0	018	0	0	0
009	0	0	0	019	1	0	1
010	1	1	0	020	1	1	1

(code: 1 = erythema)

7. In one of the example problems in Chapter 15, an instrument manufacturer ran a series of disintegration tests to compare the pass/fail rate of a new piece of equipment at two extreme temperatures. The manufacturer decided to also evaluate the influence of paddle speed as a possible confounding factor. The test was designed to collect results at two speeds, defined as fast and slow. The results were as follows:

		Test Results		
Speed of Paddle	Temperature	Pass	Fail	Totals
Fast	39°C	48	2	50
	35°C	47	3	50
		95	5	100
Slow	39°C	48	2	50
	35°C	45	5	50
		93	7	100

Without Yates' correction for continuity there is a significant relationship between the temperature and proportion of test failures ($\chi^2 = 4.35$). Could the paddle speed be a confounding factor in the design?

17

Measures of Association

To this point, most of this book has dealt with tests of differences (e.g., t-tests, F-tests, z-tests of proportions). Other tests have dealt with relationships (e.g., chi-square test of independence, correlation). This chapter focuses on other types of relationships with tests that can measure the degree of association between different types of variables. As will be seen the term "measures of association" refers to a wide variety of procedures used to evaluate the strengths of various types of relationships. One type of measure of association has already been discussed in Chapter 13 where the correlation coefficient measured the association or strength of the relationship between two or more variables where those variables involve interval or ratio data. This chapter is a complement to the previous one and will focus primarily on measures of association for nominal and ordinal types of data scales (Chapter 1 defined these types of scales).

Introduction

These measures of association require that at least one of the variables is presented in a nominal or ordinal scale and can be applied only to data from a contingency table reporting frequencies (or counts). Basically, there is a significant relationship, if the magnitude of the observed relationship is significantly different than what one would expect due to chance produced from random sampling. If there is no association, the two variables are independent and there is an absence of any predictable relationship between the variables tested. Data will be presented in contingency tables similar to those used for the chi square test of independence (Chapter 16). Chi square itself is not a measure of association, but a test of the null hypothesis that two nominal or ordinal variables are unrelated.

The strengths of the various measures of association are evaluated by their **coefficients of association**. Most coefficients of association vary from 0 (indicating no relationship) to +1.0 (a perfect positive relationship) or −1.0 (a perfect negative relationship). This is similar to the type of association for continuous data seen with the correlation coefficient (Chapter 13). As discussed in the following sections, there are various types of "perfect relationships" and "null relationships." When these specific coefficients of associate are discussed, their definitions of perfect and no relationships will be cited and this is an important criterion for choosing among the

available tests. Most coefficients of association define "perfect relationship" as monotonicity (discussed below) and consider the null relationship as statistical independence.

There are four types of "perfect linear" relationships when dealing with nominal and ordinal data (and their respective measures of association) and these are based on **monotonicity**. These types of perfect linear relationships are defined as those where there is: 1) strict monotonicity; 2) ordered monotonicity; 3) predictive monotonicity; and 4) weak monotonicity. These terms are defined below. If there is perfect strict monotonicity all other three monotonic states will also be perfect. If either the ordered monotonicity or predictive monotonicity is perfect, there will be perfect weak monotonicity. However, it is impossible to have perfect ordered monotonicity and perfect predictive monotonicity at the same time unless there is perfect strict monotonicity. None of the definitions for monotonicity is appropriate for a curvilinear relationship which is beyond the scope of this book.

Monotonicity is based on the possible pairs of cells within a contingency table. Seen below is a 3 × 4 (three-by-four) contingency table with the cells labeled *a* to *l*.

		Factor X			
		1	2	3	4
Factor Y	1	a	b	c	d
	2	e	f	g	h
	3	i	j	k	l

Data for the *X*-factor (the *X* variable) contain four levels and the *Y*-factor (*Y* variable) contains three levels of a nominal or ordinal variable. Based on possible various combinations (discussed in Chapter 2, Eq. 2.12) there should be 66 different pairs of cells in this contingency table (twelve cells taken two at a time).

$$\binom{12}{2} = \frac{12!}{2!10!} = \frac{12\times 11\times 10!}{2\times 1\times 10!} = 66$$

These pairs can be identified by combining cells across rows, down columns, or across diagonals to identify all 66 possible pairs. The symbol X_0 represents the pairs moving down the columns (*X*-factor). For the first column they would be *ae*, *ai* and *ei*. Which can be written *ae + ai + ei or a(e + i) +ei*. Expanding this for all columns there are:

$$X_0 = ae + ai + bf + bj + cg + ck + dh + dl + ei + fj + gk + hl$$

pairs and this formula can be simplified and written as follows:

$$X_0 = a(e + i) + b(f + j) + c(g + k) + d(h + l) + ei + fj + gk + hl$$

Using this same nomenclature Y_0 represents the pairs moving across each row (*Y*-factor):

$$Y_0 = a(b+c+d) + e(f+g+h) + i(j+k+l) + b(c+d) + f(g+h) + j(k+l) + cd + gh + kl$$

Thus, the columns account for 12 pairs and the rows for 18 pairs. These are also referred to as "ties by row" or "ties by column." The remaining 36 possible pairs (66 − 30) can be identified moving diagonally through the table. **Concordant pairs** (P) are those moving diagonally from upper left to lower right (this is based on the assumption that for ordinal data, values will nominally increase moving from left to right in the columns and from top to bottom on the rows):

$$P = a(f + g + h + j + k + l) + b(g + h + k + l) + c(h + l) + e(j + k + l) + f(k + l) + gl$$

Concordant pairs represent an additional 18 pairs. The discordant pairs must account for the remaining 18 pairs. **Discordant pairs** (Q) are those moving from upper right to lower left:

$$Q = d(e + f + g + I + j + k) + c(e + f + I + j) + b(e + i) + h(i + j + k) + g(I + j) + fi$$

A parallel terminology is to refer to the concordant pairs as the pairing of values along the "diagonal" (e.g., cells *af, ak, al*) and the term "off-diagonal" (e.g., cells *dg, di, dj*) for discordant pairs. Thus, as summarized in Table 17.1, all possible results presented in the previous contingency table are as follows:

Pairs by row (Y_0)	18
Pairs by column (X_0)	12
Concordant pairs (P)	18
Discordant pairs (Q)	18
Total possible pairs	66

The use of concordant and discordant pairs will be needed for many of the tests of association discussed in this chapter.

The simplest matrix for a contingency table would be the 2 × 2 design (read two by two) used in dichotomous tests of association, Figure 16.3. We have already seen the use of all possible pairs in the second formula (Eq. 16.5) presented for calculating the 2 × 2 chi square test of independence.

$$\chi^2 = \frac{n(ad - bc)^2}{(a+c)(b+d)(a+b)(c+d)}$$

Note that the numerator contains the only possible concordant and discordant pairs and the denominator is the product of the pairs by row and pairs by column:

Table 17.1 Summary of all Possible Pairs for a 4 x 3 Table

Type of Pair	Symbol	Possible Pairs	Numbers of pairs
Concordant	P	a(f+g+h+j+k+l) + b(g+h+k+l) + c(h+l) + e(j+k+l) + f(k+l) + gl	18
Discordant	Q	d(e+f+g+i+j+k) + c(e+f+i+j) + b(e+i) + h(i+j+k) + g(i+j) + fi	18
Pairs by Columns	X_0	a(e+i) + b(f+j) + c(g+k) + d(h+l) + ei + fj + gk + hl	12
Pairs by Rows	Y_0	a(b+c+d) + e(f+g+h) + i(j+k+l) + b(c+d) + f(g+h) +j(k+l) + cd + gh + kl	18

$$\chi^2 = \frac{n(ad-bc)^2}{(a+c)(b+d)(a+b)(c+d)} = \frac{n(P-Q)}{X_0 Y_0} \qquad \text{Eq. 17.1}$$

In this case the diagonal pairing is cells *a* and *d*, and the off-diagonal is cells *b* and *c*. Unfortunately, this same logic cannot be expanded for tables larger than a 2 × 2 scenario.

Recall that the chi square test of independence (Chapter 16) indicates whether a significant relationship exists (rejection of the null hypothesis of independence). Failure to reject the null hypothesis resulted in the failure to reject the assumption of statistical independence between the row and column variables. The tests in this

chapter will provide a measure of the strength of the relationship between the variables, expressed as the **coefficient of association**. Consider the following perfect linear relationship.

		Factor X		
		A	B	C
Factor Y	A	25	0	0
	B	0	25	0
	C	0	0	25

In this example, there is a perfect positive **strict monotonicity** (by definition the Q, X_0 and Y_0 equal 0); a perfect **ordered monotonicity** (defined as both Q and Y_0 equal 0); a perfect **predictive monotonicity** (defined as both Q and X_0 equal 0); and a perfect **weak monotonicity** (defined as Q equals 0). If this data were evaluated for a chi square test of independence there would be a statistically significant relationship ($\chi^2 = 150$, $p < 0.0001$). As seen later, measures of association (such as Cramer's V for nominal data or *gamma* for ordinal data) would both produce a coefficient of association equal to 1.0. Thus, the following measures of association can be thought of as determinations of how close (or far) the relationships are to a perfect linear relationship.

In addition, some of the tests discussed in this chapter are **symmetric**, meaning that not only can values be predicted for *Y*-factor from *X*-factor, but values for the *X*-factor can be predicted from *Y*-factor. In contrast **asymmetric** tests cannot be used to predict the *X*-factor from the *Y*-factor. Thus, care must be taken in the selection of the row and column variables. For consistency, if an independent variable exists, it will always be used as the columns variable.

A second reason for the use of measures of association is that the chi square test of independence is very sensitive to the sample size. When a sample size is too small, the chi square value may represent an overestimate. However, if the sample size is too large, the chi square values could be an underestimate. The use of tests such as the phi, contingency coefficient, Cramer's *V* or *gamma*, in general overcome this problem.

Dichotomous Associations

As discussed in Chapter 2, a dichotomous variable is a discrete, nominal variable with only two possible levels (e.g., control or experimental, live or die). Therefore, coefficients of association used for these tests employ 2 × 2 contingency tables. Measures of association for larger contingency tables will be presented under nominal and ordinal associations. Another term used to generically label measures of association involving two dichotomous variables is a **four-fold point correlation coefficient**.

A chi square test of independence with one degree of freedom (discussed in Chapter 16) is an example of a dichotomous test of association and employs the traditional 2 × 2 matrix (Figure 16.3). As mentioned previously, if there is an independent variable it will be presented as the column factor.

For descriptive statistics involving dichotomous data the reporting of **percent difference** is the most common and simplest method to use. The percent difference

(*%d*) is computed by subtracting the difference (measured in percent) between the columns in either row. Using the previous layout, *%d* would equal $a - b$ or $b - a$, and $c - d$ or $d - c$. Consider the following example:

Example 1:

Hospitalization Required	Initial Outpatient Therapy		
	Treatment A	Treatment B	%d
Yes	20 (50%)	10 (25%)	–25% (b – a)
No	20 (50%)	30 (75%)	+25% (d – c)

In this case there was a 25% difference in the incidence of hospitalization depending upon which treatment was selected. With Treatment B there appeared to be 25% fewer hospital admissions. In this type of association *%d* would define the "perfect association" as strictly monotonic and the "null relation" is a statistical independence between the two treatments.

Note that in a 2 × 2 table the *%d*'s are asymmetric. If numbers were changed the *%d* would still be the same. Adjusting the data:

Example 2:

Hospitalization Required	Initial Outpatient Therapy		
	Treatment A	Treatment B	%d
Yes	18 (45%)	14 (35%)	–10% (b – a)
No	22 (55%)	26 (65%)	+10% (d – c)

As noted, if the independent variable is always represented by the column percentages, the sum for each column will be 100%. If independent and dependent variables were reversed, the columns would not add up to 100% (80% and 120% in Example 2).

In additional, the percent difference allows one to state whether the independent variable makes a difference in predicting values for the dependent variable. In Example 1, if *%d* equals 25%, then knowing the independent variable (e.g., which treatment) makes a 25% difference in predicting the outcome for the dependent variable (e.g., hospitalization).

As seen in Chapter 16, evaluation of the significance for a 2 × 2 contingency table could be evaluated using either Pearson's or Yates' chi square, both using the traditional *a–b–c–d–*matrix presented earlier. Three measures of association can be used to evaluate this data: 1) the *phi*-coefficient; 2) Yule's *Q* test; and 3) Yule's *Y* test.

The **phi-statistic** (ϕ) is a chi square-based measure of association for 2 × 2 tables involving nominal or ordinal dichotomous data. Phi eliminates the impact of sample size by dividing chi square by *n* (the sample size) and taking the square root of the results:

$$\phi = \sqrt{\frac{\chi^2}{n}} \qquad \text{Eq. 17.2}$$

The chi square used in the calculation should be the Pearson's chi square (Eq. 16.5), not the Yates' correction for continuity formula (Eq. 16.6). The *phi*-value measures the strength of the relationship based on the number of cases in the discordant pair minus the number of cases in the concordant pair, adjusted for by the sample size. An equivalent formula is:

$$\phi = \frac{\left|(b)(c) - (a)(d)\right|}{\sqrt{(a+b)(c+d)(a+c)(b+d)}} \qquad \text{Eq. 17.3}$$

Phi represents the mean percent difference between the column variable and row variable where either can be considered to cause the other. Thus, the ϕ-statistic is symmetrical and it does not matter if the column is an independent or dependent variable. The ϕ-statistic defines perfect association as a perfect predictive monotonicity and the null hypothesis is a statistical independence. This test is sometime referred to as a **four–fold point correlation**.

For the previous example (Example 2) for hospitalization following treatment with Treatments A and B, the chi square value would be:

$$\chi^2 = \frac{n(ad - bc)^2}{(a+c)(b+d)(a+b)(c+d)} = \frac{80((18)(26) - (14)(22))^2}{(40)(40)(32)(48)} = 0.83$$

The *phi*-value would be:

$$\phi = \sqrt{\frac{\chi^2}{n}} = \sqrt{\frac{0.83}{80}} = 0.102$$

The alternative formula produces the same results:

$$\phi = \frac{\left|(b)(c) - (a)(d)\right|}{\sqrt{(a+b)(c+d)(a+c)(b+d)}} = \frac{\left|(14)(22) - (18)(26)\right|}{\sqrt{(40)(40)(32)(48)}} = 0.1202$$

The results make sense, since the coefficient of association (in this case ϕ) should show a weak relationship since the chi square value was not significant (critical value for rejecting the null hypothesis of independence is 3.84). If there was a significant chi square, resulting in the rejection of the null hypothesis of independent, we would expect a stronger measure of association. For example, if the chi square (for the same sample size) were 9.00 the resulting phi statistic would be much closer to 1.0:

$$\phi = \sqrt{\frac{\chi^2}{n}} = \sqrt{\frac{9.00}{80}} = 0.335$$

The resultant ϕ can be viewed as a symmetric percent difference (*%d*), measuring the percent of results seen on the diagonal. In the 2 × 2 table, the ϕ-value is identical to a correlation coefficient for the same data. It is possible to dichotomize continuous data (e.g., above and below the median value for the row variable and column variable). This type of comparison is referred to as a **tetrachoric correlation**. Phi is also referred to as the **Pearson's coefficient of mean square contingency.** Unfortunately this same name is sometimes also applied to the Pearson's contingency coefficient, which is a modification of the *phi*-statistic. For tables larger than a 2 × 2 design the maximum value for phi depends on the size of the table and can exceed 1.0. Thus, even though phi can handle larger tables, it is not practical to use for such situations. Other tests discussed in the next sessions are appropriate for a larger table involving nominal or ordinal data.

The **Yule's Q** is another symmetric measure of association based on the difference between the concordant ($P = ad$) and discordant ($Q = bc$) data pairings. Yule's Q is recommended for situations where at least one variable is ordinal and is calculated as follows:

$$Q = \frac{(ad - bc)}{(ad + bc)} = \frac{P - Q}{P + Q} \qquad \text{Eq. 17.4}$$

This represents the difference ($P - Q$) as a percentage of all nontied (column or row) pairs ($P + Q$). Once again using the example cited above for hospitalizations (Example 2), the Yule's Q would be:

$$Q = \frac{(18)(26) - (14)(22)}{(18)(26) + (14)(22)} = \frac{160}{776} = 0.206$$

Thus, the surplus of consistent data pairs over inconsistent pairs is 20.6% of all the non-tied data pairs. In this case, consistent implies consistent with the null hypothesis of independence between treatment choices and hospitalization. The Q-value approaches 1.0 under perfect weak monotonicity. Interpretation of the results can be difficult and arbitrary with measures of association and there are various ways to verbally describe the magnitude of the association. One rule of thumb (Knoke and Bohrnstedt, 1991) goes as follows:

0 – 0.249	virtually no relationship
0.25 – 0.49	weak relationship
0.50 – 0.75	moderate relationship
0.75 - 1.00	strong relationship

This same terminology could serve for other measures of association presented in this chapter. As will be seen later, the *gamma* statistic is used as a measure of association

involving tables larger than 2 × 2. The resultant *Q*-value is equal to *gamma* for a 2 × 2 table. However, the *Q*-value will often be higher than *gamma* for the dichotomized data since the process of dichotomization will tend to mask small differences that in turn lead to inconsistent pairs in *gamma*. Therefore it is not recommended to take ordinal or nominal data and force it into a dichotomous situation. It is better to evaluate the data in its original larger format (larger than a 2 × 2 configuration). Also, Yule's Q should not be used if there is a zero in any of the cells.

The **Yule's Y test** is a modification of the Yule's *Q*. It is also called **Yule's coefficient of colligation**, and uses the geometric mean of diagonal and off-diagonal pairs rather than the number of pairs seen in the *Q*-statistic.

$$Y = \frac{(\sqrt{ad} - \sqrt{bc})}{(\sqrt{ad} + \sqrt{bc})} = \frac{\sqrt{P} - \sqrt{Q}}{\sqrt{P} + \sqrt{Q}} \qquad \text{Eq. 17.5}$$

Yule's *Y* is rarely used, because there is no easily expressible interpretation. Yule's *Y* tends to estimate associations more conservatively than Yule's *Q*. Unfortunately, this measure of association has little substantive or theoretical meaning.

Also associated with the results with a dichotomous independent variable are odds ratios and relative risk ratios. These two measures will be discussed separately and in greater detail in the next chapter.

Nominal Associations

This portion of the chapter will consider tests of association where the nominal data exceed the 2 × 2 contingency table. These nominal coefficients of association may be computed for ordinal or higher levels of data, but tests designed specifically for higher types of scales have more power and are preferred to these tests. The tests presented in this section include: 1) Pearson's C; 2) Cramer's V; 3) Tschuprow's T; 4) the *lambda* statistic; and 5) the uncertainty coefficient. These procedures adjust the chi square statistic to remove the effect of sample size. Unfortunately they are not easily interpretable, but provide an index regarding the strength of the association between nominal variables.

As seen in Chapter 1, a nominal variable consists of a set of unique categories in no specific order (e.g., males–females, treatments A–B–C–D). Tests in this section measure the strength of association between variables; however, they cannot indicate a direction or describe the nature of relationship. Each measure of association for nominal data attempts to modify the chi square statistic to reduce the influence of sample size and degrees of freedom (dimensions of the table). These tests also restrict the range of possible outcome to values between 0 and 1 (with zero indicating no association linking the two variables).

Pearson's C or the **contingency coefficient** is a modification of the *phi*-statistic for contingency tables that are larger than two rows by two columns. The formula is as follows:

$$C = \sqrt{\frac{\chi^2}{\chi^2 + N}} \qquad \text{Eq. 17.6}$$

The *C*-statistic will approach a maximum of 1.0 only for large tables (e.g., 5 × 5 or larger contingency tables). Unfortunately, the *C*-statistic is influenced by the size and shape of the contingency table. In larger non-square tables, the *C*-value will never reach 1.0 and for smaller tables the *C*-value will underestimate the level of association. To correct for this underestimation there is **Sakoda's adjusted Pearson's C** (*C**). Regardless of the size of the table the *C** will vary between 0 and 1. *C** is calculated using the following modification of Pearson's *C*:

$$C^* = \frac{C}{\sqrt{\frac{k-1}{k}}} \qquad \text{Eq. 17.7}$$

where *k* equals the number of rows or columns (whichever is smaller).

As an example, let us expand on the previous problem to four different treatment levels. Notice that the treatments represent nominal categories with no particular order. As with the ϕ-statistic, both C and C* are symmetrical and either variable (row or column) can be the independent variable. Once again, for consistency, the independent variable is presented as the column factor.

	Initial Outpatient Therapy				
Hospitalization Required	Rx A	Rx B	Rx C	Rx D	
Yes	22	14	10	14	60
No	18	26	30	26	100
	40	40	40	40	160

The chi square value (Eq. 16.2) for this example would be 8.11. With three degrees of freedom (critical value = 7.815, $p < 0.05$) the result for the chi square would be statistically significant and we would reject the null hypothesis of independence between the two variables. But how strong is the relationship between the therapy and hospitalization? The resultant *C* and *C** values are:

$$C = \sqrt{\frac{\chi^2}{\chi^2 + N}} = \sqrt{\frac{8.11}{168.11}} = 0.220$$

$$C^* = \frac{C}{\sqrt{\frac{k-1}{k}}} = \frac{0.220}{\sqrt{\frac{2-1}{2}}} = \frac{0.220}{0.707} = 0.311$$

Neither *C* nor *C** is easily interpreted. It is possible to view *C* as a nominal approximation of the correlation coefficient (*r*). Both *C* and *C** define a perfect relationship as a perfect weak monotonic, and view the null hypothesis as statistical independence. For smaller tables, it is more likely that *C* (but not *C**) will be less than 1.0 regardless of monotonicity. Therefore, Pearson's C is recommended for tables smaller than a 5 × 5 design.

An alternative for tables equal to or larger than a 5 × 5 design, is **Tshuprow's T**, which is another chi square-based measure of association. It approaches 1.0 in square contingency tables (equal number of rows and columns) where the row marginal values are identical to column marginal values. The greater the deviation from a square table or the more unequal the marginal values, the more *T* will be less than 1.0. Tshuprow's T is the square root of chi square value divided by sample size *n* times the square root of the number of degrees of freedom (rows minus one times columns minus one):

$$T = \sqrt{\frac{\chi^2}{n\sqrt{(r-1)(c-1)}}} \qquad \text{Eq. 17.8}$$

Since the *T*-value is less than 1.0 for non-square tables, it is recommended for square tables. For 2 × 2 tables, *T* equals the *phi*-statistics, since the square root of $(r - 1)(c - 1)$ is one.

$$T = \sqrt{\frac{\chi^2}{N\sqrt{1*1}}} = \sqrt{\frac{\chi^2}{N}} = \phi$$

T-statistic defines a perfect linear relationship for weak monotonicity and defines a null relationship as statistical independence. As with previous tests, Tshuprow's *T* is symmetrical. Using the previous example ($\chi^2 = 8.11$) Tshuprow's *T*-value would be:

$$T = \sqrt{\frac{8.11}{160\sqrt{(3)(1)}}} = \sqrt{\frac{8.11}{277.13}} = 0.171$$

Of all the tests for nominal associations, **Cramer's V** is the most popular. Also a chi square-based measure, it has the best 0-to-1 association when row marginal values equal column marginal values (regardless of table size). Cramer's V test is used when one or both of the variables are nominally scaled. The formula is:

$$V = \sqrt{\frac{\chi^2}{Nm}} \qquad \text{Eq. 17.9}$$

where *N* is the total sample size and *m* is either $(r - 1)$ or $(c - 1)$, whichever is smaller. Cramer's *V* can be considered as a test of association between two variables

measuring the percentage of their maximum possible variation. Squaring the *V*-value is the mean square canonical correlation between the variables. If either the rows or columns contain only two levels, Cramer's *V* equals the *phi*-statistic.

$$V = \sqrt{\frac{\chi^2}{N(1)}} = \phi$$

The *V*-statistic defines a perfect linear relationship as one that has either predictive or ordered monotonicity and the null relationship is defined as statistical independence. As with previous tests, Cramer's *V* is symmetrical and either variable can be the independent (column) variable.

Using the previous example ($\chi^2 = 8.11$) Cramer's *V* is:

$$V = \sqrt{\frac{\chi^2}{Nm}} = \sqrt{\frac{8.11}{(160)(1)}} = 0.225$$

Note that each measure of association gave a slightly different value ($T = 0.171 < C = 0.220 < V = 0.225 < C^* = 0.311$).

Another type of measure of association deals with the **proportionate reduction of error** (*PRE*). *PRE* measures are generally used only when both an independent and dependent variable are present. For nominal data a *PRE* measure of association is *lambda*; for ordinal data *PRE* measurements include *gamma* and Somers' *d*. *Lambda* is discussed below and *gamma* and Somers' *d* will be discussed in the next section. Values for all three tests range between 0 and 1. They can be interpreted as follows: if for example, we have a *PRE* value equal to 0.47; by knowing the values represented by the independent variable, we are able to reduce our errors of predicting values for the dependent variable by 47%. In other words, we reduced our amount of error by 47%. *PRE* reflects the percentage reduction in errors in predicting the dependent variable given knowledge about the independent variable. With *PRE* measurements you are trying to assess whether knowing the distribution of the dependent variable in relationship to the categories for the independent variable will enable you to reduce the amount of error in predicting the distribution of the dependent variable.

The **lambda test**, also referred to as the **Goodman and Kruskal lambda**, is the first *PRE* measurement to be discussed. *Lambda* (λ) can be used for either nominal or ordinal data (two nominal variables, one nominal and one ordinal variable, or two ordinal variables). This probabilistic measurement is defined as the probability that an observation is in a category other than the most common category (the modal category). In other words, with no knowledge of the independent variable, the researcher could guess that each observation of the dependent variable will have the same value as the most frequent level. Therefore, the marginal value for this modal category is the number of correct guesses by chance alone. This creates the denominator of the lambda equation.

$$\lambda = \frac{\sum f_i - f_d}{N - f_d} \qquad \text{Eq. 17.10}$$

where N is the total sample size, f_d is marginal total of the modal category for the dependent variable, and f_i is largest frequency for each level of the i categories of the independent variable. For the example we have used in this section (hospitalization for four different therapies), the $N = 160$, $f_d = 100$, and the f_i's are 22, 26, 30, and 26 for treatments A, B, C, and D, respectively. The lambda is:

$$\lambda = \frac{\sum f_i - f_d}{N - f_d} = \frac{(22 + 26 + 30 + 26) - 100}{160 - 100} = \frac{4}{60} = 0.067$$

In this example, knowing the drug therapy reduces errors in guessing the hospitalizations by 6.7%. The denominator represents the errors made not knowing which is subtracting the modal category of the dependent variable (f_d) from the total number of observations. In other words, if the researcher did not know the distribution of the drug therapies used, then she would guess at the likelihood of hospitalization, and she would be right 100 (f_d) times and wrong 60 ($N - f_d$) times.

Lambda can be used when both variables are dependent variables. *Lambda* ranges between 0 and 1. A value of 0 means the independent variable offers no value in predicting the dependent variable. However, it does not necessarily imply statistical independence. *Lambda* reflects the reduction in error when the value for one of the variables is used to predict values of the other variable. With a 1.0, the independent variable perfectly predicts the categories of the dependent variable. For example, a *lambda* value of 0.65 indicates that the independent variable predicts 65% of the variation of the dependent variable.

The final measure of association for nominal data is the **uncertainty coefficient (UC)**, which is also referred to as **Theil's U**. The *UC* represents a percent reduction in error that accounts for the variance in the dependent variable. This variance is defined in terms of the logarithm of the ratios, thus the *UC* is sometimes referred to as the **entropy coefficient**. Both lambda and *UC* are *PRE* measures of nominal association, but *UC* is different because the formula takes into account the entire distribution rather than just the modal distribution. Therefore, it is often preferred over *lambda*. The *UC* can vary from 0 to 1. The formula for *UC(R|C)*, is the uncertainty coefficient for predicting the dependent variable (row) based on independent variable (column):

$$UC(R|C) = \frac{\left[\sum\left(\frac{r_j}{N} \cdot ln\frac{r_j}{N}\right) + \sum\left(\frac{c_k}{N} \cdot ln\frac{c_k}{N}\right) - \sum\sum\left(\frac{n_{ij}}{N} \cdot ln\frac{n_{ij}}{N}\right)\right]}{\sum\left(\frac{c_k}{N} \cdot ln\frac{c_k}{N}\right)} \qquad \text{Eq. 17.11}$$

where r_j is the margin total for each row, c_j is the margin totals for each column, and n_{ij} is the frequency within each cell. This test also could be used for ordinal data. When the U is 0, the independent variable is of no value in predicting the dependent

variable. The uncertainty coefficient is an asymmetric measure and requires that the independent variable be placed in the columns. The "uncertainty coefficient" also has a proportionate reduction in error but the formula accounts for the entire distribution not just the mode (which is used for lambda). Therefore the uncertainty coefficient is preferred over the *lambda*-statistic.

As seen, the adjusted contingency coefficient (C^*) and Cramer's V will vary between 0 and 1.0 regardless of sample size. However, the phi-, C-, and T-statistics do not. All measures that define a perfect linear relationship as strict monotonicity, require that the distribution of the marginal values be equal for the coefficient to reach 1.0. Also, note that measures of association do not assume randomly sampled data.

Ordinal Associations

Looking at higher types of measurement scales, this section focuses on ordinal data and presents four different tests for measuring the association between two variables (*gamma*, Kendall's *tau-b*, Kendall's *tau-c*, and Somers'*d*). With ordinal measurements there are two or more categories and there is some inherent order among them (e.g., a five-point Likert scale ranging from strong disagreement to strong agreement with a statement). For *PRE* measurements, *lambda* can be used for both nominal and ordinal data (two nominal variables, one nominal and one ordinal variable, or two ordinal variables), but *gamma*, the Kendall *taus* and Somers' *d* are recommended only for two ordinal variables.

The **Goodman and Kruskal's gamma**, also simply referred to as **gamma**, is a symmetric measure based on the difference between concordant pairs (P) and discordant pairs (Q). The results can range from −1 to +1. As discussed previously concordant pairs are all possible pairs going diagonally from the upper left to lower right and discordant pairs are diagonal pairs from the upper right to lower left. *Gamma* is 0 in the case of independence and is +1 if all the observations are concentrated in the upper left to lower right diagonal of the contingency table. *Gamma* is calculated as follows:

$$\Gamma = \frac{P - Q}{P + Q} \qquad \text{Eq. 17.12}$$

The sampling distribution for *gamma* is approximating normal for large samples and it is possible to compute its standard error and significance. *Gamma* can be thought of as the surplus of concordant pairs over discordant pairs. It is a percentage of all pairs ignoring ties (by row pairs and by column pairs). The *gamma* defines a perfect association as weak monotonicity. With statistical independence, *gamma* will be 0. However, *gamma* can also be 0 whenever the concordant pairs minus discordant pairs are 0. The strength of the association would commonly be verbally described; for example, a *gamma* of +0.65 would indicate a moderate, positive association between the two variables.

For 2 × 2 contingency tables, *gamma* will equals Yule's Q-statistic. If ordinal or higher data is dichotomized into two levels, Q will usually be lower than *gamma* for the original nondichotomized data. This is because the act of dichotomizing results in

the loss of information since levels of one variable are being combined. Obviously, *gamma* cannot be computed when there is only one row or one column. However, it can be computed even when cell(s) frequencies are small or zero.

There are two Kendall *tau* tests: Kendall's *tau-b* and Kendall's *tau-c*. Kendall's *tau-b* and *tau-c* should be used when both variables are on ordinal scales. The range of possible outcomes varies from −1 to +1. The tests differ in the manner in which the concordant pairs minus discordant pairs are normalized. As a measure of association the **Kendall's tau-b** is often used for 2 × 2 contingency tables, but also may be used for larger matrices associated with ordinal data. Where *gamma* was concerned with the concordant and discordant pairs, Kendall's measures of association are based on the comparison of all possible pairs for both variables for all possible pairs of cases. It evaluates the excess of concordant over discordant pairs in the numerator and uses a term in the denominator that measures the geometric mean between the number of row pairs and column pairs. These terms were defined at the beginning of this chapter. The formula for Kendall's *tau-b* is:

$$\tau_b = \frac{P - Q}{\sqrt{(P + Q + X_0)(P + Q + Y_0)}} \qquad \text{Eq. 17.13}$$

Kendall's *tau-b* will reach either +1.0 or −1.0 for square tables only (equal number of rows and columns). However, *tau-b* is 0 under statistical independence for both square and non-square tables. It is recommended to use *tau-c* for tables that are not square.

Kendall's tau-c (also referred to as **Stuart's tau-c** or **Kendall-Stuart tau-c**) is a modification of the *tau-b* for large tables and specifically for nonsquare contingency tables (the number of rows and columns are not equal). *Tau-c* is an excess of concordant pairs over discordant pairs, times an adjustment factor for the size of the contingency table:

$$\tau_c = (P - Q)\left(\frac{2m}{n^2(m-1)}\right) \qquad \text{Eq. 17.14}$$

where n is the total sample size and m is the number of row or columns, whichever is smaller. *Tau-c* is a symmetrical test and can vary from −1 (for negative relationships) to +1. Neither *tau-b* nor *tau-c* is easy to interpret; they are simply indices of the strength of the association (somewhere between −1 and +1).

The **Somers' *d*** is a modified *gamma* statistic that penalizes for tied pairs on independent variable only, for hypotheses that are directional, where x causes or predicts y; and to penalize for pairs tied on y only, in hypotheses in which y causes of predicts x. Somers' d is used with ordinal data. The formula for the hypothesis that the column variable (y) causes or can predict the row variable (x) is:

$$d_{yx} = \frac{(P - Q)}{(P + Q + Y_0)} \qquad \text{Eq. 17.15}$$

Table 17.2 Evaluation Results from Pharmacist Survey

	Years of Practice			
Education	10 or less	11 – 20	21– 30	31 or more
5 "strongly agree"	2	3	2	1
4 "agree"	2	3	3	2
3 "uncertain"	8	6	7	4
2 "disagree"	12	4	8	18
1 "strongly disagree"	8	25	17	15
	32	41	37	40

If the hypothesis is that the row variable (x) causes or predicts the column variable (y), the formula is:

$$d_{xy} = \frac{(P - Q)}{(P + Q + X_0)} \qquad \text{Eq. 17.16}$$

Somers' d is an asymmetric statistic, but by averaging d_{xy} and d_{yx} it can be made symmetrical. The symmetric d-value will be 1.0 only when both variables have strict monotonicity. Somers' d result can be similar to the findings for other measures of association. For example, for 2 × 2 table, Somers' d will be equivalent to percent difference. For square tables, *tau-b* is the geometric mean between d_{xy} and d_{yx}. An asymmetric Somers' d will be less than or equal to *gamma* or *tau-c* for the same table.

To illustrate these ordinal measures of association, the following are data associated with two ordinal sets of data. In a study, pharmacists are asked their agreement with a statement using the Likert Scale. At the same time, the years of pharmacy practice for the respondents are divided into four ordinal categories. The results are listed in Table 17.2. What is the strength of the association between these two variables? The first task would be to calculate the impacts of the concordant (P) and discordant pairs (Q):

$$P = (2)(3) + (2)(3) + (2)(2) + \ldots (4)(15) + (8)(15) = 3257$$

$$Q = (1)(3) + (1)(3) + (1)(2) + \ldots (8)(25) + (4)(8) = 2747$$

The Goodman and Kruskal's *gamma* would be:

$$\Gamma = \frac{P - Q}{P + Q} = \frac{3257 - 2747}{3257 + 2747} = \frac{510}{6004} = 0.085$$

In this example, by knowing the pharmacists' years of practice, we can reduce the error in predicting the rank (not value) of the Likert scale response by 8.5%. The *gamma* value tells us that we can reduce our predictive error by 8.5% when we use

the independent variable to predict the dependent response. Since the χ^2 statistic was not significant (failure to reject the null hypothesis, $p = 0.06$) it is not surprising that the measure of association is so small.

Even though the contingency table is not square, we will still calculate both Kendall's *taus*. For *tau-b* we need also to calculate the pairs for ties on the columns and ties on the rows. Continuing with the same example, there are 40 pairs for the columns and 30 pairs for the rows:

$$Y_0 = (2)(2)+(2)(8)+(2)(8)+\ldots(4)(15)+(18)(15) = 1857$$

$$X_0 = (2)(3)+(2)(3)+(8)(6)+\ldots(8)(18)+(17)(15) = 2409$$

Calculated earlier there were 60 pairs each for the concordant and discordant pairs. Note that the total number of pairs is 190 (60 concordant, 60 discordant, 40 ties for columns and 30 ties for rows) which is the combination of 20 cells taken two at a time.

$$\binom{20}{2} = \frac{20!}{2!\cdot 18!} = 190$$

The *tau-b* value is:

$$\tau_b = \frac{(3257-2747)}{\sqrt{(3257+2747+2409)(3257+2747+1857)}} = \frac{510}{8132.32} = 0.063$$

Because the table is not square, the more appropriate statistic would be *tau-c*. In this example the N is 150 and m equals 4 (the smaller value for the number of columns or rows). The *tau-c* is:

$$\tau_c = (3257-2747)\left(\frac{2(4)}{(150)^2(3)}\right) = (510)(0.000119) = 0.060$$

Continuing with this same example, Somers' d for the ability to predict an evaluation response (y) based on years of practice experience (x) would be:

$$d_{yx} = \frac{(P-Q)}{(P+Q+Y_0)} = \frac{(3257-2747)}{(3257+2747+1857)} = \frac{510}{7861} = 0.065$$

Conversely, if we were to use the evaluation response (y) as a predictor of the years of practice (x), the Somers' d would be:

$$d_{xy} = \frac{(P-Q)}{(P+Q+X_0)} = \frac{(3257-2747)}{(3257+2747+2409)} = \frac{510}{8413} = 0.061$$

All three tests produce similar, although not identical, results.

With Goodman and Kruskal's *gamma tau-b*, *tab-c*, and Somers'*d* it is assumed that the data are on ordinal scales. It is possible to used interval data for these tests; however some information is lost use the ordinal process and a better assessment has already been discussed in Chapter 13 (e.g., Pearson's correlation). Once again with these tests of association, one does not need to assume that the data is randomly sampled.

Nominal-by-Interval Associations

In Chapter 10 we saw that the analysis of variance typically focuses on significance differences, not associations or relationships among variables. However, with large sample sizes, levels of the discrete independent variable may be found to be significantly different on a dependent variable, but the differences may be small. In these cases researchers may wish to use a statistic to report the strength of association effects.

Eta (E), or the **correlation ratio**, is a coefficient for nonlinear association. As seen in Chapters 13 and 14, for linear relationships the more appropriate test is the correlation coefficient (*r*) or linear regression. For a linear relationship *eta* will equal *r*, but for nonlinear relationships *eta* will be larger. Therefore, the difference between *eta* and *r* can be used as a measure of the extent to which the relationship between two variables is nonlinear.

When discussing a nominal or ordinal independent variable and interval (continuous) dependent variable, the first test that should come to mind is a one-way analysis of variance (Chapter 10). *Eta* measures the strength of the relationship between these two variables based on sums of squares presented in the ANOVA table. Therefore, the ANOVA must be computed first, before the *eta*-statistic can be determined.

$$E = \sqrt{\frac{SS_B}{SS_T}} \qquad \text{Eq. 17.17}$$

where SS_B and SS_T are taken directly from the one-way ANOVA table. *Eta* may be a useful coefficient outside the context of an analysis of variance. Although the numerator and denominator in Eq. 17.17 have meanings as in the *F*-statistics for the analysis of variance, they also measure the extent to which the *x* and *y* variables are linearly or nonlinearly related. The numerator will approach the value in the denominator as *eta* will approach 1.0.

The **coefficient of nonlinear correlation** ($\boldsymbol{E^2}$) is the percent of total variance in the dependent variable that is accounted for by the variance between levels of the independent variable(*s*). This is calculated by dividing the between-groups sum of squares by the total sum of squares.

$$E^2 = \frac{SS_B}{SS_T} \qquad \text{Eq. 17.18}$$

For linear relationships, *eta* is equal to the Pearson correlation coefficient. Also, just as r^2 can be described as the percent of in the dependent variance that can be accounted for by the linear relationship, E^2 is the percent of variance explained linearly or nonlinearly by the independent variable. Thus, E^2 is analogous to r^2 in linear regression (Eq. 14.9). *Eta* defines "perfect relationship" as curvilinear and uses statistical independence as the null hypothesis. Also, by defining the perfect association as curvilinear, *eta* is not sensitive to the order of the categories in the ordinal or nominal variable.

Similar to the ANOVA, one variable must be on the interval or ratio scale (usually but not always the dependent variable). *Eta* can be computed with either variable considered the dependent variable. The second variable must be categorical (nominal or ordinal). The frequencies of each level of the nominal or ordinal variable should be large enough to give stability to the sample means for each category.

A second measure of association, where there is nominal data (independent variable) and interval/ratio data (dependent variable), is **omega-squared (ω^2)**; sometimes referred to as the **coefficient of determination**. This is the proportion of variance in the dependent variable that is accounted for by the independent variable. It is interpreted similarly to r^2 in Chapter 14 (also called the coefficient of determination) in the linear regression model:

$$\omega^2 = \frac{SS_B - (k-1)MS_W}{SS_T + MS_W} \qquad \text{Eq. 17.19}$$

where SS_B, SS_T, MS_W, and k are taken from the ANOVA table. *Omega-square* usually varies from 0 to 1, but may have negative values when the F-ratio is less than 1. *Omega-square* is a common measure for the magnitude of the effect for an independent variable. An ω^2 is considered large when the value is over 0.15, a medium effect if between 0.06 and 0.15, and a small effect if less than 0.06 (based on a conversion by Cohen, 1988). *Omega-square* is not used for random effects models. Also, due to large variability, ω^2 is not used for two-way or higher repeated measures designs.

To illustrate the use of these tests, consider the data in Table 17.3 for patients randomly assigned to receive different doses for a specific analgesic and the patients' responses to a 100-point scale for pain relief (100 = complete pain relief, 0 = no change in pain). The analysis of variance table for this data would be:

Source	DF	SS	MS	F
Between	3	2242	747.3	6.70
Within	28	3122	111.5	
Total	31	5364		

There is a significant difference in the patients' responses ($p < 0.001$); is there a curve linear relationship? The *eta* and *omega square* would be as follows:

Table 17.3 Patient Responses to Different Amounts of Analgesic

	5 mg	10 mg	12.5 mg	15 mg
	9	19	29	49
	0	15	39	29
	35	26	37	35
	21	22	23	19
	19	36	55	40
	10	47	39	33
	24	36	45	19
	16	26	51	39
Mean =	16.75	28.38	39.75	32.88
SD =	10.66	10.57	10.63	10.37

$$E = \sqrt{\frac{SS_B}{SS_T}} = \sqrt{\frac{2242}{5364}} = \sqrt{0.418} = 0.647$$

$$\omega^2 = \frac{SS_B - (k-1)MS_W}{SS_T + MS_W} = \frac{2242 - (3)(111.5)}{5364 + 111.5} = \frac{1907.5}{5475.5} = 0.348$$

If a Pearson's correlation coefficient were run on the same data, *r* would equal 0.556. Thus, in this example, *eta* is 0.647, which compares with a Pearson's *r* correlation of 0.556 for the *grouped* data. Squaring each value, we find that a linear relationship (reflected in r^2) accounts for about 30.9% of the variance, whereas the nonlinear relationship (reflected in ω^2) accounts for 41.9% of the variance.

Reliability Measurements

The last part of the chapter will be of interest to pharmacy educators and those involved with cognitive testing and/or survey research, primarily, the researcher concerned that results from such instruments are stable and have a certain degree of consistency when administered to different groups of individuals. **Reliability** is the extent to which the measurements from the entire survey instrument and those from each item within the instrument yield the same results when administered at different times, in different locations, or to different populations. Reliability coefficients, which can be calculated, are special types of correlation coefficients. For example, consider a test instrument used to collect information about study participans (e.g., survey questionnaire). The observed results or scores can be divided into the true score and the error score (the total score = true score + error score). The error score, or deviation from the true score, can be due to either systematic error (bias) or random error. The larger the error component associated with the scores, the lesser the reliability of the instrument. As described in the following paragraphs, there are several types of reliability, each measuring a different dimension of reliability.

The assumptions associated with tests for reliability are the same as those required for the correlation coefficient; the tests involve interval/ratio scales, and the data are derived from a normally distributed population. It is also desirable that the test instrument have **validity** (measures what it is intended to measure). Reliability and validity are related, but not the same. An instrument can be reliable but not valid, but it cannot be valid without being reliable. In other words, reliability is essential, but not enough to prove validity. Reliability can refer to test stability, internal consistency, or equivalency.

Test stability means that the same results will be obtained over repeated administration of the instrument. Stability is assessed by the process of test-retest reliability or parallel forms reliability. The **test-retest reliability** involves the administration of the same test to the same subjects at two or more different points in time. The appropriate length of the interval will vary based on the specific instrument and the stability of the information being evaluated. The scores for each subject are compared using a correlation coefficient (Chapter 13). In general, an $r \geq 0.70$ is acceptable. **Parallel forms reliability** is where two or more equivalent series of items or test questions are used. These parallel sets of questions are administered to the same people and the scores are compared using a correlation coefficient. The disadvantage with the parallel forms approach is that administration of two tests is required. However, the method offers an advantage for the researcher who feels that repeated administration of the same instrument (e.g., test-retest reliability) may result in "test-wiseness" on the part of the individuals taking the tests (they will perform better the second time simply because of repeated exposure to similar questions).

The homogeneity of the items is a measure of the **internal consistency reliability** of the test instrument. Such measures determine the extent to which the items in the instrument are measuring the desired skill or knowledge. In other words, is the instrument consistently measuring the same skill or knowledge? The advantage is that only one administration of the instrument is required. Sometime referred to as **split-form reliability**, these measures of internal consistency include: 1) item-total correlations; 2) split-half reliability; 3) Kuder-Richardson coefficients; and 4) Cronbach's *alpha*. These tests will be illustrated below. The closer these various correlations are to 1.0, the greater the reliability and certainty that the two forms are equivalent.

The simplest measure of internal consistency is an **item-total correlation**, where each item in the instrument is correlated to the total score. If used as a pretest to develop an instrument; those items with low correlations should be deleted from the final instrument. This type of correlation is only important if the researcher wants homogeneity of items. The **split-half method** for measuring internal consistency involves dividing the instrument into two halves (usually odd items versus even items, or first half versus second half). The scores for each split-half are calculated and differences between each half-test for each individual subject are computed. Specific methods for evaluating this type of reliability are the Spearman-Brown conversion of the correlation coefficient and Rulon's split-half method. The **Spearman-Brown formula** is applied to the correlation coefficient comparing each half: where r_{xy} is the Pearson correlation coefficient. With **Rulon's split-half method**, the variance of the differences is compared to the variance for the total scores:

Table 17.4 Original Data for Measures of Internal Consistency (scores for the even-numbered and odd-numbered questions)

Student	Odd	Even	d
1	44	46	+2
2	35	36	+1
3	47	50	+3
4	43	39	–4
5	33	39	+6
6	25	32	+7
7	39	40	+1
8	44	40	–4
9	17	23	+6
10	47	46	–1

$$\rho = 1 - \frac{S_d^2}{S^2} \qquad \text{Eq. 17.21}$$

where S_d^2 is the variance for the difference between each split half and S^2 is the total variance to the test instrument. Obviously, if each half produces the exact same results the S_d^2 will be 0 and $\rho = 1$. Both Spearman-Brown and Rulon's method will give similar results.

To illustrate these two tests, consider the data presented in Table 17.4, which evaluates student responses to the odd and even questions on a final examination. The correlation comparing the two sets of questions is very positive ($r = 0.933$). The mean and standard deviation for the entire test for these ten students are 76.5 and 17.42, respectively. Using the approach for calculating the variance for the paired t-test (Eq. 9.10), the variance for the differences between the odd and even questions is 15.57. The calculations for the two methods of internal consistency are:

$$\rho = \frac{2r_{xy}}{1 + r_{xy}} = \frac{2(0.933)}{1 + 0.933} = \frac{1.866}{1.933} = 0.965$$

$$\rho = 1 - \frac{S_d^2}{S^2} = 1 - \frac{15.57}{(17.42)^2} = 0.949$$

The most commonly used measures of internal consistency involving dichotomous results (yes/no, true/false), are two methods developed by G.F. Kuder and M.W. Richardson at the University of Chicago in the late 1930s: the Kuder-Richardson 20 (*KR20*) and Kuder-Richardson 21 (*KR21*). The *KR20* and *KR21* are calculated as follows:

$$\rho_{KR20} = \left(\frac{k}{k-1}\right)\left(1 - \frac{\sum pq}{S^2}\right) \quad \text{Eq. 17.22}$$

$$\rho_{KR21} = \left(\frac{k}{k-1}\right)\left(1 - \frac{\overline{X}(k-\overline{X})}{k \cdot S^2}\right) \quad \text{Eq. 17.23}$$

where k is the number of test items (e.g., questions), p is the proportion of correct responses per question for each individual, $\overline{X}$ is the mean score for all persons tested, and S^2 is the total variance to the test instrument. The higher the *KR* value, the stronger the relationship between the individual items in the instrument. The *KR21* is similar to the *KR20*, but easier to compute; unfortunately the *KR20* is considered a more accurate measure. The *KR21* is a rough approximation because it involves the mean for all subjects rather than the proportion of successes and failures for each individual. The *KR21* is always less than the *KR20* unless the items are all equal in difficulty, in which case the *KR20* will equal *KR21*. Both methods are based on the consistency of responses to all the items in a single instrument.

Examples of the use of *KR20* and *KR21* are presented below using the data in Table 17.5. The table presents the results for 20 students completing a ten-item test and each item is scored as a correct or incorrect response. Listed in the lower section of the table are the p (proportion of correct answers), q (proportion of incorrect answers), and their product (pq). The sum of these products (Σpq) is 1.57. The mean for the test scores is 7.25, with a variance of 5.88. Thus, the calculations for both Kuder-Richardson measures of reliability are:

$$\rho_{KR20} = \left(\frac{10}{10-1}\right)\left(1 - \frac{1.57}{5.88}\right) = (1.111)(0.733) = 0.814$$

$$\rho_{KR21} = \left(\frac{10}{10-1}\right)\left(1 - \frac{(7.25)(10-7.25)}{(10)(5.88)}\right) = (1.111)(0.661) = 0.734$$

The reason for this high reliability becomes visually obvious if the students are ranked in order of the scores and the questions are ranked in order of their difficulty (Table 17.6). Note the clustering of correct answers (1) in the upper left and incorrect answers (0) in the lower left. For this example, the more difficult the question, the more likely that the poorer students will respond with incorrect answers.

Another commonly used measure of reliability is **Cronbach's alpha**. It measures how consistently individuals respond to the items within an instrument and can be used for nondichotomous responses (e.g., Likert scales). Cronbach's *alpha***,** also called the **reliability coefficient**, measures the extent to which responses to items, obtained at the same time, correlate with each other. It is a measure of the level of mean intercorrelation weighted by the variances and can be thought of as the average of all possible split-half estimates. In addition to estimating the reliability of the items for the average correlation, the Cronbach's *alpha* also takes into account the number

Table 17.5 Original Data for Example Problem for KR-20 and KR-21

	Instrument Items*										
Student	A	B	C	D	E	F	G	H	I	J	Score
1	1	1	1	1	1	1	1	1	1	0	9
2	1	1	1	1	0	0	1	1	1	0	7
3	1	1	0	0	0	0	1	1	0	0	4
4	0	1	1	1	1	1	1	1	1	1	9
5	1	1	1	1	1	0	1	1	1	1	9
6	1	1	1	1	1	1	1	1	1	1	10
7	1	0	0	0	0	0	1	0	1	0	3
8	1	1	1	1	1	0	1	1	1	1	9
9	1	1	1	1	1	0	1	0	0	1	7
10	1	1	1	1	1	1	1	1	1	1	10
11	1	1	0	1	0	0	1	1	0	0	5
12	1	0	0	0	0	0	1	1	0	0	3
13	1	1	1	1	1	1	1	1	1	1	10
14	0	1	1	1	1	1	1	1	1	1	9
15	1	1	1	1	0	0	1	1	1	0	7
16	1	1	0	1	0	0	1	1	0	0	5
17	1	1	0	1	1	0	1	1	1	0	7
18	1	1	1	1	1	0	1	1	1	1	9
19	1	1	1	1	1	0	1	1	1	1	9
20	1	0	0	1	0	0	1	1	0	0	4
Σ =	18	17	13	17	12	6	20	18	14	10	
p =	.90	.85	.65	.85	.60	.30	1.0	.90	.70	.50	
q =	.10	.15	.35	.15	.40	.70	0	.10	.30	.50	
pq =	.09	.13	.23	.13	.24	.21	0	.09	.21	.25	
S^2 =	.09	.13	.24	.13	.25	.22	.00	.09	.22	.26	

* Code: 1 = correct answer; 0 = incorrect answer.

of questions in the instrument. The general theory is that the larger the number of questions, the more reliable the instrument. Cronbach's *alpha* makes no assumptions about what one would obtain at a different point in time (e.g., test-retest reliability). The Cronbach's *alpha* formula is:

$$\rho_\alpha = \left(\frac{k}{k-1}\right)\left(1 - \frac{\sum S_i^2}{S^2}\right) \qquad \text{Eq. 17.24}$$

where k is the total number of questions or items in the instrument, S_i^2 is the variance for each individual item and S^2 is the variance for the total score. Thus, the more

Table 17.6 Sorted Data for Example Problem for KR-20 and KR-21

	Instrument Items*										
Student	G	A	H	D	B	I	C	E	J	F	Score
10	1	1	1	1	1	1	1	1	1	1	10
6	1	1	1	1	1	1	1	1	1	1	10
13	1	1	1	1	1	1	1	1	1	1	10
4	1	0	1	1	1	1	1	1	1	1	9
19	1	1	1	1	1	1	1	1	1	0	9
8	1	1	1	1	1	1	1	1	1	0	9
5	1	1	1	1	1	1	1	1	1	0	9
14	1	0	1	1	1	1	1	1	1	1	9
18	1	1	1	1	1	1	1	1	1	0	9
1	1	1	1	1	1	1	1	1	0	1	9
9	1	1	0	1	1	0	1	1	1	0	7
15	1	1	1	1	1	1	1	0	0	0	7
17	1	1	1	1	1	1	0	1	0	0	7
2	1	1	1	1	1	1	1	0	0	0	7
11	1	1	1	1	1	0	0	0	0	0	5
16	1	1	1	1	1	0	0	0	0	0	5
3	1	1	1	0	1	0	0	0	0	0	4
20	1	1	1	1	0	0	0	0	0	0	4
12	1	1	1	0	0	0	0	0	0	0	3
7	1	1	0	0	0	1	0	0	0	0	3
Σ =	20	18	18	17	17	14	13	12	10	6	

* Code: 1 – correct answer; 0 – incorrect answer.

consistent within-subject responses (individual variances), the greater the variability between subjects (total variance), the larger the Cronbach's alpha. Also, *alpha* will be higher if there is homogeneity of variances among questions. The generally accepted cut-off for Cronbach's *alpha* is 0.70 or greater for an item to be considered in the instrument (Nunnally and Bernstein, 1994). To illustrate Cronbach's *alpha*, we can use the same data from the *KR20* and *KR21* example. Note that the last row in Table 17.5 is the variance for each test item, the sum of which is 1.655 (ΣS_i^2) and as noted previously the variance for the test scores in 5.88. For this example the Cronbach's *alpha* is:

$$\rho_\alpha = \left(\frac{10}{10-1}\right)\left(1-\frac{1.655}{5.88}\right) = (1.111)(0.719) = 0.799$$

Test equivalence is the last measure of reliability for a test or survey instrument. It is the consistency of the agreement among various observers, or data collectors, using the same measurement or among alternative forms of the instrument. One

measure is the parallel forms approach previously discussed. The second is **interrater reliability**, which requires the administration of the same instrument to the same people by two or more raters (interviewers or observers) to establish the extent of consensus between the various raters. For nominal or ordinal data, this consensus is measured as the number of agreements divided by total number of observations. Consensus for interval or ratio scales is measured using the correlation coefficient between the scores for pairs of raters. Because the reliability coefficient makes no assumptions about mean scores for the individual raters, a t-test of the significance of r (Eq. 13.8) can be used to determine if interrater means are significantly different. Thus, for data involving interval or ratio, a Pearson's correlation coefficient can be employed. **Intraclass correlation (ICC)** can be used to measure interrater reliability. Even though the correlation coefficient can be used to measure the test-retest reliability, the ICC is recommended when sample size is small (<15) or when there are more than two tests being evaluated. It is the ratio of between-groups variance to total variance. The ICC process is described by Shrout and Fleiss (1979) and Ebel (1951).

For nominal or ordinal data one would use a different measure of agreement between two raters, **Cohen's kappa**. The two variables that contain the ratings must have the same range of values (creating a matrix with an even number of rows and columns). The *kappa* statistic normalizes the difference between the observed proportions of cases where both raters agree with the expected proportions by chance alone. This is accomplished by dividing it by the maximum difference possible for the marginal totals. The t-value is the ratio of the value of *kappa* to its asymptotic standard error when the null hypothesis (e.g., *kappa* = 0) is true. Obviously, if there are an equal number of categories for both raters, the contingency table will always be square. Consider the example of raters classifying an outcome into one of three possible categories (either nominal or ordinal). If the raters were in perfect agreement all results would fall on the diagonal.

		Rater One			
		A	B	C	
Rater Two	A	**30**	0	0	30
	B	0	**25**	0	25
	C	0	0	**15**	15
		30	25	15	70

Realistically there would probably be some differences between the observer responses:

		Rater One			
		A	B	C	
Rater Two	A	**20**	5	5	30
	B	6	**16**	3	25
	C	4	4	**7**	15
		30	25	15	70

Using the method described in Chapter 16 for the chi square test of independence, it is possible to calculate the expected values for each cell if the two raters' responses are independent of each other.

		Rater One A	Rater One B	Rater One C	
	A	**12.9**	10.7	6.4	30
Rater Two	B	10.7	**8.9**	5.4	25
	C	6.4	5.4	**3.2**	15
		30	25	15	70

The chi square for this particular set of data is 22.01, which would result in the rejection of the null hypothesis of independence between the two raters. The follow-up questions might ask how strong is the relationship between these two observers? Is there reliability between the two individuals raters?

Since the diagonal values indicate the strength of the agreement we use the diagonal values (or concordant items) in calculating Cohen's *kappa*. In this example, the observed data for the concordant items (f_0) sum up to 43 (20 + 16 + 7) and the sum of the concordant items by chance alone (f_C) or for the expected results under independence is 25 (12.9 + 8.9 + 3.2). The excess in observed results compared to the number of chance occurrences as 43 – 25 = 18. Similarly, the expected number of nonconcordant numbers is N minus the expected concordant items (f_C), which is 70 – 25 = 45. Cohen's *kappa* is simply the ratio of the two differences:

$$\kappa = \frac{f_O - f_C}{N - f_C} \qquad \text{Eq. 17.25}$$

Note that the actual frequency counts, not proportions, are used for the Cohen's *kappa*. For this example the results would be:

$$\kappa = \frac{45 - 25}{70 - 25} = \frac{18}{45} = 0.40$$

In other words, 40% of the results are concordant or the judges are in agreement 44.4% of the time. If there were perfect agreement between the two observers (first table), the results would be:

$$\kappa = \frac{70 - 25}{70 - 25} = \frac{45}{45} = 1.0$$

Thus, similar to other "coefficients of association" the measure of association is the proximity of the *kappa* to a perfect association of 1.0. *Kappa* values greater than 0.80 are considered very good, 0.61–0.80 are good, 0.41–0.60 are moderate, 0.21–0.40 are fair and <0.21 are poor (Altman, 1991).

Table 17.7 Summary of Measures of Association by Type of Scale

Dependent Variable	Second or Independent Variable	2 × 2 Table	Table Larger than 2 × 2	
			Square	Not Square
Nominal	Nominal	*Phi*	Pearson C ($\leq 4 \times 4$) Pearson C* Tshuprow's ($> 4 \times 4$)	Cramer's V Theil's U *Lambda*[*]
	Ordinal	Yule's Q	Cramer's V *Lambda*[*]	Cramer's V *Lambda*[*]
Ordinal	Nominal	Yule's Q	*Tau-c* *Lambda*[*]	*Tau-c* *Lambda*[*]
	Ordinal	*Tau-b*	*Tau-b* Somers' *d* gamma	*Tau-c* *gamma* *Lambda*[*] Somers' *d*
Interval or ratio	Nominal or ordinal	*Eta* Eta^2		
	Interval or ratio	Correlation coefficient		

[*] No independent variable.

Summary

Measures of association are used to estimate both the strength (strong, moderate, or weak) and the direction (positive/negative) of the relationship. The selection of the appropriate test is based on the type of data, hypothesis being tested and the properties of the various measures (nominal, ordinal, or index/ratio). Various textbooks provide rules interpreting for strength of the coefficient of association. Table 17.7 presents a summary of the tests presented above and the types of variables for which each is most appropriate.

References

Altman, D.G. (1991). *Practical Statistics for Medical Research*, Chapman and Hall, London, p. 404.

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*, Second edition, L. Erlbaum Associates, Hillsdale, NJ, pp. 284-288.

Ebel, R.L. (1951). "Estimation of the reliability of ratings," *Psychometrika* 16:407-424.

Knoke, D. and Bohrnstedt, G.W (1991). *Basic Social Statistics*, F.E. Peacock Publishers, p. 126.

Kuder G.F. and Richardson, M.W. (1937). "The theory of the estimation of test reliability," *Psychometrika* 2:151-160.

Nunnally, J.C. and Bernstein, I. (1994). *Psychometric Theory,* 2nd edition, McGraw–Hill, New York, pp. 277.

Shrout, P.E. and Fleiss, J.L. (1979). "Intraclass correlations: Uses in assessing rater reliability," *Psychological Bulletin* 86:420-428.

Suggested Supplemental Readings

Bohrnstedt, G.W. and Knoke, D. (1982). *Statistics for Social Data Analysis*, F.E. Peacock Publishers, Inc., Itasca, IL, pp. 283-314.

Cronbach, L.J. (1951) "Coefficient alpha and the internal structure of tests," *Psychometrika* 16:297-333.

Goodman, L.A. (1979). *Measures of Association for Cross Classifications*, Springer-Verlag, New York.

Liebetrau, A.M. (1983). *Measures of Association*, Sage Publications. Newbury Park, CA.

Miller, M.B. (1995). "Coefficient alpha: A basic introduction from the perspectives of classical test theory and structural equation modeling," *Structural Equation Modeling* 2(3):255-273.

Reynolds, H.T. (1977). *The Analysis of Cross-Classifications*. The Free Press, New York, pp. 34-61.

Example Problems (Answers are provided in Appendix D)

1. Using the following information: a) calculate the various measures of association for a 2 × 2 design; and b) indicate which results are best, given the types of variables involved.

   Assume an equal number of males and females are treated with the same medication for a specific illness and the outcome is either success or failure. Is there a relationship between patient gender and therapeutic outcome?

	Females	Males	
Success	45	30	75
Failure	5	20	25
	50	50	100

2. Using the following information: a) calculate the various measures of association for a 3 × 3 design; and b) indicate which results are best, given the types of variables involved.

   Patients are randomly divided into three groups and treated with one of three medications for high cholesterol. After six months of therapy they are assessed to determine if they met their desired cholesterol goal, did not meet goal, or were changed to a different treatment regimen. Is there a relationship between treatment and therapeutic outcome?

	Treatment A	Treatment B	Treatment C	
At goal	56	46	35	137
Not at goal	30	18	18	66
Discontinued	13	20	37	70
	99	84	90	273

3. Using the following information: a) calculate the various measures of association for a 3 × 5 design; and b) indicate which results are best, given the types of variables involved.

   A survey of pharmacists in different practice settings asks their level of agreement with a series of questions. Listed below are their responses to one question. Is there an association between practice setting and response to the question?

	Practice Setting			
Evaluation	Retail	Hospital	Long-Term Care	
5 “strongly agree”	10	2	4	16
4 “agree”	12	2	6	20
3 “uncertain”	24	12	14	50
2 “disagree”	36	20	28	84
1 “strongly disagree”	18	64	48	130
	100	100	100	300

18

Odds Ratios and Relative Risk Ratios

The previous three chapters have dealt with discrete results and this chapter will continue our discussion of such outcomes. The last two chapters have focused on descriptive statistics presented in contingency tables and inferentially evaluated using a variety of tests, both looking for statistical independence and measures of association. This chapter will focus on ratio measures, which have become increasingly more common in the literature over the last few decades, including odds ratios and relative risk ratios. The chapter will conclude with similar procedures looking at Mantel-Haenszel relative risk ratios and logistic regression.

Probability, Odds, and Risk

Commonly used methods for evaluating the importance of observed dichotomous outcomes (e.g., success/failure, live/die) are odds ratios and relative risk ratios. As discussed in Chapter 2, **probability** is the chance that something will occur (e.g., tossing a fair coin once, the probability of a head is 0.50). In contrast, **odds** for a given outcome is the ratio of the probability of a specific outcome occurring divided by the probability of that same outcome not occurring (e.g., tossing a fair, the odds of a head occurring is 1 = 0.5/0.5 or even odds of 1). **Risk** is more closely associated with probability, in that risk is the number of a negative (or positive) outcomes divided by the total number of possible outcomes (e.g., a coin is tossed 100 times and a tail occurs 60 times, the risk of a tail is 0.60 = 60/100). It is important to understand which of these outcomes to report under given situations and conditions.

Odds and relative risks are most commonly used as ratios when comparing two levels of an independent variable (e.g., treatment group versus control group). Both the odds ratio and the relative risk compare the likelihood of an event between two groups. The odds ratio compares the relative odds of two different events occurring. The relative risk compares the probability of two different events occurring. The relative risk is closer to what most individuals think of when they think of the relative likelihood of two events. As discussed in the following sections, ratios are created between the two groups. Both the odds ratio estimator and relative risk estimator employ a 2 × 2 contingency table (similar to the layouts seen in the previous two chapters, Figure 16.3), usually with the ratio between the two outcomes in each column. Some research designs, for example the case-control design, prevent

computing a relative risk because the design involves the selection of research subjects based on outcome measurements rather than exposure. However, with retrospective case control studies it is possible to calculate and interpret an odds ratio.

Odds Ratio

An odds ratio is used when retrospective data are being analyzed and involves unpaired samples. Because the data are gathered after the fact, meaningful calculations between the proportions is not possible, as will be described later when discussing relative risk. The best summary for such data is to calculate the odds ratio, which is an approximate risk.

Calculation of odds and odds ratio involves a binary dependent variable with two possible outcomes (e.g., success or failure, positive or negative results). For example, assume that an event has a 75% chance of occurring (success) and a 25% chance of not occurring (failure). The probabilities of success and failure are $p = 0.75$ and $q = 1 - p = 0.25$, respectively. Thus the odds of observing or not observing the specific event are calculated as follows:

$$odds(success) = \frac{p}{q} \qquad \text{Eq. 18.1}$$

$$odds(failure) = \frac{q}{p} \qquad \text{Eq. 18.2}$$

With this particular example the odds of success or failure are:

$$odds(success) = \frac{0.75}{0.25} = 3.00$$

$$odds(failure) = \frac{0.25}{0.75} = 0.33$$

As noted in the chapter introduction, odds and probability are not the same. In this example the probability of a success is 0.75, but the of odds of success are 3 to 1 and the odds of failure are 0.33 to 1. This makes sense; if we randomly select one sample from all possible outcomes there is a three times greater chance of selecting a success than a failure.

In the search for causes of specific diseases, epidemiologists are interested in the risks of certain behaviors or characteristics on the causes of these diseases. Outcomes (e.g., yes or no for a specific disease, disability, or death) are compared against potential risk factors (e.g., predisposing characteristics, exposure to disease or pollutants, risk-taking behavior). The design of such comparisons is presented below:

		Exposure: Yes (+)	Exposure: No (−)	
Outcome	Yes (+)	a	b	a + b
	No (−)	c	d	c + d
		a + c	b + d	n

Using this design, a cross-sectional study can be undertaken where an overall sample of the population is collected regardless of the outcomes or factors involved. For example, a cross-section of individuals living in the Midwest is compared for the incidence of chronic lung disease and compared to smoking histories. The previous 2 × 2 model can be employed, when two levels of a predictor (independent) variable are compared with two possible outcomes. Results of the hypothetical study of chronic lung disease are found in Table 18.1.

The odds of developing an outcome (e.g., disease present) in the group exposed to the risk factor are referred to as the experimental event odds (*EEO*). The odds of developing chronic lung disease for smokers would be the odds of the outcome of interest being present (chronic lung disease) in those with the risk factor present (smokers):

$$EEO = \frac{a}{c} \qquad \text{Eq. 18.3}$$

The odds of developing the outcome in the unexposed (control) group are the control event odds (*CEO*). In this case, the odds of developing chronic lung disease without the risk factor (smoking) present would be:

$$CEO = \frac{b}{d} \qquad \text{Eq. 18.4}$$

The odds ratio for developing the outcome in the experimental group is the experimental event odds divided by the control event odds, or the ratio of the odds for the risk factor present divided by the odds where the risk factor is absent:

Table 18.1 Example of Odds Ratio Data

		Risk Factor: Smoker	Risk Factor: Nonsmoker	
Chronic Lung Disease	Present	84	133	217
	Absent	68	215	283
		152	348	500

$$OR = \frac{EEO}{CEO} = \frac{a/c}{b/d} \qquad \text{Eq. 18.5}$$

The results would indicate the number of times the experimental group is more likely to develop the disease. For the data presented in Table 18.1 the odds of developing chronic lung disease for smokers is as follows:

$$odds(\ present\ ) = \frac{a}{c} = \frac{84}{68} = 1.235$$

The odds of developing chronic lung disease for a nonsmoker is:

$$odds(\ absent\ ) = \frac{b}{d} = \frac{133}{215} = 0.619$$

The odds ratio (*OR*) for developing chronic lung disease, comparing smokers to nonsmokers is:

$$OR = \frac{a/b}{c/d} = \frac{1.235}{0.619} = 1.995$$

Thus, based on the results of this retrospective study, the odds of developing chronic lung disease for smokers is approximately two times greater than nonsmokers.

When analyzing a case-control retrospective clinical trial, there are no differences between the proportion of outcomes of interest or their relative risks. Thus, the best way to summarize the data is to report the odds ratio. When the event rate is small, odds ratios are very similar to relative risks.

Confidence intervals can be created to determine if a calculated odds ratio is significant or not. With previous tests we were concerned with zero appearing within the interval (zero difference between the observed results). But with ratios we will be concerned about the location of the value one rather than zero. Consider two outcomes that have the exact same odds of occurring, $a/b = 0.50$ and $c/d = 0.50$; the odds ratio would be one:

$$OR = \frac{a/b}{c/d} = \frac{0.50}{0.50} = 1.0$$

Thus, one would indicate absolutely no difference. So with ratio-type tests we create a confidence interval and if one is within the interval there is no statistically significant difference in the two levels of our independent variable. If one cannot fall within the interval there is a significant difference. If the population odds ratio (θ) has an outcome of one, there is no significant relationship between the independent variable and the outcome.

$$H_0: \theta = 1$$
$$H_1: \theta \neq 1$$

An outcome with an odds ratio less than *one* indicates that the factor was effective in reducing the odds of a negative outcome. When the odds ratio is greater than one, there is an increase in the likelihood of the negative outcome occurring. To test the null hypothesis, it is possible to create a confidence interval, similar to previous intervals, using the best estimate (based on the sample) plus or minus a reliability coefficient times an error term. To calculate the standard error term, data is converted to the natural logarithm, because the distribution of the natural logarithm of θ ($ln\ \theta$) converts data to more of a normal distribution for smaller sample sizes than the original distribution of θ. After finding the confidence interval for $ln\ \theta$, data can be transformed back to a confidence interval for θ. The estimated error term for the sample based on $ln\ \theta$ is:

$$\hat{\sigma}_{ln(OR)} = \sqrt{\frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}} \qquad \text{Eq. 18.6}$$

Using this **log-odds ratio** is more convenient than trying to work with the odds ratio itself. The confidence interval for $ln\ \theta$ is:

$$ln\,\theta = ln_{(OR)} \pm Z_{1-\alpha/2}(\hat{\sigma}_{ln(OR)}) \qquad \text{Eq. 18.7}$$

Each $ln\ \theta$ is converted back to θ by

$$\theta = e^{ln\,\theta} \qquad \text{Eq. 18.8}$$

where e is the base of the natural logarithm and equals 2.718. Using the previous example we can test the significance of chronic lung disease with the associated risk factor of smoking:

$$\hat{\sigma}_{ln(OR)} = \sqrt{\frac{1}{84} + \frac{1}{133} + \frac{1}{68} + \frac{1}{215}} = 0.197$$

The *ln* of 1.995 is 0.691 for our best estimate:

$$ln\,\theta = 0.691 \pm 1.96(0.197)$$

$$0.305 < ln\,\theta < 1.077$$

$$e^{0.305} = 1.357 \quad and \quad e^{1.077} = 2.936$$

$$1.357 < \theta < 2.936$$

Thus, since *one* is not within the interval, there is a significant difference in the odds ratio, with smokers 1.36 to 2.94 times more likely than nonsmokers to develop chronic lung disease.

Alternatively, it is possible to establish a ratio between the *ln OR* and error term, then refer to a table for the normal standardized distribution (Appendix B, Table B2) to determine the *p*-value associated with the *z*-value from the ratio.

$$z = \frac{ln\frac{(ad)}{(cb)}}{\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}} = \frac{ln\ OR}{\sigma_{log(OR)}} \qquad \text{Eq. 18.9}$$

In the previous example this would result in the following:

$$z = \frac{0.691}{0.197} = 3.51$$

The *z*-value of 3.51 is not listed on Table B2, but can be calculated using Excel command *fx=(1–NORMSDIST(x))*2*. In this case $p = 0.00045$.

The odds ratio also can be useful in the interpretation of the results of logistic regression analysis, which will be discussed later in this chapter. Odds ratio can also be used in making covariate adjustments. It is relatively easy to adjust an odds ratio for potentially confounding variables. Such adjustments are more difficult with relative risk ratios.

Relative Risk

A second type of ratio is the risk ratio or **relative risk ratio** (*RR*). For prospective studies, the *RR* involves sampling subjects with and without the risk factor (or experimental condition) and to evaluate the development of a certain condition or outcome over a period of time. Where the term "odds" was associated with the ratio of the number of success in an outcome with an event to the number of failures, the term "risk" is the ratio of people experience negative outcomes compared to the total number within the group. With respect to proportions, odds equal *np/nq* or *p/q*, whereas risk is *np/np + nq* or *p/p + q*, which equals *p*. Where odds was calculated as the number of positive outcomes divided by the number of negative outcomes, risk is the number of negative outcomes divided by the total number of outcomes.

One could think of an odds ratio as a measure of the odds of suffering some fate or outcome. Whereas, the risk ratio gives you the percentage difference in outcomes between two groups or conditions. These two ratios (*OR* and *RR*) can be compared; however, the risk ratio is easier to interpret. One can think of an odds ratio as an approximate relative risk. However, an odds ratio is used more commonly because an odds ratio is more closely related to logistic regression and linked to other procedures. Also, relative risk requires that the contingency table have a specific orientation (factors in the columns and outcomes in the rows), an odds ratio offers more

flexibility because the results will be the same even if the table is rotated by 90 degrees.

Using the same 2 × 2 matrix seen with odds ratios, the **experimental event rate** (*EER*) is the risk associated with developing a specific outcome for the group exposed to the risk factor:

$$EER = \frac{a}{a+c} \qquad \text{Eq. 18.10}$$

and the **control event rate** (*CER*) is the risk associated with the outcome for the unexposed control group:

$$CER = \frac{b}{b+d} \qquad \text{Eq. 18.11}$$

The relative risk (*RR*) is the experimental event rate divided by the control event rate:

$$Relative\ Risk = \frac{EER}{CER} = \frac{a/(a+c)}{b/(b+d)} \qquad \text{Eq. 18.12}$$

Algebraically this can be simplified to:

$$Relative\ Risk = \frac{ab+ad}{ab+bc} \qquad \text{Eq. 18.13}$$

The relative risk predicts the likelihood of a given outcome associated with the experimental factor (e.g., there is a 1.5 greater probability of cancer in individuals exposed to a given risk factor). Relative risk can be any value greater than or equal to zero. If the *RR* = 1 there is no association between the factor and the outcome (independence). An *RR* greater than one indicates a positive association or an increased risk that the outcome will occur with exposure to that factor. If *RR* is less than one there is a negative association, or protection against the outcome. The relative risk is our best estimate of the strength of the factor-outcome association.

The complement of the risk ratio is the **relative risk reduction** (*RRR*).

$$RRR = 1 - RR \qquad \text{Eq. 18.14}$$

It can also be defined as the risk rate in the treatment group minus the risk rate in the control group, divided by the risk rate in the control group.

$$RRR = \frac{\left(\frac{a}{a+c}\right) - \left(\frac{b}{b+d}\right)}{\left(\frac{b}{b+d}\right)} \qquad \text{Eq. 18.15}$$

Table 18.2 Example of Relative Risk

		Risk Factor		
		Mask	No Mask	
Respiratory Function	Negative	6	12	18
	Positive	19	13	32
		25	25	50

Relative risk means that the treatment group has a certain percentage of the risk compared to the control group. Relative risk reduction indicates that treatment reduces risk by a certain proportion compared to the control group.

In a prospective study, volunteers are assigned to two groups and the relative risk is the ratio of the proportion of cases having a positive outcome in the two groups. For example, workers in a chemical production facility are divided into two groups: one group working unprotected in the existing conditions and the other group required to wear protective masks. After a period of time, workers in such a follow-up or longitudinal study would be evaluated on respiratory function tests. After two years the respiratory function tests are compared to baseline (values at the beginning of the study). The results are either positive (no change or an improvement in test results) or negative (a decrease in scores on the respiratory function tests). These results are seen in Table 18.2. The relative risk for developing poorer (negative) respiratory function results for those wearing a mask compared to those without the mask would be calculated as follows:

$$EER = \frac{a}{a+c} = \frac{6}{25} = 0.24$$

$$CER = \frac{b}{b+d} = \frac{12}{25} = 0.48$$

$$Relative\ Risk = \frac{a/(a+c)}{b/(b+d)} = \frac{0.24}{0.48} = 0.5$$

or:

$$RR = \frac{ab+ad}{ab+bc} = \frac{(6)(12)+(6)(13)}{(6)(12)+(12)(19)} = 0.5$$

As indicated earlier, a relative risk of less than one indicates that the intervention (in this example wearing a protective mask) was effective in reducing the risk of the outcome (decreased respiratory function). But how does one evaluate the significance of the relative risk ratio? Two methods are available, either a confidence interval or chi square test of independence. The hypotheses associated with determining the

relative risk is:

$$H_0: \quad RR_{Population} = 1$$
$$H_1: \quad RR_{Population} \neq 1$$

Similar to previous tests, a confidence interval is constructed and if 1 is within the interval, the researcher cannot reject the null hypothesis. However, if 1 is not within the interval, the null hypothesis can be rejected and one can conclude that the relative risk is significant. The interval is constructed as follows:

$$RR_{Population} = RR_{Sample}\left(e^{\pm Z_{RR}}\right) \qquad \text{Eq. 18.16}$$

where: $e = 2.718$ and

$$Z_{RR} = Z_{1-\alpha/2}\sqrt{\frac{1}{a} - \frac{1}{a+c} + \frac{1}{b} - \frac{1}{b+d}} \qquad \text{Eq. 18.17}$$

For the previous example involving protective masks, the 95% confidence interval is calculated as follows:

$$Z_{RR} = (1.96)\sqrt{\frac{1}{6} - \frac{1}{25} + \frac{1}{12} - \frac{1}{25}} = (1.96)(0.41) = 0.80$$

$$RR_{Population} = 0.5(2.7183)^{\pm 0.80}$$

$$0.5(2.7183)^{-0.80} < RR_{Population} < 0.5(2.7183)^{+0.80}$$

$$0.22 < RR_{Population} < 1.11$$

Since the value one is within the confidence interval it can be concluded that working with or without the protective masks does not appear to significantly influence respiratory function.

A second way to test for significance is to perform a chi square analysis for our 2 × 2 table, with one degree of freedom, using the same hypotheses associated with risk:

$$H_0: \ RR_{Population} = 1$$
$$H_1: \ RR_{Population} \neq 1$$

The null hypothesis is independence between the factor and the outcome. As they become closely related, the *RR* will increase and there is a greater likelihood that the difference is not due to chance alone and H_0 is rejected. For this test we will employ the Yates' correction for continuity equation. In this example (Eq. 14.5):

Table 18.3 Modified results for Previous Example

		Risk Factor		
		Mask	No Mask	
Respiratory Function	Negative	6	14	18
	Positive	19	11	32
		25	25	50

$$\chi^2 = \frac{n(|ad - bc| - .5n)^2}{(a+b)(c+d)(a+c)(b+d)}$$

$$\chi^2 = \frac{50[|(6)(13) - (12)(19)| - (0.5)(50)]^2}{(18)(32)(25)(25)} = 2.17$$

With a chi square less than $\chi^2_1 = 3.84$, we fail to reject H_0 and assume that there is not a significant association between wearing a mask (as a risk factor) and decreased pulmonary function test results (the outcome).

To prove that the statistical results are the same, let us slightly modify the results, so the results are just barely significant at 95% confidence. Consider the alternative results presented in Table 18.3, where the *RR* is 0.571. In this scenario the relative risk ratio confidence would be significant, because the value of one is not within the possible confidence interval (with 95% confidence):

$$0.197 < RR_{Population} < 0.934$$

The chi square test of independence would be significant because the calculated value is greater than the critical value of 3.84:

$$\chi^2 = \frac{50[|(6)(11) - (14)(19)| - (0.5)(50)]^2}{(18)(32)(25)(25)} = 4.25$$

In this particular case the Yates' correction for continuity was used since it gives a better approximation of the confidence interval calculated with the relative risk (Table 18.4).

As we have seen, in the case of a simple clinical trial comparing a treatment group to a control group, the relative risk ratio is the probability of an event in the experimental group divided by the probability of the event in the control group. Subtracting the relative risk for the experimental group from the relative risk for the control group produced the **absolute risk reduction** (*ARR*).

$$ARR = CER - EER \qquad \text{Eq. 18.18}$$

Table 18.4 Comparison of Chi Square Results with Relative Risk

Matrix (a,b,c,d)	Relative Risk CI	Yates' Chi Square	Pearson Chi Square
12,6,13,19	0.89 < RR < 4.48	2.17	3.13
13,6,12,19	0.98 < RR < 4.79	3.06	4.16*
14,6,11,19	1.07 < RR < 5.09*	4.08*	5.33*
12,6,13,19	0.89 < RR < 4.48	2.17	3.13
12,5,13,20	0.99 < RR < 5.81	3.21	4.37*
12,4,13,21	1.12 < RR < 8.05*	4.50*	5.88*

* Significant with $p < 0.05$.

If the *ARR* is zero, the treatment is neither beneficial nor harmful. If the *ARR* is positive the intervention has had an advantageous effect on the outcome. Often the *ARR* is stated as the inverse of the decimal. This is termed the "**number needed to treat**" (*NNT*) and represents the number needed to prevent one adverse event.

$$NNT = \frac{1}{ARR} \qquad \text{Eq. 18.19}$$

In our previous example of chemical workers, the absolute risk reduction is:

$$ARR = 0.48 - 0.24 = 0.24$$

And the number needed to treat is:

$$NNT = \frac{1}{0.24} = 4.2 \approx 5$$

For every five chemical workers using protective face masks, prevention of one case of decreased respiratory function is possible.

Graphic Displays for Odds Ratios and Relative Risk Ratios

Often graphics are used to illustrated results from either odds ratios or relative risk ratios. These are used when evaluating multiple predictor variables or when comparing multiple studies, for example, in a meta analysis. The estimate of the *OR* or *RR* is denoted by a circle (sometimes a square or diamond) and horizontal lines to each side of the circle represent the confidence interval for the population θ or $RR_{Population}$ (Figure 18.1). In this illustration, Factors A, B, and D are not significant because one is a possible outcome (within the confidence intervals), Factor C is the only significant predictor variable and represents the results seen earlier in the chapter with the *OR* for smoking and developing chronic lung disease.

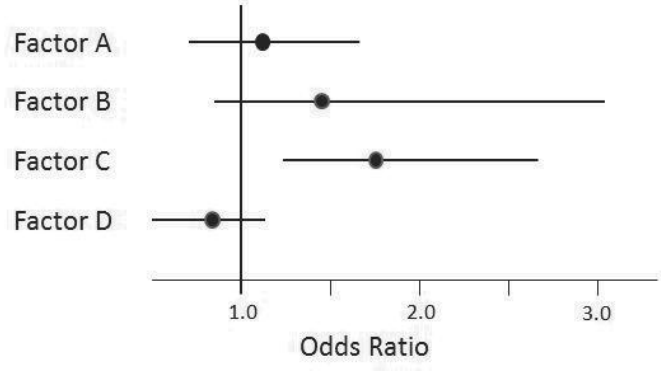


Figure 18.1 Example of graphic illustration for odds ratios.

Mantel-Haenszel Estimate of Relative Risk

In Chapter 16 we discussed the Mantel-Haenszel test for evaluating a potential confounding third variable for a 2 × 2 chi square test of independence. This procedure can be modified for dealing with odds and risk ratios. The **Mantel-Haenszel relative risk ratio**, sometimes referred to as the **Mantel-Haenszel common odds ratio,** is a method for calculating risk while controlling for a third potentially confounding variable. It removes the confounding that can result from a possible second independent variable and estimates the *RR* without the effect of a third variable. It involves stratification of our original data into levels for the third variable. The Mantel-Haenszel relative risk (RR_{MH}) is calculated as follows:

$$RR_{MH} = \frac{\sum \frac{a_i(c_i + d_i)}{N_i}}{\sum \frac{c_i(a_i + b_i)}{N_i}} \qquad \text{Eq. 18.20}$$

where a_i, b_i, ... N_i represent results at each individual strata or level. The test statistic produces an overall risk ratio controlling for the third variable. For example, consider gender as a possible confounding variable for a study comparing the incidence of Type II diabetes in overweight volunteers versus normal weight volunteers. After ten years of following initially healthy volunteers, the results presented in Table 18.5 were observed. For this example the relative risk of developing Type II diabetes in overweight and normal weight volunteers, controlling for gender is:

$$RR_{MH} = \frac{\frac{4(46+36)}{100} + \frac{22(28+48)}{100}}{\frac{46(4+14)}{100} + \frac{28(22+2)}{100}} = \frac{20.00}{15.00} = 1.333$$

Table 18.5 Relative Risk of Diabetes between Two Weight Categories and Controlling for Gender

Gender	Developed Diabetes	Over-Weight	Normal Weight	Totals
Male	Yes	4	14	18
	No	46	36	82
		50	50	100
Female	Yes	22	2	24
	No	28	48	76
		50	50	100

The results are interpreted similar to the *RR* discussed in the previous section. Without controlling for gender, the *RR* would have equaled 1.625 and is not statistically significant with a 95% confidence interval of 0.940 to 2.838. It is possible to test the null hypothesis that there is no association between the exposed and unexposed groups using the following formula.

$$\chi^2_{MH} = \frac{\left(\sum a_i - \sum \frac{a_i(c_i + d_i)}{N_i}\right)^2}{\sum \frac{(a_i + b_i)(c_i + d_i)(a_i + c_i)(b_i + d_i)}{N_i{}^2 (N_i - 1)}} \qquad \text{Eq. 18.21}$$

The resulting statistic is compared to the critical χ^2 with one degree of freedom (3.8415). If the calculated statistic is greater than 3.8415 the null hypothesis is rejected and there is a significant association between the exposure and resultant outcome. For the example presented above, the Mantel-Haenszel relative risk may have been closer to one, but the chi square results indicate that there is a significant difference when gender is considered as a confounding variable.

$$\chi^2_{MH} = \frac{\left((4 + 22) - \left[\frac{4(82)}{100} + \frac{22(76)}{100}\right]\right)^2}{\frac{18 \cdot 82 \cdot 50 \cdot 50}{100^2(99)} + \frac{24 \cdot 76 \cdot 50 \cdot 50}{100^2(99)}} = \frac{36.00}{8.33} = 4.32$$

Logistic Regression

Logistic regression is the appropriate regression model to use when the dependent variable is a dichotomous outcome (e.g., live or die, pass or fail a criteria). This binary logistic regression can be thought of as a regression analysis where the dependent variable response is a so-called dummy variable (coded *zero* or *one*). The

dummy variable is used in mathematical manipulation and results in means and standard deviations that are meaningless in terms of quantifiable measures. In the traditional least squares model in regression and the formula for linearity (Eq. 14.2) was:

$$y = a + \beta x + e$$

where y is the dependent variable, x is the independent variable, a is the coefficient for the constant, β is the coefficient on the independent variable(s), and e is the random error term. This might be extended to logistic regression by making y the dummy dependent variable (one if the outcome occurs, zero if it does not), However there are several problems with this model, including: e is not normally distributed when there is a dichotomous outcome; homogeneity of variance does not exist among different levels of the independent variable; and the predictive probability associated with the independent variable(s) can be greater than one or less than zero. Use of the log-odds model solves these problems.

Logistic regression analysis allows us to examine the relationship between a dependent discrete variable with two possible outcomes, and one or more independent (predictor) variables. In logistic regression the independent variable(s) may be continuous or discrete. Also, unlike regression analysis, it may not be possible to order the levels of the independent variable. This method is especially useful in epidemiological studies involving a binary dependent variable, where we wish to determine the relationship between outcomes and exposure variables (e.g., age, smoking history, obesity, presence or absence of given pathologies). Such binary outcomes include the presence or absence of a disease state or survival given a particular disease state. The use of odds and odds ratios for the evaluation of outcomes is one of the major advantages of logistic regression analysis.

Logistic regression can involve a single independent variable or several different predictor variables. To begin with a simple analogy to a simple regression model, with only one independent variable and one dichotomous dependent variable, consider the following example. Assume 156 patients undergo endoscopy examinations, and based on predefined criteria, are classified into two groups based on the presence or absence of gastric ulcer(s). For this specific dichotomous outcome the majority of patients (105) are found to have gastric ulcers present and the remaining 51 are diagnosed as ulcer free. Researchers are concerned that smoking may be associated with the presence of gastric ulcers, through the swallowing of chemicals found in smoking products. These same individuals are further classified as either smokers or nonsmokers. The results of the endoscopic examinations, based on the two variables, are presented in Table 18.6. The odds ratio (Eq. 18.5) for having a gastric ulcer given that a person is a smoker is:

$$OR = \frac{a/c}{b/d} = \frac{60/23}{45/28} = 1.623$$

Thus, the odds are 1.6 times greater for a smoker to exhibit a gastric ulcer (EEO = 2.609) than a nonsmoker (CEO = 1.607). The 95% confidence interval for the

Table 18.6 Outcomes from Endoscopic Examinations

	Risk Factor		
Gastric Ulcer(s)	Smokers	Nonsmokers	
Present	60	45	105
Absent	23	28	51
	83	73	156

population, based on these results (Eq. 18.7) would be 0.828 to 3.183 (not significant because the value one falls within the interval). The outcomes seen in Table 18.6 represent a 2 × 2 contingency table are similar to ones previously discussed in Chapters 16 and 17 and for which we already have several tests to analyze the data (e.g., chi square and measures of association). Where the chi square tested the relationship between the two discrete variables, the odds ratio focuses on the likelihood that the act of smoking can be used as a predictor of an outcome of gastric ulcers. Unfortunately odds ratios are only concerned with 2 × 2 contingency tables and only one dependent, or predictor, variable. Logistic regression can be used when there are two or more levels of the independent variable.

If regression analysis were used on scores of one for success and zero for failure using a fitted process, the resultant value would be interpreted as the predicted probability of a successful outcome. However, as indicated above, with dichotomous results the outcomes or predicted probabilities could exceed one or fall below zero (as discussed in Chapter 2, $0 \leq p(E) \leq 1$). In logistic regression, the equations involve the natural logarithm (*ln*) of the probabilities associated with the possible outcomes. These logarithms associated with the probabilities are referred to as the **log odds** or **logit**.

$$logit = ln \frac{\pi_{i1}}{\pi_{i2}} \qquad \text{Eq. 18.22}$$

where π_{i1} is the probability of the first possible outcome of the dichotomous outcome (presence) and π_{i2} is the probability of the second outcome (absence) at *i*th lead level of the predictor variable (smoking). These odds are based on the probabilities of being in any given cell of the matrix based on the total number of observations. The probability (π_{11}) of the presence of a gastric ulcer and being a heavy smoker is 60/156 = 0.385 and the second possible outcome for heavy smokers (π_{12} – absence of ulcer) is 23/156 = 0.147. The result would be the following probabilities, where the sum of all possible outcomes is one ($\sum p$=1.00):

	Risk Factor	
Gastric Ulcer(s)	Smoker	Nonsmoker
Present	0.385	0.288
Absent	0.147	0.179

Therefore, for smokers the logit would be:

$$logit(S) = ln\frac{0.385}{0.147} = ln(2.62) = 0.96$$

and for nonsmokers:

$$logit(\bar{S}) = ln\frac{0.288}{0.179} = ln(1.61) = 0.48$$

By using the logit transformation the transformed proportion values can range from minus infinity and plus infinity (logit(1) = +∞, logit(0.5) = 0, and logit(0) = −∞). In this particular example, the larger the logit value the greater the likelihood that the action (smoking) will serve as a predictor of the outcome (gastric ulcer).

	Risk Factor	
Gastric Ulcer(s)	Smoker	Nonsmoker
Present	60	45
Absent	23	28
Logit	0.96	0.48

A second way to express the logit model is a modification of Eq. 18.22:

$$logit = ln\frac{\pi_{i1}}{\pi_{i2}} = \mu + \alpha_i \qquad \text{Eq. 18.23}$$

where μ is a constant and α_i is the effect at the ith level. In our previous example of smoker versus nonsmokers the effect could be defined as the difference between the two logits:

$$\alpha = ln\frac{\pi_{11}}{\pi_{12}} - ln\frac{\pi_{21}}{\pi_{22}}$$

The difference of two logarithms is the logarithm of the ratio:

$$\alpha = ln\frac{\pi_{11}}{\pi_{12}} - ln\frac{\pi_{21}}{\pi_{22}} = ln\left(\frac{\pi_{11}\cdot\pi_{22}}{\pi_{12}\cdot\pi_{21}}\right) \qquad \text{Eq. 18.24}$$

In this case α is also the natural logarithm of the odds ratio:

$$OR = \frac{\pi_{11}\cdot\pi_{22}}{\pi_{12}\cdot\pi_{21}} \qquad \text{Eq. 18.25}$$

Table 18.7 Outcomes from Endoscopic Examinations with Three Levels of Smokers

	Gastric Ulcer(s)		
	Present	Absent	
Heavy smokers	19	7	26
Light smokers	41	16	57
Nonsmokers	45	28	73
	105	51	156

In this example the odds ratio is:

$$OR = \frac{\pi_{11} \cdot \pi_{22}}{\pi_{12} \cdot \pi_{21}} = \frac{(0.385)(0.179)}{(0.288)(0.147)} = 1.63$$

The advantage of using the logistic regression analysis is we can expand the number of our levels of the independent variable to more than just two. Using the above example, assume that the researcher instead classified the smokers as light and heavy smokers and found the results in Table 18.7. Logits can be calculated for each of the levels seen in Table 18.7. For example the logit for heavy smokers would be:

$$logit(HS) = ln\frac{19/156}{7/156} = ln\frac{0.122}{0.045} = ln(2.711) = 0.997$$

In this particular example, the larger the logit value the greater likelihood that the action (smoking) will serve as a predictor of the outcome (gastric ulcer). Listed below are the logit numbers for all three levels of smokers:

	Gastric Ulcer(s)		
	Present	Absent	Logit
Heavy smokers	19	7	0.997
Light smokers	41	16	0.937
Nonsmokers	45	28	0.476

An advantage with logistic regression is that it does not require the assumption of normality or homogeneity of variance.

What if the researchers are interested in a possible third confounding variable, such as stress, alcohol intake or socioeconomic class? Multiple logistic regression offers procedures and interpretations similar to those found with multiple linear regression, except the transformed scale is based on the probability of success of a particular outcome. Also, many of the procedures used for multiple linear regression can be adapted for logistic regression analysis. In Chapter 14 the plane for multiple regression was defined as follows (Eq. 14.30):

$$y_j = a + \beta_1 x_1 + \beta_2 x_2 + \beta_2 x_2 + ... + \beta_j x_j + e_j$$

and the regression model could measure the effects of one or more predictor variables (x_i) on a single dependent continuous outcome (y_i). Logistic regression analysis allows us to examine the relationship between a dependent discrete variable with two possible outcomes, and one or more independent variables (continuous or discrete). The logit model can be described by either of the two following equivalent formulas:

$$ln\left(\frac{p}{1-p}\right) = ln\, OR = a + \beta x + e \qquad \text{Eq. 18.26}$$

$$\frac{p}{1-p} = OR = exp(a + \beta x + e) \qquad \text{Eq. 18.27}$$

where *ln* is the natural logarithm and *exp* is the natural exponential function (2.718). Thus, logistic regression can be thought of as a nonlinear transformation of the linear regression model. The "logistic" distribution will be s-shaped similar to the cumulative frequency polygon (Figure 4.13) and similar to other probability outcomes ($0 \le p \le 1$). This probability can be calculated modifying Eq. 18.27:

$$p = \frac{1}{1 + exp-(a + \beta x)} \qquad \text{Eq. 18.28}$$

The functional form defined in the previous equation is the logistic function, thus the term **logistic model**.

The coefficient β is approximated by the coefficient from our sample data *b*. Note in linear regression the *b*-values represent slope coefficients and indicate the rate of change in *y* as *x* changes. However, in logistic regression the *b*-values represent the rate of change in the log odds as *x* changes. The formula in Eq. 18.28 can be expanded for multiple independent variables:

$$p = \frac{1}{1 + exp^{[-(a+\beta_1 x_1 + \beta_2 x_2 + ... + \beta_k x_k)]}} \qquad \text{Eq. 18.29}$$

where *a* is the intercept or constant coefficient and b_1 through b_k are the regression coefficients. In this case the chi square test (instead of the ANOVA) will determine the significance of the predicted outcome. Other variables that can be dichotomized (such as gender, race, age groupings) can use this coding system. Using this zero or one coding system it is possible to use odds and odds ratios for the evaluation of outcomes and this is one of the major advantages of logistic regression.

The **maximum likelihood estimation** (MLE) is a statistical method for determining the slope coefficients (*b*) and is a nonlinear least squares determination for nonlinear equations. Its determination is well beyond the scope of this book and

Table 18.8 Example of a Computer Output for Logistic Regression

Variable	Coefficient	Standard Error	Chi Square	Probability
Intercept	+0.601	0.955	6.443	0.011
Factor A (β_1)	+0.835	0.125	10.486	0.001
Factor B (β_2)	−0.284	0.103	2.667	0.102
Factor C (β_3)	−1.567	0.870	36.244	<0.0001
Factor D (β_k)	+0.307	0.942	3.128	0.077

involves computer iterations. Results of computer manipulation are presented in output tables similar to Table 18.8. The *b*-values (approximations of the βs) are in the "Coefficient" column and their associated error terms in the "Standard Error" column. The "intercept," sometimes referred to as the constant, is the point where the plane crosses the *y*-axis for the dependent variable. In Table 18.8, a chi square and its associated *p*-value are calculated for each of the specific independent variables (factors). The chi square indicates the significant association of the factor to the prediction of the binary outcome. Some computer software will also generate the odds ratio and 95% confidence interval for each factor. Using Eq. 18.27, it is possible to estimate the odds ratio by using the sample logistic coefficient:

$$OR = exp(a + bx) \qquad \text{Eq. 18.30}$$

and calculate the odds ratio by raising the *exp* to the power of the logistic coefficient:

$$OR = exp^{b} \qquad \text{Eq. 18.31}$$

In the previous example in Table 18.8, the individual OR can be calculated for each of the four factors. For example, Factor C would have an odds ratio of

$$OR_C = exp^{-1.567} = 0.209$$

For illustrative purposes, let us assume that Table 18.8 represents risk factors associated with patients seen in the emergency room and admitted to the hospital. Let us assume that a patient is seen in the ER and has Factors A, C, and D, but not Factor B in the table. What is the probability of admission for this patient? In this case our estimate of the constant (*a*) would be the intercept of 0.601. Using the Eq. 18.30, the estimated prediction of admission would be:

$$p = \frac{1}{1 + exp^{[-\{0.601+(0.835)(1)+(-0.284)(0)+(-1.567)(1)+(+0.307)(1)\}]}}$$

$$p = \frac{1}{1 + exp^{[-0.176]}} = \frac{1}{1 + 1.192} = \frac{1}{2.192} = 0.456$$

In this example the patient would have a probability of 0.456 of being admitted based on the factors presented. As will be seen in Chapter 19, this regression model can be used as part of evidence-based medicine.

If the dependent variable has more than two possible outcomes it is termed a **multinominal logistic regression** and when the multiple levels can be presented in a rank order it becomes an **ordinal logistical regression**. Application usually requires significant computer manipulation of the data to calculate the regression coefficients and goes beyond the scope of this book. A more extensive introduction to the topic of multiple logistic regression can be found in Forthofer and Lee (1995), Agresti (2002), or Kleinbaum et. al. (1982).

References

Agresti, A. (2002). *Categorical Data Analysis*, Wiley-Interscience, New York, pp. 182-191.

Forthofer, R.N. and Lee, E.S. (1995). *Introduction to Biostatistics: A Guide to Design, Analysis and Discovery*, Academic Press, San Diego, pp. 440-444.

Kleinbaum, D.G., Kupper, L.L., and Morgenstern, H. (1982). *Epidemiologic Research: Principles and Quantitative Methods*, Lifetime Learning Publications, Belmont, CA, pp. 448-456.

Suggested Supplemental Readings

Agresti, A. (2002). *Categorical Data Analysis*, Wiley-Interscience, New York.

Fisher, L.D. and van Belle, G. (1993). *Biostatistics: A Methodology for the Health Sciences*, John Wiley and Sons, Inc., New York, pp. 631-647.

Forthofer, R.N. and Lee, E.S. (1995). *Introduction to Biostatistics: A Guide to Design, Analysis and Discovery*, Academic Press, San Diego, pp. 440-444.

Kleinbaum, D.G. (2002). *Logistic Regression: A Self-Learning Text*, Springer-Verlag, New York.

Example Problems (Answers are provided in Appendix D)

1. In a retrospective study of 170 randomly selected patients, the researcher is interested in determining if several factors (family history of hyperlipidemia, presence of hypertension, presence of diabetes, and smoking) might significantly influence the odds of meeting the cholesterol level goals set for them by their

physicians based on institutional standards. The evaluation for patients with and without hypertension was as follows:

		Hypertension		
		Yes	No	
Met goal	Yes	60	24	84
	No	57	29	86
		117	53	170

2. A total of 750 women were followed for a period of ten years following radical mastectomy. A comparison of their survival rates versus whether there was axial node involvement at the time of the surgery is presented below:

		Nodal Involvement		
		Yes	No	
Outcome in 10 years	Dead	299	107	406
	Alive	126	218	344
		425	325	750

   a. Based on this one study, what is the relative risk of death within ten years following a mastectomy and positive nodes? Is the relationship between survival and node involvement statistically significant?

   b. The researchers are concerned about the presence of estrogen receptors because this factor (estrogen-positive or estrogen-negative patients) may have confounded the results of the study. Based on the following outcomes, what is the relative risk of death within ten years and does estrogen receptor status appear to confound the results?

Estrogen Receptors	Outcome	Node (+)	Node (−)	Totals
Positive	Dead	179	26	205
	Alive	100	148	248
		279	174	453
Negative	Dead	120	81	201
	Alive	26	70	96
		146	151	297

3. Modifying Problem 5 in Chapter 16, assume that containers that contained a moisture level <2000 are defined as successes. Using logistic regression, identify which amount of torque applied to the container closures would have the greatest likelihood of success?

Torque (inch-pounds):	Success (<2000)	Failure (≥2000)	
21	26	24	50
24	31	19	50
27	36	14	50
30	45	5	50
	138	62	200

4. During a cholera outbreak in a war-devastated country, records for one hospital were examined for the survival of children contracting the disease. These records also reported the children's nutritional status. Was there a significant difference in the survival rate based on nutritional status?

	Nutritional Status	
	Poor (N_1)	Good (N_2)
Survived (S_1)	72	79
Died (S_2)	87	32

19

Evidence-Based Practice: An Introduction

The chapter will introduce the topic of evidence-based practice, which involves estimating the probability of a specific outcome. This determination involves historical data and information about the "goodness" of diagnostic tests or procedures. Having this information the clinician can estimate the probabilities of certain outcomes based on positive or negative diagnostic test results.

Determining whether a patient is likely to have a specific disease or condition usually begins with an *a priori* probability for an occurrence. This is often the prevalence (or pretest probability) of the disease in a specific population. Most diagnostic tests are not perfect, but the results of the test(s) will be used to increase or decrease our estimate of the likelihood (posttest probability) of the disease. This process is sometime referred to as the **refining probability.** The most important reason pharmacists and other health professionals order a test is to help refine probability and make a decision about the best approach to treating the patient. This refining probability is the process of modifying our estimate of the probability that a disease or condition is present through the results observed on some diagnostic test(s). As will be developed in this chapter, probabilities are critical in predicting the likelihood for a particular disease in a given patient. This prediction will be based on the prevalence of the disease and the likelihood ratio associated or a modification of conditional probability resulting from a diagnostic test, which is affected by the test's sensitivity and specificity.

Sensitivity and Specificity

Conditional probability was important when we discussed the chi square test of independence. Based on Eq. 2.6 the probability of some level of variable *A* given a certain level of variable *B* was defined as

$$p(A) \text{ given } B = p(A \mid B) = \frac{p(A \cap B)}{p(B)}$$

and if the two discrete variables are independent of each other, then the probability of each level of *A* should be the same regardless of which *B* characteristic it contains.

		The Real World	
		Positive	Negative
Test Results	Positive	Sensitivity	False Positive
	Negative	False Negative	Specificity

Figure 19.1 Contingency table for determining sensitivity and specificity.

$$P(A_1/B_1) = P(A_1/B_2) = P(A_1/B_3) \ldots = P(A_1/B_K) = P(A_1)$$

These points will be revisited in this chapter where more complex tests involving frequency data are discussed.

If we develop a specific test or procedure to identify a certain characteristic or attribute (e.g., presence or absence of a disease), it is important that such a test produces the correct results. **Sensitivity** is defined as the probability that the test we use to identify a specific outcome will identify that outcome when it is truly present. If we are evaluating a diagnostic test for a specific disease, it will produce a **true positive result** if the patient actually has the disease. In the case of chemical analysis, a method will detect a specific compound if that material is present. In contrast, **specificity** is the probability that the test or method will produce a negative result when the given outcome is not present. Once again, using the example of a diagnostic test, the test will present a **true negative result** when the patient does not have the specific condition that the test is designed to detect. We can depict these results in Figure 19.1. This is similar to the figure seen in Chapter 8 for hypothesis testing where potential errors exist in the lower left and upper right quadrants. In a "perfect" world we would expect sensitivity and specificity to both have a probability of 1.00 (with all the outcomes in the upper left and lower right quadrants). Unfortunately in the real world sensitivity and specificity will usually have probabilities less than 1.00. Just like hypotheses testing, errors can occur. Continuing with our example of a diagnostic test, if administered to a "healthy" person it is possible that a positive result might occur. This would be called a **false positive result**. If the test were administered to a patient known to have the disease, but it fails to detect the condition, the results would be deemed a **false negative**. Obviously, we want our test to have high sensitivity and specificity; resulting in a low probability of either false positive or false negative results.

Before a diagnostic or analytical test is used in practice, it is important to evaluate these rates of error (false positives and false negatives) that are possible with the test. In the case of an analytical procedure, mixtures can be produced with and without the material that we wish to detect and then test to determine whether or not the material is identified by the test.

Using a medical diagnostic test we will illustrate this process. Assume we have developed a simple procedure for identifying individuals with HIV antibodies. Obviously we want our test to have a high probability of producing positive results if the person has the HIV infection (sensitivity). However, we want to avoid producing extreme anxiety, insurance complications, or even the potential for suicide, from a

		Study Volunteers		
		HIV(+)(D)	HIV(−)($\overline{D}$)	
Results of Diagnostic Procedure	Positive (T)	97	40	137
	Negative ($\overline{T}$)	3	360	363
		100	400	500

Figure 19.2 Results of testing with a new HIV diagnostic.

false positive result (1.0 − p (specificity)). Therefore we pretest on a random sample of patients who have the presence or absence of HIV antibodies based on the current gold standards for this diagnostic procedure. Assume we start with 500 volunteers with 100 determined to be HIV-positive and the remaining 400 as HIV-negative based on currently available procedures. We administer our diagnostic procedure and find the results presented in Figure 19.2.

Similar to the symbols used in Chapter 2, let us identify the true diagnostic status of the patient with the letter D (disease) for the volunteers who are HIV(+) and $\overline{D}$ (no disease) for volunteers who are HIV(−). We will use the letter T to indicate the results from our new diagnostic procedure: T for a positive test result and $\overline{T}$ for a negative test result.

Suppose we randomly sample one of the 100 HIV(+) volunteers. What is the probability that the person will have a positive diagnostic result from our test? Using conditional probability (Eq. 2.6) and the outcomes expressed as proportions (Figure 19.3) we calculate the results to be:

$$p(T \mid D) = \frac{p(T \cap D)}{p(D)} = \frac{0.194}{0.200} = 0.970$$

This meets our definition of sensitivity, the probability that a person will give a positive test if he or she has the disease. Thus, the sensitivity for a diagnostic test is 97%. In a similar manner, if we sample one patient from our 400 HIV(−) patients, What is the probability that our test results will be negative?

		Study Volunteers		
		HIV(+)(D)	HIV(−)($\overline{D}$)	
Results of Diagnostic Procedure	Positive (T)	0.194	0.080	0.274
	Negative ($\overline{T}$)	0.006	0.720	0.726
		0.200	0.800	1.000

Figure 19.3 Results for Figure 19.2 expressed as proportions.

$$p(\overline{T} \mid \overline{D}) = \frac{p(\overline{T} \cap \overline{D})}{p(\overline{D})} = \frac{0.720}{0.800} = 0.900$$

In this example the result is 90% and meets our definition of specificity as the probability that a person will give a negative test result if he or she does not have the disease. Identical results can be obtained if we work vertically within our table by dividing the frequency within each cell by the sum of the respective column.

$$Sensitivity = \frac{97}{100} = 0.970$$

$$Specificity = \frac{360}{400} = 0.900$$

Conditional probabilities can be used to calculate the probability of a false negative rate (probability of a negative result given the presence of the disease):

$$False\ negative = p(\overline{T} \mid D) = \frac{p(\overline{T} \cap D)}{p(D)} = \frac{0.006}{0.200} = 0.030$$

or a false positive rate (probability of a positive result given no disease):

$$False\ positive = p(T \mid \overline{D}) = \frac{p(T \cap \overline{D})}{p(\overline{D})} = \frac{0.080}{0.800} = 0.100$$

Because they are complementary, the same results can be obtained by subtracting the results for sensitivity and specificity from the total for all possible outcomes (1.00):

$$False\ negative = 1 - p(sensitivity) = 1.000 - 0.970 = 0.030$$

$$False\ positive = 1 - p(specificity) = 1.000 - 0.900 = 0.100$$

Two-by-Two Contingency Table

As seen previously, the sensitivity is the ability of a test (diagnostic or analytical) to detect a condition for which it is testing. For example, with a diagnostic test, if sensitive, it will give a positive result for a patient who actually has the given disease or condition. Using our previous layout for a 2 × 2 chi square design (Figure 16.3), it is possible to label a similar table for outcomes from a diagnostic test (Figure 19.4). In this model we would hope most of the test results fall in either the *a* or *d* cells of the table. Similar to conditional probability, the upper left cell (*a*) represents the frequency of **true positives** (*TP*). The lower right cell (*d*) represents the frequency

		Real World: Present	Real World: Absent	
Test Results	Present	a	b	a + b
	Absent	c	d	c + d
		a + c	b + d	n

Figure 19.4 Modification of a 2 × 2 contingency table for sensitivity and specificity.

of **true negatives** (*TN*). Both are desirable outcomes; unfortunately some patients may test as **false positives** (*PF*, cell *b*) or **false negatives** (*FN*, cell *c*). Ideally, there would be a low false positive rate and low false negative rate, meaning a low incidence of incorrect results. As an alternative to the calculations for conditional probability, the frequency counts from the contingency table can be used to calculate the sensitivity and specificity of a test using the following formula:

$$sensitivity = \frac{TP}{TP + FN} = \frac{a}{a + c} \qquad \text{Eq. 19.1}$$

$$specificity = \frac{TN}{TN + FP} = \frac{d}{d + b} \qquad \text{Eq. 19.2}$$

In addition the probability of a false positive or false negative result can also be calculated directly from a 2 × 2 contingency table:

$$p(false\ positive\ \ results) = \frac{FP}{FP + TN} = \frac{b}{b + d} \qquad \text{Eq. 19.3}$$

$$p(false\ \ negative\ \ results) = \frac{FN}{FN + TP} = \frac{c}{c + a} \qquad \text{Eq. 19.4}$$

Notice in Figure 19.4, for sensitivity and specificity, we are dealing once again with information vertically in our 2 × 2 contingency table. Using the previous example we get the exact same results employing this method:

$$Sensitivity = \frac{97}{97 + 3} = 0.970$$

$$Specificity = \frac{360}{40 + 360} = 0.900$$

$$p(false\ positive\ results) = \frac{40}{40+360} = 0.100$$

$$p(false\ negative\ results) = \frac{3}{97+3} = 0.030$$

For this example, the probability that the diagnostic test will indicate the presence of disease, when the disease is actually present (sensitivity or a true positive rate) is 0.970 or 97%. The probability that the diagnostic test will indicate an absence of the disease when the disease is actually absent (specificity or a true negative rate) is 0.900 or 90%.

The sensitivity and specificity of diagnostic procedures or commercially available tests are often available through medical literature or the manufacturer's product information. Both methods give identical results. Conditional probabilities can be thought of as definitional formulas and the contingency table approach as computational formulas.

Defining Evidence-Based Practice

Also referred to as **evidence-based medicine,** evidence-based decision making or evidence-based analysis is the process of using the best evidence in the literature to provide the best care for an individual patient.. With evidence-based practice, instead of making predictions about a population, we use statistics to apply population information to decisions about individual patients. What the practitioner is attempting to do is update his or her information about a specific patient based on previous knowledge plus diagnostic test information.

Using sensitivity and specificity alone, one cannot determine the value of a diagnostic test for a specific patient. It also requires the practitioner's index of suspicion (or the pretest probability) that the patient might have the disease. Used together, these facts can provide an estimate of the probability of disease (or absence of disease) for a specific patient. The pretest (or *a priori*) probability is the probability that a patient has the disease before undergoing a test. The best estimate of this probability is the **prevalence** of the disease or condition in that specific population. To estimate prevalence we need an understanding of the historical probability of a particular condition.

The pretest probability can be estimated by either professional experience or published scientific studies. The latter is probably more reliable, but the value of the former cannot be overlooked. Published studies in the medical and pharmacy literature are invaluable in therapeutic decision making. One of the commonly used hierarchical structures for information in evidence-based practice is the "4S" model. This is usually represented as a pyramid or triangle with four subdivisions. The first, at the widest base portion of the triangle is "studies," followed by "syntheses," "synopses," and at the top of the pyramid "systems" (Brian, 2001). The "studies" level represents original studies and clinical trials. These studies are primary literature sources and, in ascending order of importance, include: 1) case studies; 2) cases series; 3) retrospective and prospective cohort studies; 4) clinical trials; 5)

Table 19.1 Information Sources for Decision Making with Evidence-Based Practice

Level	Resource	URL
Systems	UptoDate	http://www.uptodate.com
	Physicians' Information and Education Resource (PIER)	http://pier.acponline.org/info/index.htm
	FIRSTConsult	http://www.firstconsult.com/
Synopses	Database of Abstracts of Reviews of Effectiveness (DARE)	http://www.york.ac.uk/inst/crd/index.htm
	Bondolier	http://www.medicine.ox.ac.uk/bondolier
	ACP Journal Club	http://www.acpjc.org/
Syntheses	Cochrane DSR (Database of Systematic Reviews)	http://www.libraries.iub.edu/index.php?pageId=400&resourceId=1307912
	Ovid Medline	http://www.ovid.com
Studies	PubMed/MEDLINE	http://www.ncbi.nlm.nih.gov/PubMed/
	CINAHL	http://www.cinahl.com/index.html
	OTSeeker	http://www.otseeker.com/
	PEDro	http://www.pedro.fhs.usyd.edu.au/index.html
	WebMD	http://webmd.com

randomized clinical trials; and 6) blinded randomized clinical trials. The "syntheses" level involves systematic reviews or meta analyses of relevant studies. The "synopses" level includes resources that evaluate and discuss the implications of selected studies or reviews. They are usually brief abstracts reviewing important study findings. Finally, the highest level in this hierarchy is the "systems" level; this is pre-evaluated evidence-based practice information with clinical advice on relevance of the information. With the Internet and electronic retrieval sources for these types of information, searching for information has become much easier for the practitioner. Some currently electronic resources and their URLs are listed in Table 19.1.

Using the information that can be obtained from the references sources listed in the previous paragraph, coupled with the tests described below, can result in a posttest probability. This posterior probability is the probability that a patient has the disease, given the results of the diagnostic procedure.

The threshold model can be used to estimate the probability of a patient having a disease and the value of treatment. The model is based on a continuous probability line from 0 to 1 (Figure 19.5). The testing threshold is that point on this continuum where no difference exists between the value of not treating the patient and performing a diagnostic test. The treatment threshold is that point on the continuum where no difference exists between the value of performing the test and treating the

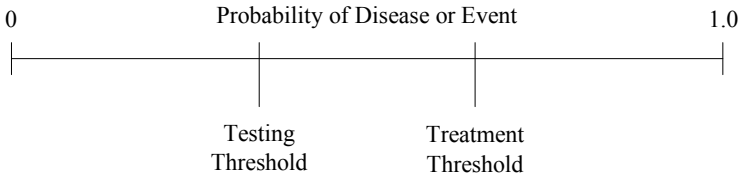


Figure 19.5 The threshold model.

patient without doing the diagnostic test. This model was originally proposed by Pauker and Kassirer (1980). This model can be used to assist practitioners in making decisions based on the risks and benefits associated with ordering tests and therapeutic interventions. The questions that must be answered are: 1) what is the probability that a given patient has the disease; and 2) where on this continuum does the probability lie? The diagnostic test results may have varying effects on our estimate of the probability of disease. Clinicians will make choices on whether to treat or not treat a disease by considering if the results have crossed a treatment threshold.

As seen in the previous section, in order to calculate the sensitivity and specificity for a test, the information in the columns requires that we already know who had the disease or condition. However, in day-to-day clinical decision making what we really are interested in is what a positive or negative test result will mean to an individual patient being tested and the probability (given a positive or negative test result) that he or she will have the disease or condition. Using the test sensitivity and specificity, along with the estimated prevalence of the disease or condition, the calculation of a refined probability of a specific outcome can be accomplished by different methods: 1) Bayes' theorem; 2) a 2×2 contingency table and application of the likelihood ratio; or 3) a decision tree. This chapter will focus first the two approaches; the decision tree approach is discussed in Shlipak (1998).

Frequentist versus Bayesian Approaches to Probability

As discussed in previous chapters, probability theory is the body of knowledge that enables us to make determinations about uncertain events. The populist or **frequentist approach** was presented in Chapter 2 where the probability p of an uncertain event A, written $p(A)$, is defined by the frequency of that event based on *previous* observations. As seen in Chapter 2, using a fair deck of cards, the probability of drawing a queen at random is 0.077 (four queens out of 52 cards). In health care, based on prior knowledge of a disease state, one could estimate the probability that an individual will develop that disease. This is based on the prevalence of the particular disease.

This frequentist approach to the probability of an uncertain event is helpful, if we have accurate information about past instances of that event or disease. However, what if no historical database exists? In these situations we need to consider an alternative approach. Using our previous example of a new HIV diagnostic test, since there is no previous experience with this kit, we cannot use the frequentist approach

to define our degree of confidence in correct test results for this uncertain event.

As seen in the beginning of this chapter, conditional probability was defined with respect to joint probability (Eq. 2.6):

$$p(A) \text{ given } B = p(A \mid B) = \frac{p(A \cap B)}{p(B)}$$

Conditional probability $p(A|B)$ can also be calculated without reference to the joint probability $p(A \cap B)$. Rearranging the previous formula we can calculate $p(A \cap B)$:

$$p(A \cap B) = p(A|B) \cdot p(B) \qquad \text{Eq. 19.5}$$

because of symmetry we can also create:

$$p(A \cap B) = p(B|A) \cdot p(A) \qquad \text{Eq. 19.6}$$

Substituting for $p(A \cap B)$ we remove the need for the information about this intercept term and create what is called **Bayes' rule or Bayes' theorem**:

$$p(A|B) = \frac{p(B|A) \cdot p(A)}{p(B)} \qquad \text{Eq. 19.7}$$

Bayes' rule (British clergyman, Thomas Bayes, 1702-1761) provides a mechanism for updating our estimate of the probability of A based on evidence provided by B. Our final estimate $p(A/B)$ is calculated by multiplying our prior estimate $p(A)$ and the likelihood $p(B/A)$ that B will occur if A is true. In many situations computing $p(A|B)$ is difficult to do directly. However, we might have direct information about $p(B|A)$. One of the strengths of Bayes' rule is that it enables us to compute $p(A|B)$ in terms of $p(B|A)$. Bayes' theorem has become the basis for **Bayesian statistics**. It involves the evaluation of data using a utility function (which is probability-based) and then maximizing the expected utility.

With the frequentist approach, statistical methods attempt to provide information about outcomes or effects through the use of easily computed p-values. However, as seen in previous chapters there are problems surrounding the use of p–values, including statistical versus clinical significance, one–tailed versus two–tailed tests, and difficulty in interpreting confidence intervals and null hypotheses associated with Type I and II errors. In contrast, the Bayesian approach can provide probabilities that are often of greater interest to clinicians, for example, the probability that treatment X is similar to treatment Y or the probability that treatment Y is at least 10% better than treatment X. These methods may be simpler to use and understand in monitoring ongoing trials. However, at the same time, Bayesian methods are controversial in that they require assumptions about prior probabilities and sometimes the calculations are more complex, even though the concepts are simpler. Good sources of information about Bayesian statistics include Lee (1997) and Press (1989). These are listed in the suggested readings at the end of this chapter.

Predictive Values

Using the previous example of our HIV diagnostic test, let us apply Bayes' theorem. Based on the initial trial results with our diagnostic test, which had a sensitivity of 97% and specificity of 90%, what is the probability that a single individual who has the HIV antibody in the general population will test positive with the new diagnostic? This would be the question of interest in evidence-based practice. Assuming only our sample of 500 volunteers the answer would be:

$$p(D \mid T) = \frac{p(D \cap T)}{p(T)} = \frac{0.194}{0.274} = 0.708$$

Sensitivity and specificity are evaluators for the test procedure. However, we are more interested in the ability to detect a disease or condition based on the test results; specifically, the probability of disease given a positive test result (called the **predicted value positive, *PVP***) and the probability of no disease given a negative test result (termed the **predicted value negative, *PVN***). In other words, we are interested in the general population and want to know the probability that a person having the HIV antibody will give a positive result on our test. In order to accomplish this we can expand upon Bayes' theorem.

$$p(A \mid B) = \frac{p(B \mid A) \cdot p(A)}{p(B)}$$

As discussed in Chapter 2, an event can be expressed as the sum of probabilities of the intersection of the event with all possible outcomes of a second event:

$$p(B) = \sum p(B \cap A_i)$$

With conditional probabilities the relationship can be expressed as

$$p(B) = \sum p(B \mid A_i) p(A_i)$$

Substituting our symbols for disease the result is:

$$p(T) = p(T \mid D) p(D) + p(T \mid \overline{D}) p(\overline{D})$$

The result is used in the denominator of Bayes' theorem to produce what is termed the predicted value positive:

$$PVP = p(D \mid T) = \frac{p(T \mid D)p(D)}{p(T \mid D)p(D) + p(T \mid \overline{D})p(\overline{D})} \qquad \text{Eq. 19.8}$$

It is possible also to determine the probability of not having HIV antibodies given a negative diagnostic result or a *PVN*:

$$PVN = p(\overline{D} \mid \overline{T}) = \frac{p(\overline{T} \mid \overline{D})p(\overline{D})}{p(\overline{T} \mid \overline{D})p(\overline{D}) + p(\overline{T} \mid D)p(D)} \qquad \text{Eq. 19.9}$$

These predictive values will help redefine probability in the patient's specific population and will provide information on the likelihood a disease is present or absent in a specific patient. If a disease or condition is either extremely rare, or conversely, very common, then only an extremely definitive test is likely to change the posttest probabilities. However, midrange probabilities (between 0.20 and 0.80) can change greatly on the basis of even a reasonably definitive test.

If we apply these equations to the results for our 500 volunteers we should expect to calculate the same result as seen in the first conditional probability in this section. Using the proportions in Figure 19.3, based on other gold standard tests, we know the prevalence of the disease specific to only our volunteers is:

$$p(D) = 0.200 \qquad \text{and} \qquad p(\overline{D}) = 0.800$$

Using conditional probabilities we were able to calculate the probabilities for true positives (sensitivity) and true negatives (specificity)

$$p(T \mid D) = 0.970 \qquad \text{and} \qquad p(\overline{T} \mid \overline{D}) = 0.900$$

and calculate the probabilities of false positive and false negative results:

$$p(\overline{T} \mid D) = 0.030 \qquad \text{and} \qquad p(T \mid \overline{D}) = 0.100$$

Applying this information the *PVP* and *PVN* for our sample can be calculated:

$$PVP = p(D \mid T) = \frac{p(T \mid D)p(D)}{p(T \mid D)p(D) + p(T \mid \overline{D})p(\overline{D})}$$

$$PVP = \frac{(0.97)(0.20)}{(0.97)(0.20) + (0.10)(0.80)} = 0.708$$

$$PVN = p(\overline{D} \mid \overline{T}) = \frac{p(\overline{T} \mid \overline{D})p(\overline{D})}{p(\overline{T} \mid \overline{D})p(\overline{D}) + p(\overline{T} \mid D)p(D)}$$

$$PVN = \frac{(0.90)(0.80)}{(0.90)(0.80) + (0.03)(0.20)} = 0.992$$

Similar to the previous equations using for a 2 × 2 contingency table (Figure 19.4), the equations for *PVP* and *PVN* can be simplified. The *PVP* is the proportion of patients with a positive test result who actually have the disease or condition:

$$PVP = \frac{TP}{TP + FP} = \frac{a}{a + b} \qquad \text{Eq. 19.10}$$

The *PVN* is the percent of patients with a negative result who do not truly have the condition or disease:

$$PVN = \frac{TN}{FN + TN} = \frac{d}{c + d} \qquad \text{Eq. 19.11}$$

Notice in these two equations we are dealing with horizontal information presented in the 2 × 2 contingency table. Using the information in Figure 19.2 we find the same results using either set of formulas:

$$PVP = \frac{a}{a + b} = \frac{97}{137} = 0.708$$

$$PVN = \frac{d}{c + d} = \frac{360}{363} = 0.992$$

If we define the proportion of patients with the disease (in this case 100 out of 500 volunteers) as a prevalence, we can further rewrite Eqs. 19.8 and 19.9 to be stated as follows:

$$PVP = \frac{(sensitivity)(prevalence)}{[(sensitivity)(prevalence)] + [(1 - specificity)(1 - prevalence)]} \qquad \text{Eq. 19.12}$$

$$PVN = \frac{(specificity)(1 - prevalance)}{[(specificity)(1 - prevalence)] + [(1 - sensitivity)(prevalence)]} \qquad \text{Eq. 19.13}$$

Without going through the entire derivation of these two formulas, we will prove the equations using the data from Figure 19.4. With our knowledge of the associated sensitivity and specificity from these volunteers, we can calculate *PVP* and *PVN* where prevalence is 0.20 (100 out of 500 volunteers):

$$PVP = \frac{(sensitivity)(prevalence)}{[(sensitivity)(prevalence)] + [(1 - specificity)(1 - prevalence)]}$$

$$PVP = \frac{(0.97)(0.20)}{(0.97)(0.20)+(1-0.90)(1-0.20)} = 0.708$$

$$PVN = \frac{(\text{specificity})(1-\text{prevalance})}{[(\text{specificity})(1-\text{prevalence})]+[(1-\text{sensitivity})(\text{prevalence})]}$$

$$PVN = \frac{(0.90)(0.80)}{(0.90)(0.80)+(0.03)(0.20)} = 0.992$$

Using Eqs. 19.12 and 19.13 we have simplified our equation to requiring only three pieces of information: sensitivity and specificity of the diagnostic test and the prevalence of the disease or condition. In the previous case our prevalence was based on our knowledge of only 500 volunteer in the study. To extend these equations for the general population or a subpopulation we will use an estimate of the prevalence of a given disease. Prevalence is the probability of persons in a defined population having a specific disease or characteristic of interest. For illustrative purposes, let us assume that a review of the literature revealed that the prevalence of HIV antibodies (*D*) in the general U.S. population is 5%. We would replace the previous $p(D)$ and $p(\overline{D})$ with the information for the U.S. population and recalculate the *PVP* to be:

$$PVP = \frac{(\text{sensitivity})(\text{prevalence})}{[(\text{sensitivity})(\text{prevalence})]+[(1-\text{specificity})(1-\text{prevalence})]}$$

$$PVP = \frac{(0.970)(0.050)}{(0.970)(0.050)+(0.100)(0.950)} = 0.338$$

Thus, based on initial trials with our diagnostic test, there is only a 33.8% chance that an individual with HIV antibodies will be identified using our test. The negative predictive value is:

$$PVN = \frac{(\text{specificity})(1-\text{prevalance})}{[(\text{specificity})(1-\text{prevalence})]+[(1-\text{sensitivity})(\text{prevalence})]}$$

$$PVN = \frac{(0.90)(0.95)}{(0.90)(0.95)+(0.03)(0.05)} = 0.998$$

However, based on these same initial measures of sensitivity and specificity, there is a 99.8% chance that a patient with a negative test result actually does not have the disease. Therefore, selectivity and sensitivity of a procedure can be applied to a known prevalence to predict the ability to detect specific outcomes.

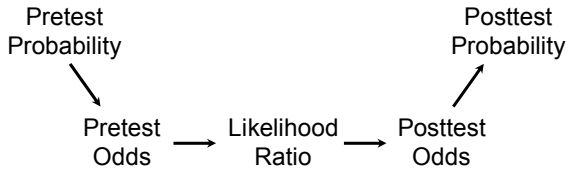


Figure 19.6 Redefining probability using the likelihood ratio.

disease. Therefore, selectivity and sensitivity of a procedure can be applied to a known prevalence to predict the ability to detect specific outcomes.

Notice in the previous examples we were dealing with dichotomous results (pass or fail, present or absent). Such dichotomies will be used for the following tests that are expansions of the chi square test of independence.

Likelihood Ratios

An alternative, equivalent process for redefining pretest probability and create new posttest probability involves the likelihood ratio(s). Using this method we combine the likelihood ratio with information about the prevalence of the disease or condition to determine the posttest probability of disease. This is illustrated in Figure 19.6. Unfortunately, the direct relationship needs one final modification, namely, incorporating odds into the calculations.

As mentioned earlier, **pretest probability** or **prior probability** is a term used to describe the probability of an event occurring based on previous experience with that event. It is the probability of a given disease prior to performing any diagnostic procedure. Clinically this might be referred to as the practitioner's **index of suspicion**. For example, based on national data, the probability of a *certain disease* occurring in otherwise healthy individuals is 0.020. This prior probability is used with a likelihood ratio to calculate a **posttest probability** or **posterior probability**. This posttest result is the probability that a specific patient has the disease, given the result of the diagnostic procedure for that patient is positive. Calculating the likelihood ratio is based on the sensitivity and specificity of the diagnostic procedure used to determine the latter probability.

If an individual gives a positive test result, how many times more likely is this individual to actually have the disease present? As discussed previously, to evaluate the success or failure of a diagnostic procedure, the sensitivity is defined as the proportion of individuals with a given disease that are correctly identified as positive by the diagnostic test. The specificity is that proportion of individuals without disease, that is correctly identified as negative, by the diagnostic test. A diagnostic test may be very useful in one specific population, but could possibly be worthless for screening in a different population. This is determined by the **likelihood ratio,** which is dependent on both the sensitivity and specificity of the test:

$$LR^{+} = \frac{Sensitivity}{1 - Specificity} \qquad \text{Eq. 19.14}$$

The resultant value for the likelihood ratio can range from zero to infinity. The LR^{+} is the likelihood of a particular test result in someone *with* disease divided by the likelihood of the same test results in someone *without* the disease. If a calculated likelihood ratio is 8.0, then the individual with a positive test result is eight times more likely to have the disease than someone with a negative test result. This is sometimes referred to as the **likelihood ratio for a positive result** and symbolized as LR^{+}.

It is also possible to calculate the **likelihood ratio for a negative result**:

$$LR^{-} = \frac{1 - Sensitivity}{Specificity} \qquad \text{Eq. 19.15}$$

Similar to the description above, the LR^{-} is the likelihood of a negative test result in someone *with* disease divided by the likelihood of the same test results in someone *without* the disease. In this case an LR^{-} of 8.0 would indicated that an individual patient with a negative test result is eight times more likely to not have the disease than another patient with a positive test result.

These two likelihood ratios do not require a 2 × 2 contingency table and are easy to calculate if information in the literature provides only sensitivity and specificity for a diagnostic test or procedure. But looking at Eqs. 19.14 and 19.15 we can see where they can be derived from such a table:

$$LR^{+} = \frac{sensitivity}{1 - specificity} = \frac{\dfrac{a}{a+c}}{\dfrac{b}{b+d}} \qquad \text{Eq. 19.16}$$

$$LR^{-} = \frac{1 - specificity}{sensitivity} = \frac{\dfrac{c}{a+c}}{\dfrac{d}{b+d}} \qquad \text{Eq. 19.17}$$

Using data from our previous example of the HIV diagnostic test, with a sensitivity of 0.970 and specificity of 0.900, the two likelihood ratios would be:

$$LR^{+} = \frac{Sensitivity}{1 - Specificity} = \frac{0.970}{0.100} = 9.70$$

$$LR^{-} = \frac{1 - Sensitivity}{Specificity} = \frac{0.030}{0.900} = .0033$$

Table 19.2 Impact of Likelihood Ratios of the Posttest Probability

Likelihood Ratio	Posttest Probability
0	No disease
0.1	Lower incidence
1	No change
10	Higher incidence
∞	Disease is certain

Modified from: Go, A.S. (1998). "Refining Probability: An Introduction to the Use of Diagnostic Tests" (1998). *Evidence-Based Medicine: A Framework for Clinical Practice*, Friedland, D.J., ed., Appleton and Lange, Stamford, CT, p. 24.

or taking the results directly from Figure 19.2:

$$LR^{+} = \frac{\frac{a}{a+c}}{\frac{b}{b+d}} = \frac{\frac{97}{100}}{\frac{40}{400}} = \frac{0.970}{0.100} = 9.70$$

$$LR^{-} = \frac{\frac{c}{a+c}}{\frac{d}{b+d}} = \frac{\frac{3}{100}}{\frac{360}{400}} = \frac{0.030}{0.900} = 0.033$$

In this case, if a patient has a positive test result, he or she is 9.7 time more likely to have the disease than a patient with a negative result. How does one interpret the likelihood ratio with respect to the value of a diagnostic test? In general, the greater the LR^{+}, the better the test at diagnosing the disease or condition. Likelihood ratios equal to or greater than 10 are considered to be useful. In contrast, the smaller the negative LR^{-}, the better the test at excluding the disease or condition. Negative likelihood ratios equal to or less than 0.1 are considered useful.

An advantage to using likelihood ratios is they can be derived from knowing only the test sensitivities and test specificities. The likelihood ratio can be used as a quick estimate of the posttest probability and the amount of certainty of the disease being present (Table 19.2). Another advantage, as will be seen later: if independent tests are involved they can be multiplied together to calculate a single estimate of a specific patient outcome.

The likelihood ratio combines information about test sensitivity and specificity and provides an indication of how much the odds of the presence of a disease or condition change based on a positive or a negative diagnostic result. However, in order to apply the likelihood ratio, one needs to know the pretest odds (also a ratio). With this information in hand, one can multiply the pretest odds by the likelihood ratio to calculate the posttest odds.

Table 19.3 Comparison of Probabilities to Odds

Probability	Odds
0.01	1 to 99
0.05	1 to 19
0.10	1 to 9
0.20	1 to 4
0.25	1 to 3
0.33	1 to 2
0.50	1 to 1
0.66	2 to 1
0.75	3 to 1
0.80	4 to 1
0.90	9 to 1
0.95	19 to 1
0.99	99 to 1

As discussed in Chapter 18, odds is the number of times a given outcome occurs, divided by the number of times that specific event does not occur, which differs from probability (the number of outcomes divided by the total number of events). The use of odds is another way of calculating the likelihood or probability of an event that is fairly easy to understand and can be useful in applying the likelihood ratio. Equation 18.1 could be modified to express odds as follows:

$$odds = \frac{p}{1-p} \qquad \text{Eq. 19.18}$$

By manipulating this equation, a probability can be calculated for any odds:

$$probability = \frac{odds}{odds+1} \qquad \text{Eq. 19.19}$$

Some examples of the conversion from probability to odds are presented in Table 19.3.

With respect to the pretest odds involving the likelihood ratio, such a value is calculated by dividing the probability of the condition (prevalence, based on the literature), by the complementary probability of not having the condition:

$$Pretest\ odds = \frac{p(D^+)}{p(D^-)} \qquad \text{Eq. 19.20}$$

Thinking of these likelihood ratios in terms of odds ratios (Chapter 18), the LR^+ represents how much the odds of having a disease increases in the presence of

positive test results. The LR^- indicates how much the odds of the disease decrease when the test result is negative.

Consider the administration of a diagnostic test; if the researcher has specific information about anticipated odds of an outcome before the test, it can be multiplied by the likelihood ratio to create the posttest odds for that outcome:

$$odds_{post} = (odds_{pre})(LR^+) \qquad \text{Eq. 19.21}$$

As seen in the equation, the magnitude of the likelihood ratio will have a direct effect on the magnitude of the posttest probability. These posttest odds represent the chance that a specific patient with a positive test result actually has a disease. Thus, if the researcher can combine the likelihood ratio with information about the prevalence of a specific disease, characteristics of the patient population, and information about the particular patient (represented as the pretest odds), it is possible to predict the odds of the disease being present. This forms the basis of evidence-based practice.

The calculation of the posttest probability is presented in Figure 19.6. The steps required to calculate the redefined probability are:

1. Estimate the pretest probability (prevalence).
2. Convert the pretest probability to pretest odds (Eq. 19.20).
3. Multiply the likelihood ratio by the pretest odds to create the posttest odds (Eq. 19.21).
4. Convert the posttest odds to a posttest probability (Eq. 19.19).

Using this process we can calculate the probability of a patient having HIV if he or she tests positive to our new diagnostic test. As defined in a previous section the prevalence of HIV infections is 5% or a pretest probability of 0.050. The pretest odds would be:

$$odds_{pre} = \frac{p}{1-p} = \frac{0.050}{0.950} = 0.0526$$

Multiplying the pretest odds by the likelihood ratio calculated in the previous section, the posttest odds would be:

$$odds_{post} = (odds_{pre})(LR^+) = (0.0526)(9.70) = 0.5102$$

Finally, the conversion of the posttest odds to a posttest probability would be:

$$posttest\ \ probability = \frac{odds_{post}}{odds_{post} + 1} = \frac{0.5102}{1.5102} = 0.338$$

Therefore, a positive diagnostic result for our new test would mean the patient has a 34% chance of truly being infected. The result is identical to the value we calculated

for the *PVP* using Bayes' theorem. Thus, either approach would give us the same answer.

The post probability for not having the disease, given a negative test result can be calculated in a similar manner, substituting the $1 - prevalence$ for the pretest probability. However, in this case the LR^- is used as the likelihood ratio and becomes the denominator in the equation.

$$odds(-)_{post} = \frac{odds(-)_{pre}}{LR^-} \qquad \text{Eq. 19.22}$$

Using our HIV example once again:

$$odds(-)_{pre} = \frac{p}{1-p} = \frac{0.950}{0.050} = 19.0$$

$$odds(-)_{post} = \frac{odds(-)_{pre}}{LR^-} = \frac{19.0}{0.033} = 575.76$$

$$posttest\ \ probability(-) = \frac{odds(-)_{post}}{odds(-)_{post} + 1} = \frac{575.76}{576.76} = 0.998$$

In this case the posttest probability is equal to the PVN using Bayes' rule. The terms *predicted value positive* and *posttest probability* or *posterior probability* of having the disease are synonymous. Similarly, the predicted value negative and posttest probability of not having the disease or condition are also synonymous.

A third approach to determining posttest probability would be through the use of a visual graphic. Go (1998) provides a simple visual method for calculating posttest probability using a nomogram. It represents a simplification of Fagan's earlier nomogram in the *New England Journal of Medicine* (1975). On three vertical axes are pretest probability, likelihood ratio, and posttest probability. Using a ruler (or similar straightedge) to line up the pretest probability and the likelihood ratio, this straightedge crosses the third line at a value for the posttest probability (labeled as percentages).

As mentioned previously, starting with the pretest odds, it is possible to combine the results from multiple tests to produce final posttest odds. This process can be used if there are multiple diagnostic criteria, since one of the useful properties of likelihood ratios is that they may be used in sequence. Therefore, determination of the posttest probability can involve combining likelihood ratios, but only if the diagnostic tests are independent and not influenced by the outcomes of the other tests (Figure 19.7). Thus, we can keep modifying the posttest probability on the basis of a series of test results.

$$odds_{post} = (odds_{pre})(LR_1^+)(LR_2^+)...(LR_k^+) \qquad \text{Eq. 19.23}$$

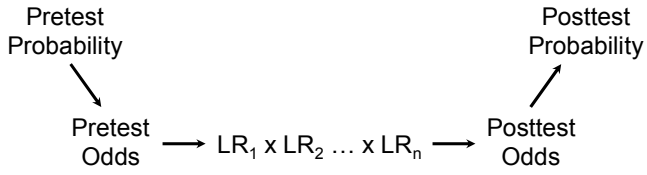


Figure 19.7 Redefining probability for multiple independent tests.

One additional advantage of the likelihood ratio is that it can be used for continuous or ordinal data and measure the magnitude of these results.

References

Brian, H.R. (2001). "Of studies, syntheses, synopses and systems: the '4S' evolution of services for finding current best evidence," *American College of Physicians Journal Club* 134(2):A11-A13.

Fagan, T.J. (1975). "Nomogram for Bayes's theorem," *New England Journal of Medicine* 293:257.

Go, A.S. (1998). "Refining probability: an introduction to the use of diagnostic tests," *Evidence-Based Medicine: A Framework for Clinical Practice*, Friedland, D.J., ed., Appleton and Lange, Stamford, CT, p. 27.

Pauker, S.G. and Kassirer, J.P. (1980). "The threshold approach to clinical decision making," *New England Journal of Medicine* 302:1109-1117.

Shlipak, M.G. (1998). "Decision analysis," *Evidence-Based Medicine: A Framework for Clinical Practice*, Friedland, D.J., ed., Appleton and Lange, Stamford, CT, pp. 35-57.

Suggested Supplemental Readings

Bland M. and Peacock, J. (2000). *Statistical Questions in Evidence-Based Medicine*, Oxford University Press, Oxford, UK.

Friedland, D.J., ed. (1999). *Evidence-Based Medicine: A Framework for Clinical Practice*, Appleton and Lange, Stamford, CT.

Forthofer, R.N., Lee, E.S. and Hernandez, M. (2006). *Introduction to Biostatistics: A Guide to Design, Analysis and Discovery*, Second edition, Academic Press, San Diego, pp. 80-87.

Lee, P.M. (2012). *Bayesian Statistics: An Introduction*, John Wiley and Sons, New York.

Mayer, D. (2009). *Essential Evidence-Based Medicine*, Second edition, Cambridge University Press, Cambridge, UK.

Sackett, D.L. (2005). *Evidence-Based Medicine: How to Practice and Teach EBM*, Third edition, Elsevier, London.

Example Problems (Answers are provided in Appendix D)

1. Returning to the first example in the problem set for Chapter 2, we employed 150 healthy female volunteers to take part in a multicenter study of a new urine testing kit to determine pregnancy. One-half of the volunteers were pregnant, in their first trimester. Based on test results with our new agent we found the following:

		Study Volunteers		
		Pregnant	Not Pregnant	
Test Results for Pregnancy	Positive	73	5	78
	Negative	2	70	72
		75	75	150

   What are the specificity and selectivity levels of our test?

2. One test for occult blood in the feces has a sensitivity of 52% and a specificity of 91% for colorectal cancer. At the same time the estimated incidence of colorectal cancer in the U.S. for 40-59 year-old males is 0.87%. What is the probability of colorectal cancer in a 52 year-old male with a positive result with this occult blood test? Use both Bayesian and non-Bayesian approaches.

3. Assume that we suspect 15% of the patients with a given risk factor will develop a particular disease. From the literature (or online databases) we see that the test has a sensitivity of 0.75 and specificity of 0.80. If a specific patient with the given risk factor tests positive, what is the probability that she will develop the given disease? Alternatively, if the same patient has a negative response to the test, what is the probability that she will not develop the disease?

20

Survival Statistics

In certain clinical studies, the researcher may wish to evaluate the progress of patients over a certain time period and observe their responses to therapeutic intervention(s). Patients are monitored from the time they enter the study until some well-defined event. This event is often death, but may include other outcomes such as time to hospitalization, organ failure or rejection, or the next seizure. They could also be positive outcomes such as time to recovery, to discharge from the hospital, return to normal renal function, or cessation of symptoms.

At first glance it would seem possible to compare two or more survival rates using previously discussed statistics such as the t-test or analysis of variance to compare the mean survival times. Unfortunately these methods may not work for two reasons. First, up to this point in the book, all the statistical procedures have involved "complete" observations. There were measurable outcomes for all the people or items associated with the data. With survival data we may not know the ultimate outcome for all the potential measures because the study may end before all subjects reach the well-defined event. The second reason is that the survival times usually do not follow a normal distribution and are positively skewed. Therefore they could be considered nonparametric tests (which will be covered in greater detail in the next chapter. Nonparametric alternatives to the t-test and ANOVA (such as the Mann-Whitney or Kruskal-Wallis tests) could be used if all the people in the study reach the well-defined endpoint. Unfortunately, in many cases, study result will be evaluated before all the patients have died or reached the outcome of interest. Therefore, a new set of statistical tests is needed to evaluate data that measure the amount of time elapsing between the two events. These types of evaluations are referred to as survival statistics.

Survival statistics, or survival analysis, is part of a larger group of tests referred to generically as "time-to-event" models. For production or industrial data, the end date might be defined as "time-to-failure" for a particular application. The examples used in this chapter will focus on clinical events and primarily "time-to-death" analysis. However, time-to-failure data could be handled with similar methods.

Although these tests can be used to assess any well-defined event, by convention these are all referred to as survival statistics. Often survival data involves the creation of a graphic representation of the outcomes from the study, referred to as survival curves. This chapter will consider the two most commonly used methods for

evaluating survival curves: 1) actuarial (life) tables and 2) the product limit method (illustrated by the Kaplan-Meier procedure). The tests are similar and can be used not only to create survival curves, but estimated confidence limits about the curves and median survival times.

Once survival curves are established, different conditions (e.g., treatment versus active control) can be compared using statistical tests. The log-rank and Cochran-Mantel-Haenszel test statistics will be presented for this type of comparison.

Censored Survival Data

The amount of time that elapses between the point at which the subject enters the study and experiences the well-defined terminal event is the **survival time**. The collection of these survival times is referred to as the **survival data** and these data will be used to create survival curves and make decisions about the relative importance of the sample information and possible predictor variable(s). For survival data the outcomes are binary discrete results (e.g., survival or death, hospitalized or not hospitalized, pass or fail criteria) and usually measured as an estimate of time with specific types of therapeutic interventions or under different conditions. However, as mentioned in the introduction, in most cases not all the patients will begin the study at exactly the same point in time and some patients may not have reached the terminal event before the study is concluded. This is one of the primary reasons why we need special methods to analyze survival data.

Using the definition above, the survival time cannot be calculated for patients who have not reached the terminal event by the closing date of the study. Also, for longer studies some patients may have been lost on follow-up and their health status may be unknown. These incomplete observations are called **censored data,** or censored survival times, and are divided into two types: 1) those alive at the end of the study (if death is the endpoint), these are labeled as "withdrawn alive"; and 2) those patients whose status could not be assessed, labeled as "lost-to-follow-up" (this may be due to actual loss or noncompliance). For example, Figure 20.1 illustrates the data for the survival time of eight patients in a clinical trial. The *x*-axis represents the dates when patients entered the trial and left the trial. Note that patients A, B, and D provide complete survival information. Unfortunately, patient C was lost-to-follow-up during the second year he was enrolled. Also, patients E and F, whose entry times are not simultaneous and were still in the study when data analysis was performed are said to be **progressively censored**. We know that they survived up to a certain time but we do not have any useful information about what happened after the time of data analysis. Patients C, E, and F represent censored data; their inclusion in the data analysis would artificially lower the average survival time because there is incomplete survival information. Even with censored data it is possible to analyze the survival times of these patients. Survival analysis is not restricted to only those who reach the definitive event, but incorporates data from all the patients enrolled in the study.

Patients who die from causes other than the disease being studied (e.g., sudden coronary or automobile accident) might be handled as either censored data or deaths. Both approaches have merit, and the investigator should determine how such data will be handled prior to starting the study.

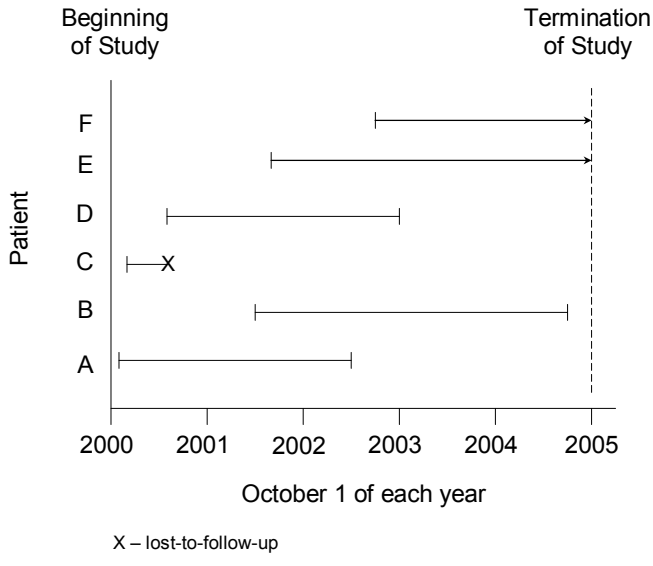


Figure 20.1 Illustration of various survival times.

Life Table Analysis

Life table analysis, also referred to as **actuarial analysis**, is a type of survival analysis involving time lines that are divided into equally spaced intervals and numbers of outcomes are observed for each interval. For example, intervals may be every 60 days, presented in 6-month intervals or as 1-year time periods. To illustrate the use of the various survival analyses, consider the fictional clinical trial where we followed 30 patients diagnosed with Stage IV melanoma over a 5-year period. Beginning on October 1, 2000, as the newly diagnosed patients (meeting very specific inclusion and exclusion criteria) entered the study, they were randomly assigned to the current gold standard for treatment (control) or the gold standard plus a new RAF kinase inhibitor (experimental). The study was terminated on September 30, 2005 and data was analyzed for the 5-year period (Table 20.1). Five patients represent censored data (patients 2, 19, 27, 29, and 30). For this and the following section we will ignore whether the patients were in the control or experimental group and first evaluate the congregate data.

The actuarial method is simpler to calculate than the product limit method discussed in the next section and at one time was the predominant method used in survival analysis. In some of the older literature it is referred to as the **Cutler-Ederer method** (Cutler and Ederer, 1958). The actual time intervals chosen for the analysis are arbitrary, but should be selected so there are a reasonable number to evaluate and should not include a large number of censored observations in any one interval. Also, the interval widths should be equidistant. Since our example data includes 5 years, we will evaluate survivals using 6-month intervals in our actuarial table. First, the data for all 30 patients are rank ordered by length of survival (Table 20.2). Note at

Table 20.1 Survival Data for Patients Enrolled in Study with Stage IV Melanoma

Patient	Entered Study	Ended Study	Survival (months)	Result*	Group
1	10/2/2000	5/28/2003	31.8	DOD	Control
2	10/7/2000	12/1/2001	13.8	LTF	Control
3	10/14/2000	12/12/2002	25.9	DOD	Experimental
4	11/15/2000	1/3/2004	37.6	DOD	Control
5	11/19/2000	9/19/2004	46.0	DOD	Experimental
6	12/12/2000	9/1/2002	20.6	DOD	Control
7	1/13/2001	4/26/2004	39.4	DOD	Experimental
8	2/1/2001	1/29/2004	35.9	DOD	Experimental
9	3/15/2001	9/23/2003	30.3	DOD	Control
10	3/21/2001	10/14/2004	42.8	DOD	Experimental
11	6/23/2001	7/16/2004	36.8	DOD	Experimental
12	7/14/2001	2/18/2003	19.2	DOD	Experimental
13	9/11/2001	6/2/2005	44.7	DOD	Experimental
14	11/11/2001	4/20/2002	5.3	DOD	Control
15	12/1/2001	10/17/2002	10.5	DOD	Control
16	3/4/2002	8/15/2004	29.4	DOD	Control
17	3/21/2002	4/15/2005	36.8	DOD	Control
18	4/30/2002	9/15/2005	40.5	DOD	Experimental
19	6/11/2002	9/30/2005	39.7	WA	Experimental
20	8/14/2002	10/23/2002	2.3	DOD	Control
21	9/21/2002	3/4/2005	29.4	DOD	Control
22	12/6/2002	7/2/2003	6.8	DOD	Control
23	3/14/2003	9/15/2005	30.1	DOD	Experimental
24	3/18/2003	8/2/2005	28.5	DOD	Experimental
25	5/11/2003	4/17/2005	23.2	DOD	Control
26	5/28/2003	7/26/2004	14.0	DOD	Experimental
27	7/13/2003	9/30/2005	26.6	WA	Control
28	7/15/2003	10/16/2004	15.1	DOD	Experimental
29	8/1/2003	9/30/2005	26.0	WA	Control
30	8/23/2003	9/30/2005	25.3	WA	Experimental

* Study results: DOD = dead of disease; WA = withdrawn alive; LTF = lost-to-follow-up.

this point the actual enrollment dates become irrelevant and only the length of time to the event is considered for analysis. Next a table is created indicating the results for each interval in the study (Table 20.3). In this table n_i is the number of patients in the study at the beginning of the interval, d_i is the number of patients who reached the event during the interval (in this example death was the terminal event) and w_i is the number of patients who were "withdrawn" from the study during the interval (either through lost to follow-up or alive at the point of data analysis). The fifth column, n_i',

Table 20.2 Congregate Results for Survival Example

Subject	Time (months)	Censored	Subject	Time (months)	Censored
20	2.3	N	16	29.4	N
14	5.3	N	21	29.4	N
22	6.8	N	23	30.1	N
15	10.5	N	9	30.3	N
2	13.8	Y	1	31.8	N
26	14.0	N	8	35.9	N
28	15.1	N	11	36.8	N
12	19.2	N	17	36.8	N
6	20.6	N	4	37.6	N
25	23.2	N	7	39.4	N
30	25.3	Y	19	39.7	Y
3	25.9	N	18	40.5	N
29	26.0	Y	10	42.8	N
27	26.6	Y	13	44.7	N
24	28.5	N	5	46.0	N

Table 20.3 Life Table for Congregate Results for Melanoma Patients

Months	n_i	d_i	w_i	n_i'	q_i	p_i	$\hat{S}_i$	SE ($\hat{S}_i$)
0.0 - 6.0	30	2	0	30	0.0667	0.9333	0.9333	0.0455
6.1 - 12.0	28	2	0	28	0.0714	0.9286	0.8667	0.0621
12.1 - 18.0	26	2	1	25.5	0.0784	0.9216	0.7987	0.0735
18.1 - 24.0	23	3	0	23	0.1304	0.8696	0.6945	0.0850
24.1 - 30.0	20	4	3	18.5	0.2162	0.7838	0.5443	0.0941
30.1 - 36.0	13	4	0	13	0.3077	0.6923	0.3769	0.0954
36.1 - 42.0	9	5	1	8.5	0.5882	0.4118	0.1552	0.0748
>42.0	3	3	0	3	1.0000	0.0000	…	…

represents the number of observations correcting for the number of withdrawals during the interval:

$$n_i' = n_i - \frac{w_i}{2} \qquad \text{Eq. 20.1}$$

Using the information in the first five columns of Table 20.3 the proportion terminating in any *ith* interval is calculated using the following formula:

$$q_i = \frac{d_i}{n_i'} \qquad \text{Eq. 20.2}$$

and the proportion surviving would be the complement of those terminating the study during any given interval:

$$p_i = 1 - q_i \qquad \text{Eq. 20.3}$$

These results are expressed in the sixth and seventh columns of Table 20.3. The actuarial method evaluates the number of patients at the beginning of the interval but not at the end. It assumes that patients are randomly removed from the study throughout any one interval; therefore, withdrawal is measured halfway through the time represented by the interval. Patients ending the study are given credit for surviving half of the interval. Therefore, in the error term, the denominator (Eq. 20.2) is reduced by half of the number of patients who withdrew during the period. If the *ith* interval is conditional on a previous event ($ith - 1$), the probability of their joint occurrence is determined by multiplying the probabilities of the two conditional events. Thus, the cumulative probability of surviving interval i along with all the previous intervals is calculated by multiplying *ith* p_i, and all previous p_is:

$$\hat{S}_i = p_i \cdot p_{i-1} \cdot p_{i-2} \cdot \ldots \cdot p_1 \qquad \text{Eq. 20.4}$$

which can be also written as:

$$\hat{S}_i = \Pi(p_i) \qquad \text{Eq. 20.5}$$

where Π is the symbol for product, similar to Σ for sum. The eighth column represents the cumulative proportions of survival ($\hat{S}_i$) for our sample data, which is the called the sample survival function, which is our best estimate of the population **survival function** (S_i). The survival function, (synonyms are survivor function or survivorship function) is our best estimate of the probability of surviving past a given time point:

$$S_i = p(T > t_i) \qquad \text{Eq. 20.6}$$

where T is the time of death and t_i is the time under consideration. In other words, the survival function is the probability that the death or other well-defined event will occur later than some specific time interval.

An important point is that the event of interest can only happen once for each patient or object (in the case of time-to-failure studies). If an event can occur multiple times, then the **recurring event model** (or **repeated event model**) can be employed and results are often relevant to system reliability. These measurements involve a reliability function and fall beyond the scope of this book.

Survival Curve

Usually results are presented as a survival curve, rather than as a table (e.g., Table 20.3). The survival function (S_i) is the parameter for the population(s) we are

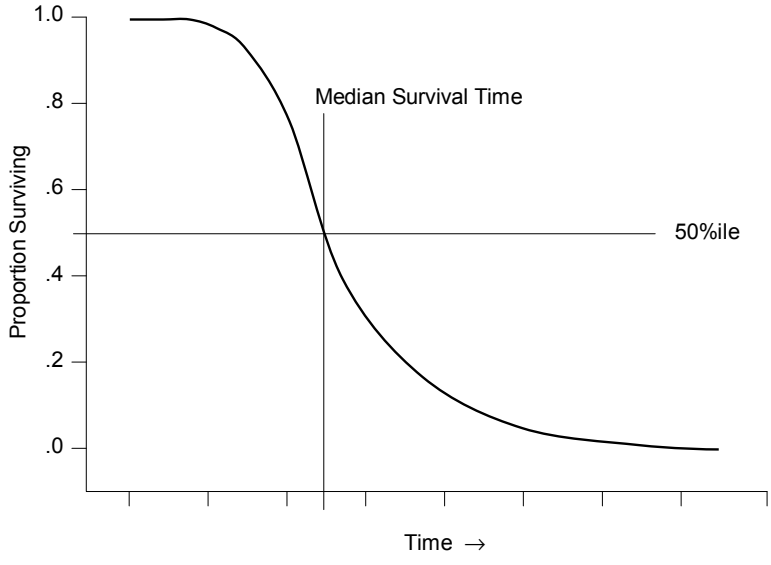


Figure 20.2 Hypothetical survival curve.

studying. Figure 20.2 illustrates a hypothetical survival function where at time zero (t_i = 0) 100% of the patients are alive (p_i = 1.00). The proportion of patients surviving will gradually decrease over time and at some endpoint none of the patients will be alive (p_i = 0). In survival analysis we estimate this curve based on sample data. The curve for the sample data is created plotting the cumulative proportions of survival on the *y*-axis and time on the *x*-axis (Figure 20.3). Instead of a smooth curve represented by population data, the curve for sample data is a series of steps downward at the end of each interval using our best estimate the sample survival functions ($\hat{S}_i$). Once again, we begin a t_i = 0 with p_i = 1.00 (all patients are alive or have not yet reached some other well-defined event), and the results from our example are illustrated in Figure 20.3. The beginning of the *y*-axis, time zero, does not refer to a particular month or year, it is the time at which each subject was entered into the study.

A common measure of survival is the **median survival time**, which is the point in time where 50% of the patients reach the well-defined endpoint in the study. Using the sample survival curve, it is relatively simple to determine the median survival time visually by drawing a horizontal line at the 0.50 point on the *y*-axis. Where it meets the survival curve, moving vertically to the *x*-axis defines the median survival time. This is illustrated in our hypothetical model (Figure 20.2). In our example problem the horizontal line in Figure 20.3 meets the curve at 36 months, which is the median survival time for the congregate data. If the p = 0.50 line intercepts at a horizontal line, the average of the two extremes of that horizontal line is defined as the median survival time. It is possible to have no median survival if the survival curve fails to reach 0.50 by the end of the study.

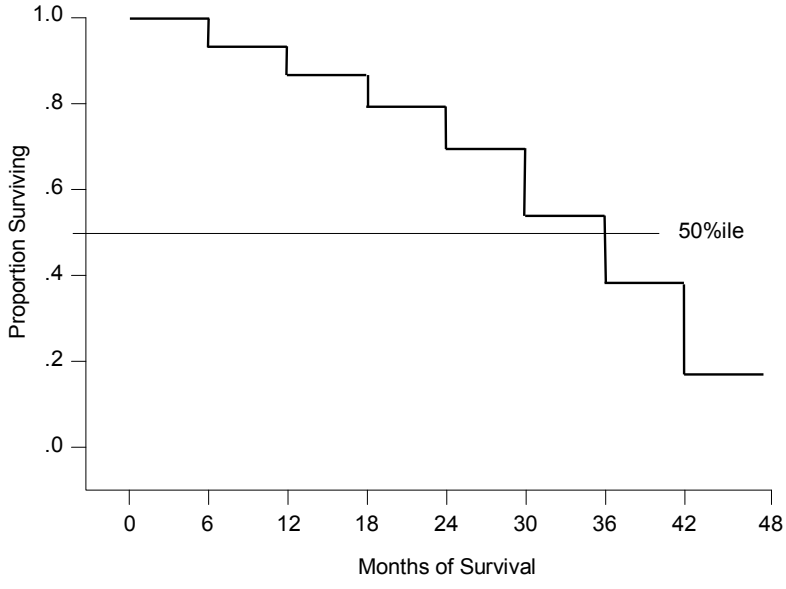


Figure 20.3 Actuarial life table curve with median line.

Often these curves include dotted or dashed lines on either side of the survival curve that represent confidence bands. Normally these confidence intervals will become wider as time progresses, illustrating a decreased confidence in the estimate due to decreasing sample sizes. Applying the survival function defined above and data presented in Table 20.3, it is possible to calculate an estimated standard error term for each interval:

$$SE(\hat{S}_i) = \hat{S}_i \sqrt{\sum \frac{q_i}{n_i'(p_i)}} \qquad \text{Eq. 20.7}$$

For example the standard error for the 30.1- and 36.0-month interval would be:

$$SE(\hat{S}_i) = 0.3769 \sqrt{\frac{0.0667}{30(0.9333)} + \frac{0.0714}{28(0.9286)} + \ldots \frac{0.3077}{13(0.6923)}}$$

$$SE(\hat{S}_i) = 0.3769(0.2532) = 0.0954$$

The results for all the intervals are presented in the last column of Table 20.3. This formula assumes a reasonably large sample size and only a relatively small number of censored observations. It is assumed that the proportion of survival at any interval is approximately normally distributed. Using this error term from Eq. 20.7 and a desired level of confidence represented by the $z_{1-\alpha/2}$ reliability coefficient (e.g., 1.96 for 95%

confidence) it is possible to define the confidence bands:

$$S_i = \hat{S}_i \pm z_{1-\alpha/2} \cdot SE(\hat{S}_i) \qquad \text{Eq. 20.8}$$

Continuing with the same example, the 95% confidence interval for an interval of 30.1 to 36.0 months would be:

$$S_i = 0.3769 \pm 1.96(0.0954) = 0.3769 \pm 0.1870$$

$$0.1899 < S_i < 0.5639$$

With 95% confidence the true p_i for surviving 30.1 to 36.0 months, based on our sample on only 30 patients is somewhere between a probability of 0.1899 to 0.5639. The resulting confidence interval is very large because of the small sample size for patients still living after 36 months of therapy. Because the p_i-value cannot be greater than 1.0 or less than zero, some intervals may need to be truncated. For example, for the first interval (up to 6.0 months) the upper confidence bond would be greater than 1.0.

$$S_i = 0.9333 \pm 1.96(0.0455) = 0.9333 \pm 0.0893$$

$$0.8440 < S_i < 1.0226$$

Therefore, the ceiling for the probability would be truncated to 1.000.

$$0.8440 < S_i < 1.000$$

Based on the study results, patients would have a 0.8440 or greater probability of surviving at least 6 months. The results for all the confidence intervals are listed in Table 20.4. These are graphically represented in Figure 20.4.

Table 20.4 Confidence Intervals for Life Table for Melanoma Patients

Months	Lower Band	$\hat{S}_i$	Upper Band	Band Width
0.0 to 6.0	0.8440	0.9333	1.0000	…
6.1 to 12.0	0.7451	0.8667	0.9883	0.2433
12.1 to 18.0	0.6547	0.7987	0.9427	0.2881
18.1 to 24.0	0.5279	0.6945	0.8611	0.3333
24.1 to 30.0	0.3598	0.5443	0.7288	0.3690
30.1 to 36.0	0.1899	0.3769	0.5639	0.3740
36.1 to 42.0	0.0087	0.1552	0.3017	0.2931
>42.0	…	…	…	…

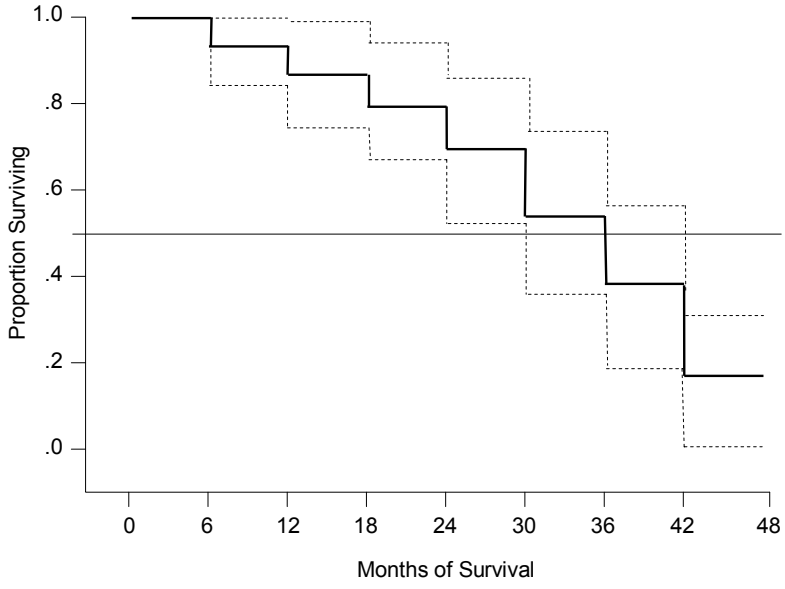


Figure 20.4 Actuarial curve with 95% confidence bands.

The 95% confidence bands can be used to estimate the median survival time by locating where the 0.50 line crosses the two vertical bands around the survival curve. For our example in Figure 20.4, the 95% confidence intervals for the median would be 24 and 48 months.

For the various survival statistics (actuarial or product-limit methods) it is possible to calculate an estimated population median, mean, standard error term and confidence interval for all the data in the study. But these calculations are difficult to do by hand and there are a variety of approaches, some involving log transformation of the data. The most commonly used approach has been proposed by Brookmeyer and Crowley (1982).

There are two major assumptions for using the actuarial time. First, that an individual withdrawal during a specific interval, on the average, occurs at the midpoint of the interval. This is a problem with the censored patients in that we do not know their actual length of survival either within the interval or after that interval. The advantage of using the Kaplan-Meier method (discussed in the following section) is that it overcomes the problem of an averaged midpoint. The second assumption is that even though the survival in a specific interval (i) depends on survival in all previous periods, the probability of survival at one specific interval is independent of the probability of survival at any of the other periods.

Kaplan-Meier Procedure

As will be discussed below, this procedure involves successive multiplications of individual estimated probabilities (the survival functions $\hat{S}_i$) and for this reason the **Kaplan-Meier procedure** is sometimes referred to as the **product-limit method** of estimating survival probabilities. Similar to the actuarial table curve, the Kaplan-Meier survival curve plots the proportion of survival as a function of time. However, unlike the previous method, with the product limit method each death is a downward step in the curve, rather than considering the number of deaths within a specific time interval. Each time one patient dies, there is a subsequent decrease in the p_i and $\hat{S}_i$.

Because the Kaplan-Meier procedure is based on the ranking of all the individual survival times, it may be mathematically tedious to apply to large data sets (greater than 100 patients). However, with the aid of computer programs it would be the preferred method for determining survival curves and subsequent statistics. Because withdrawals or censored patients are ignored, the procedure involves fewer calculations than the actuarial method. These calculations involve determining the proportions of patients in a sample who survive for various lengths of time (p_is). However, at times when a patient is censored (withdrawn), the survival curve does not step down since no one has died. Step- downs in the curve only occur only with a death and the survival curve changes precisely at the time points when patients die. With the Kaplan-Meier method, censored observations have not been excluded from the analysis. They are used to determine the number of patients at risk for each time of relapse. If censored withdrawals were excluded from the survival analysis, the estimate of the survival probabilities (p_i) for the remaining observations would be different.

The first step in the Kaplan-Meier procedure is to list the times-to-event in rank order. For our previous example problem on melanoma patients, data has already been ranked and previously presented in Table 20.2. A new table is created to calculate various probabilities, similar to the actuarial table. In this table (Table 20.5) the first column shows the times-to-event in rank order. The second column is the number of patients in the remaining previous period (n_{i-1}) who are beginning the new period (for the first period this would be the number of patients entering the study). The third column is the number of patients censored during the interval (w_i) and the fourth represents the number of patients at risk (n_i), which is the number of patients beginning the period less the number censored.

$$n_i = n_{i-1}' - w_i \qquad \text{Eq. 20.9}$$

Note again, that the interval ends only with a death or other well-defined endpoint. The fifth column shows patients-who-died-events during the period (usually one, unless more died at the exact same duration of time in the study). The sixth column is the number of patients remaining at the end of the period:

$$n_i' = n_i - d_i \qquad \text{Eq. 20.10}$$

Table 20.5 Determination of Cumulative Survival for Kaplan-Meier Example

Event (Months)	n_{i-1}'	w_i	n_i	d_i	n_i'	p_i	$\hat{S}_i$
2.3	30	0	30	1	29	0.9667	0.9667
5.3	29	0	29	1	28	0.9655	0.9333
6.8	28	0	28	1	27	0.9643	0.9000
10.5	27	0	27	1	26	0.9630	0.8667
14.0	26	1	25	1	24	0.9600	0.8320
15.1	24	0	24	1	23	0.9583	0.7973
19.2	23	0	23	1	22	0.9565	0.7627
20.6	22	0	22	1	21	0.9545	0.7280
23.2	21	0	21	1	20	0.9524	0.6933
25.9	20	1	19	1	18	0.9474	0.6568
28.5	18	2	16	1	15	0.9375	0.6158
29.4	15	0	15	2	13	0.8667	0.5337
30.1	13	0	13	1	12	0.9231	0.4926
30.3	12	0	12	1	11	0.9167	0.4516
31.8	11	0	11	1	10	0.9091	0.4105
35.9	10	0	10	1	9	0.9000	0.3695
36.8	9	0	9	2	7	0.7778	0.2874
37.6	7	0	7	1	6	0.8571	0.2463
39.4	6	0	6	1	5	0.8333	0.2053
40.5	5	1	4	1	3	0.7500	0.1539
42.8	3	0	3	1	2	0.6667	0.1026
44.7	2	0	2	1	1	0.5000	0.0513
46.0	1	0	1	1	0	0.0000	…

Once again, d_i is the number of patients who reached the endpoint during the interval and are no longer in the study. The seventh column is the probability of survival at the end of the period with the number of patients at risk divided by the number surviving at the end of the period:

$$p_i = \frac{n_i'}{n_i} \qquad \text{Eq. 20.11}$$

The eighth column is the cumulative survival determined each time a patient dies (Eq. 20.4 or Eq. 20.5). Note the n_i' used in the Kaplan-Meier method automatically accounts for censored patients by reducing the numerator. The survival curve is then created similar to the actuarial curve, plotting the cumulative probability on the *y*-axis and the time on the *x*-axis. Data from Table 20.5 is presented in Figure 20.5. Note that unlike the actuarial curve, the widths of the periods will vary and are dependent on the survival times of the individual patients in the study. Also, note that in both curves presented in Figures 20.3 and 20.5, the curve does not reach the value $p_i = 0$. Some

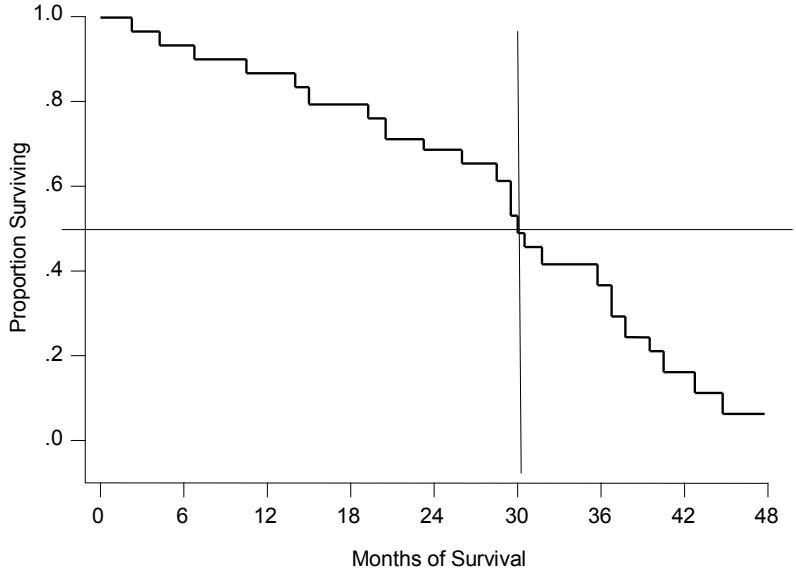


Figure 20.5 Kaplan-Meier curve for patients with melanoma.

textbooks and computer software packages may present a vertical line at the end of the survival curve extending to zero at the point of the last observation. This approach would be appropriate if there were no censored data. However, when there is censored data there are still individuals or objects that have not reached the well-defined endpoint; therefore, a better representation of the data to end with is a horizontal line at the smallest value greater than zero.

The standard error calculation for the cumulative survival estimate $\hat{S}_i$ is similar to the error term for the actuarial:

$$SE(\hat{S}_i) = \hat{S}_i \sqrt{\sum \frac{d_i}{n_i(n_i - d_i)}} \qquad \text{Eq. 20.12}$$

Calculations for the standard error terms for the various periods for our example problem on Stage IV melanoma patients are presented in Table 20.6. Similar to the actuarial life table, to extrapolate from our sample information to a population, a survival curve is more informative when it includes confidence intervals. The standard error term in the last column of Table 20.6 can be used in Eq. 20.8 to determine the bands for these intervals, the result of which are presented in Table 20.7. Once again, like the actuarial method, calculations may create bands that exceed 1.00 or are less than zero. The bands may need to be adjusted to 1.00 or zero to reflect the possible limits of statistical probability. If there are censored patients, the right side of a survival curve represents fewer patients than the left side, and the confidence

Table 20.6 Determination of Standard Errors for Kaplan-Meier Example

Event (Months)	n_i	d_i	$d_i/n_i(n_i-d_i)$	$\sum d_i/n_i(n_i-d_i)$	$\hat{S}_i$	SE($\hat{S}_i$)
2.3	30	1	0.0011	0.0011	0.9667	0.0328
5.3	29	1	0.0012	0.0024	0.9333	0.0455
6.8	28	1	0.0013	0.0037	0.9000	0.0548
10.5	27	1	0.0014	0.0051	0.8667	0.0621
14.0	25	1	0.0017	0.0068	0.8320	0.0686
15.1	24	1	0.0018	0.0086	0.7973	0.0740
19.2	23	1	0.0020	0.0106	0.7627	0.0785
20.6	22	1	0.0022	0.0127	0.7280	0.0822
23.2	21	1	0.0024	0.0151	0.6933	0.0853
25.9	19	1	0.0029	0.0181	0.6568	0.0883
28.5	16	1	0.0042	0.0222	0.6158	0.0918
29.4	15	2	0.0103	0.0325	0.5337	0.0962
30.1	13	1	0.0064	0.0389	0.4926	0.0971
30.3	12	1	0.0076	0.0465	0.4516	0.0973
31.8	11	1	0.0091	0.0556	0.4105	0.0968
35.9	10	1	0.0111	0.0667	0.3695	0.0954
36.8	9	2	0.0317	0.0984	0.2874	0.0901
37.6	7	1	0.0238	0.1222	0.2463	0.0861
39.4	6	1	0.0333	0.1556	0.2053	0.0810
40.5	4	1	0.0833	0.2389	0.1539	0.0752
42.8	3	1	0.1667	0.4056	0.1026	0.0654
44.7	2	1	0.5000	0.9056	0.0513	0.0488
46.0	1	1	0.0011	0.0011	…	…

interval will become wider as time progresses and eventually collapse at zero survival. The confidence bands for our example problem using the Kalpan-Meier procedure are presented in Table 20.7.

The median survival time can be determined by locating the time at which the cumulative survival proportion is equal to 0.50. Like the actuarial table curve, this can be visually estimated on a time plot by identifying the corresponding value on the *x*-axis for 0.50 on the *y*-axis. If this point occurs at a vertical line on the plot, the extreme values at the ends of the vertical line are averaged.

Visual Comparison of Two Survival Curves

In most research situations, the investigator will be interested in comparing the survival curves for two or more groups of patients (e.g., a control versus experimental group). Visually comparing the two curves is the simplest method. In our original example of Stage IV melanoma, the patients received two therapies: 1) the gold standard (control) and 2) the gold standard plus new RAF kinase inhibitor (experimental). To compare these two therapies, a Kaplan-Meier table for each

Table 20.7 Confidence Bands for Kaplan-Meier Table for Melanoma Patients

Months	Lower Band	$\hat{S}_i$	Upper Band
2.3	0.9024	0.9667	1.0000
5.3	0.8441	0.9333	1.0000
6.8	0.7926	0.9000	1.0000
10.5	0.7450	0.8667	0.9883
14.0	0.6976	0.8320	0.9664
15.1	0.6524	0.7973	0.9423
19.2	0.6089	0.7627	0.9164
20.6	0.5669	0.7280	0.8891
23.2	0.5262	0.6933	0.8605
25.9	0.4839	0.6568	0.8298
28.5	0.4359	0.6158	0.7957
29.4	0.3452	0.5337	0.7222
30.1	0.3022	0.4926	0.6830
30.3	0.2608	0.4516	0.6424
31.8	0.2209	0.4105	0.6002
35.9	0.1825	0.3695	0.5564
36.8	0.1107	0.2874	0.4641
37.6	0.0775	0.2463	0.4151
39.4	0.0466	0.2053	0.3639
40.5	0.0065	0.1539	0.3014
42.8	0.0000	0.1026	0.2307
44.7	0.0000	0.0513	0.1470
46.0	…	…	…

therapy is prepared (Table 20.8) and plotted (Figure 20.6).

Visually we can see a difference between the two curves, with the experimental appearing to represent a better survival curve. If a line were drawn at $p_i = 0.50$, it would indicate that the median survival for the experimental groups was 36.8 months; whereas, the median for the control group was only 29.4 months. Thus, one quick comparison is to evaluate the median survival times. One then must question whether these differences are due simply to chance or whether the difference between the two groups is statistically significant. To answer this question we will need to employ hypothesis testing. In this example, the null hypotheses would be that there is no difference between the two survival functions:

$$H_0: \ S_i\,(\text{control}) = S_i\,(\text{experimental})$$
$$H_1: \ S_i\,(\text{control}) \neq S_i\,(\text{experimental})$$

In testing the hypotheses we are interested in three pieces of information about each patient: 1) which treatment they received (experimental or control); 2) the length of time the patient was enrolled in the study; and 3) if the experience is the defined event

Table 20.8 Cumulative Survival for Experimental and Control Groups

Event (Months)	n_{i-1}'	w_i	n_i	d_i	n_i'	p_i	$\hat{S}_i$	SE($\hat{S}_i$)
Results for the Experimental Group:								
0-14.0	15	0	15	1	14	0.9333	0.9333	0.0644
14.1-15.1	14	0	14	1	13	0.9286	0.8667	0.0642
15.2-19.2	13	0	13	1	12	0.9231	0.8000	0.0641
19.3-25.9	12	1	11	1	10	0.9091	0.7273	0.0693
26.0-28.5	10	0	10	1	9	0.9000	0.6545	0.0690
28.6-30.1	9	0	9	1	8	0.8889	0.5818	0.0686
30.2-35.9	8	0	8	1	7	0.8750	0.5091	0.0680
36.0-36.8	7	0	7	1	6	0.8571	0.4364	0.0673
37.9-39.4	6	0	6	1	5	0.8333	0.3636	0.0664
39.5-40.5	5	1	4	1	3	0.7500	0.2727	0.0787
40.6-42.8	3	0	3	1	2	0.6667	0.1818	0.0742
42.9-44.7	2	0	2	1	1	0.5000	0.0909	0.0643
44.8-46.0	1	0	1	1	0	0.0000	…	…
Results for the Control Group:								
0-2.3	15	0	15	1	14	0.9333	0.9333	0.0644
2.4-5.3	14	0	14	1	13	0.9286	0.8667	0.0878
5.4-6.8	13	0	13	1	12	0.9231	0.8000	0.1033
6.9-10.5	12	0	12	1	11	0.9167	0.7333	0.1142
10.6-20.6	11	1	10	1	9	0.9000	0.6600	0.1241
20.7-23.2	9	0	9	1	8	0.8889	0.5867	0.1302
23.3-29.4	8	2	6	2	4	0.6667	0.3911	0.1424
29.5-30.3	4	0	4	1	3	0.7500	0.2933	0.1363
30.4-31.8	3	0	3	1	2	0.6667	0.1956	0.1210
31.9-36.8	2	0	2	1	1	0.5000	0.0978	0.0919
36.9-37.6	1	0	1	1	0	0.0000	…	…

of interest (in this case death) or had been censored from the study (either lost to follow-up or alive at the end of the study).

Tests to Compare Two Levels of an Independent Variable

One possible method for testing the hypotheses for comparing two survival curves is called the **log-rank test**. The log-rank test provides an objective comparison of the two survival curves to determine if they are statistically significantly different. We test the null hypothesis that there is no difference in survival experience between the two populations. The alternative hypothesis is that the difference between the two populations is significant and not due to chance variation.

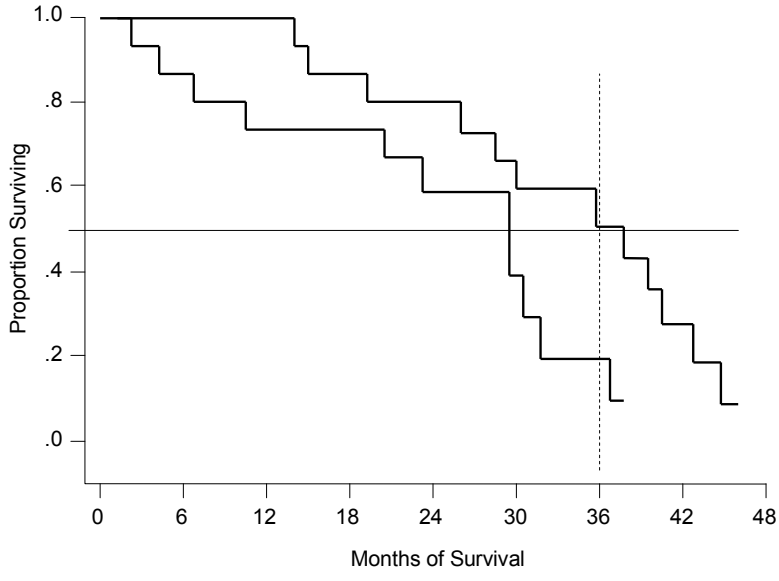


Figure 20.6 Kaplan-Meier curve for experimental and control groups.

H_0: Survival (level 1) = Survival (level 2)
H_1: Survival (level 1) ≠ Survival (level 2)

Unfortunately, as seen in previous chapters, synonyms or multiple names for the same test are commonplace in statistics. This is true also with survival statistics and the log-rank test has been given numerous names in literature, including the **Mantel log-rank test**, the **Cox-Mantel test**, the **Mantel-Cox test**, **Cox-Mantel log-rank test**, the **Cochran-Mantel-Haenszel test**, or the **CMH test**. Technically the log-rank test and CMH-type tests are different but produce equivalent results. One is interpretable based on the chi square distribution and one by the standardized normal distribution. Both methods will produce approximately the same *p*-value. In this section we will test the null hypothesis using both methods. CMH test will be discussed first because we are already familiar with this test.

In order to compare two survival curves it is assumed that: 1) patients are randomly assigned to the different groups; 2) the times are independent measures; 3) there are consistent criteria throughout the time of the study; 4) the baseline survival rate is not changing over time (inclusion and exclusion criteria remain constant); and 5) on the average, survival of censored patients would be the same as patients reaching the endpoint of the study. At each time interval (actuarial or product-limit method) there is a comparison of the number of observed deaths for each group with the expected number of deaths if the null hypothesis was true.

In Chapter 16 we used the Mantel-Haenszel test to evaluate a possible third confounding variable for a chi square test of independence. To avoid confusion we

	Results at t_i		
	Group 1	Group 2	
Number of deaths	a_i	b_i	$a_i + b_i$
Number of patients still alive	c_i	d_i	$c_i + d_i$
"At risk" at the beginning	$a_i + c_i$	$b_i + d_i$	n_i

Figure 20.7 Contingency table for each stratum for the CMH test.

will discuss the calculations for the CMH test and refer to it as such. However, the calculations are identical to the Mantel-Haenszel test. The calculations of the Cochran-Mantel-Haenszel test are cumbersome and use the same equations as the Mantel-Haenszel test (Eq. 16.16 to Eq. 16.19). For readability, we will renumber the equations and include them in this paragraph. First, the survival times until death for both groups are combined (omitting censored times) and each time constitutes a stratum of our matrix. Each stratum represents time t_i and we construct a 2 × 2 contingency table for each point in time (Figure 20.7). In each table the first row contains the number of observed deaths and the second row contains the number of patients still living. The columns represent the results for the two groups. The CMH formula is:

$$\chi^2_{CMH} = \frac{\left[\sum \frac{a_i d_i - b_i c_i}{n_i}\right]^2}{\sum \frac{(a+b)_i (c+d)_i (a+c)_i (b+d)_i}{(n_i - 1)(n_i^2)}} \qquad \text{Eq. 20.13}$$

To simplify this equation, for each stratum compute the expected frequency for the upper left-hand cell (deaths in Group 1):

$$e_i = \frac{(a_i + b_i)(a_i + c_i)}{n_i} \qquad \text{Eq. 20.14}$$

Then for each stratum the v_i intermediate is computed:

$$v_i = \frac{(a_i + b_i)(c_i + d_i)(a_i + c_i)(b_i + d_i)}{n_i^2 (n_i - 1)} \qquad \text{Eq. 20.15}$$

Finally, the Cochran-Mantel-Haenszel statistic is calculated by summing the results for each interval and creating a chi square statistic:

	Interval ending 2.3 months			Interval ending 14.0 months		
	Exper.	Control		Exper.	Control	
Deaths	0	1	1	1	0	1
Remaining	15	14	29	14	10	24
At risk	15	15	30	15	10	25

Figure 20.8 Example of two strata for example CMH test.

$$\chi^2_{CMH} = \frac{[\Sigma(a_i - e_i)]^2}{\Sigma v_i} \qquad \text{Eq. 20.16}$$

The results are compared to the chi square critical value (Table B15) with one degree of freedom (χ^2=3.84). If the calculated Cochran-Mantel-Haenszel statistic exceeds 3.84 there is a significant difference between the two curves. If 3.84 or less, one fails to identify a difference.

The test could be used for comparing two curves using either the actuarial or product-limit methods. For our previous example of patients with Stage IV melanoma, we will use the Kaplan-Meier results. Each of the possible 23 intervals identified in the combined curves (Table 20.8 and Figure 20.6) are evaluated to determine their respective e_i and v_i values. For example, at the end of the first time period there would still be 29 patients alive, 15 in the experiment and 14 in the control group. These results appear on the left side of Figure 20.8. By the end of the fifth interval, 24 patients are still alive, 10 in the control group and 14 in the experimental group. The results for all 23 intervals are presented in Table 20.9. As mentioned, censored data is not included in the calculations, with the exception of the appropriate reduction in the n_i values (as seen by the drops in the total number of patients at risk in intervals 5, 10, 11, and 19). Using the sums for Table 20.9, the Cochran-Mantel-Haenszel statistic is:

$$\chi^2_{CMH} = \frac{[\Sigma(a_i - e_i)]^2}{\Sigma v_i} = \frac{(-5.677)^2}{7.323} = 7.393$$

Since the results are greater than 3.84, we reject the null hypothesis and show that the two curves are statistically different. The exact *p*-value is 0.0065 and can be determined using Excel (**CHIDIST** with Excel 2003; **CHISQ.DIST.RT** with Excel 2010).

Initial preparation for the log-rank test is similar to the CMH test. The first step is to calculate the expected value (e_i) for cell a_i in a 2 × 2 contingency table for each point on the Kaplan-Meier or actuarial curve (Eq. 20.14). These have already been calculated for our example problem and presented in the eighth column of Table 20.9. Next the sum of the differences between the observed and expected results is calculated for a_i:

$$U_L = \sum (a_i - e_i) \qquad \text{Eq. 20.17}$$

Table 20.9 Determination of Strata and CMH Test for Example Data

Event (Months)	a_i	b_i	c_i	d_i	n_i	e_i	$a_i - e_i$	v_i	$e_i(b_i)$
2.3	0	1	15	14	30	0.500	0.500	0.250	0.500
5.3	0	1	15	13	29	0.517	0.483	0.250	0.483
6.8	0	1	15	12	28	0.536	0.464	0.249	0.464
10.5	0	1	15	11	27	0.556	0.444	0.247	0.444
14.0	1	0	14	10	25	0.600	–0.600	0.240	0.400
15.1	1	0	13	10	24	0.583	–0.583	0.243	0.417
19.2	1	0	12	10	23	0.565	–0.565	0.246	0.435
20.6	0	1	12	9	22	0.545	0.455	0.248	0.455
23.2	0	1	12	8	21	0.571	0.429	0.245	0.429
25.9	1	0	10	8	19	0.579	–0.579	0.244	0.421
28.5	1	0	9	6	16	0.625	–0.625	0.234	0.375
29.4	0	2	9	4	15	1.200	0.800	0.446	0.800
30.1	1	0	8	4	13	0.692	–0.692	0.213	0.308
30.3	0	1	8	3	12	0.667	0.333	0.222	0.333
31.8	0	1	8	2	11	0.727	0.273	0.198	0.273
35.9	1	0	7	2	10	0.800	–0.800	0.160	0.200
36.8	1	1	6	1	9	1.556	–0.556	0.302	0.444
37.6	0	1	6	0	7	0.857	0.143	0.122	0.143
39.4	1	0	5	0	6	1.000	–1.000	0.000	0.000
40.5	1	0	3	0	4	1.000	–1.000	0.000	0.000
42.8	1	0	2	0	3	1.000	–1.000	0.000	0.000
44.7	1	0	1	0	2	1.000	–1.000	0.000	0.000
46.0	1	0	0	0	1	1.000	–1.000	0.000	0.000
Sums	13	12				17.677	–5.677	4.359	7.323

For our example problem this has already been reported by the summation reported at the bottom of the eighth column in Table 20.9. If the U_L is relatively small there is probably no difference between the two levels of the independent variable. If the U_L is large the null hypothesis will be rejected and the groups being compared will be deemed statistically different. But how large should the U_L be to determine significance? To answer this question we need some measure of data variability. This is provided by calculating an error term. This measurement is determined using the following equation and assumes that the sampling distribution is approximately normal:

$$S_{U_L} = \sqrt{\sum \frac{(a_i + c_i)(b_i + d_i)(a_i + b_i)[n_i - (a_i + b_i)]}{n_i^2 (n_i - 1)}} \qquad \text{Eq. 20.18}$$

With an estimate of the differences between the observed and expected values and a measure of variability, we can calculate a ratio between the two measures:

$$z = \frac{U_L}{S_{U_L}} \qquad \text{Eq. 20.19}$$

The resultant value can be interpreted using the standardized normal distribution. As discussed in Chapter 16, since the data is based on a discrete sampling distribution and evaluating the results is based on a continuous distribution, we may wish to be more conservative in our decision making process. Once again we use a Yates' correction to make this adjustment:

$$z_{Yates} = \frac{|U_L| - 0.5}{S_{U_L}} \qquad \text{Eq. 20.20}$$

With adjustment in the numerator of the equation, the z_{Yates} will always be smaller and more difficult to reject the null hypothesis.

Using this approach, let us once again look at the sample problem of the Stage IV melanoma. From Table 20.9 we know that

$$U_L = \sum (a_i - e_i) = -5.677$$

The error term is

$$S_{U_L} = \sqrt{\sum \frac{(a_i + c_i)(b_i + d_i)(a_i + b_i)[n_i - (a_i + b_i)]}{n_i^2 (n_i - 1)}}$$

$$S_{U_L} = \sqrt{\frac{15 \cdot 15 \cdot 1 \cdot (30-1)}{30^2 \cdot (30-1)} + \frac{15 \cdot 14 \cdot 1 \cdot (29-1)}{29^2 \cdot (29-1)} + \ldots \frac{1 \cdot 0 \cdot 1 \cdot (1) \cdot}{1^2 \cdot (1-1)}} = 2.088$$

and the ratio is

$$z = \frac{U_L}{S_{U_L}} = \frac{-5.677}{2.088} = -2.719$$

A z-value of -2.719 represents p = 0.0065 [calculated using Excel code (**1 – NORMSDIST(ABS(–2.719)))*2** or **NORM.S.DIST** with Excel 2010]. This p-value is exactly the same as the p-value from our earlier calculation of the CMH tests.

If we wish to apply Yates' correction the result would be

$$z_{Yates} = \frac{|U_L| - 0.5}{S_{U_L}} = \frac{|-5.677| - 0.5}{2.088} = 2.479$$

The *p*-value associated with this *z*-value is 0.0125. Thus, with the CMH test, the log rank test, and Yates' correction on the log rank test we would reject the hypothesis and conclude there is a significant difference. The addition of the RAF kinase inhibitor to the regimen produced a significantly longer survival time.

Both the log rank and CMH tests involve a series of 2 × 2 contingency tables. From this information an odds ratio for survival could be calculated for each contingency table during the times studied. It is recommended that the CMH test statistic be used only when the odds ratios are similar across the various 2 × 2 tables or intervals for the survival distributions (Frothofer, p. 341). Recall the odds ratio equals experimental event odds divided by the control event odds (Chapter 18). If the plots of the two survival curves cross one another, then the odds ratios will not be similar across all the tables. The estimate of a pooled odds ratio can be used for descriptive purposes:

$$OR = \frac{\sum\left(\frac{a \cdot d}{n}\right)}{\sum\left(\frac{b \cdot c}{n}\right)} \qquad \text{Eq. 20.21}$$

For the example we have been using throughout this chapter and presented in Table 20.10, the odds ratio would be:

$$OR = \frac{\sum\left(\frac{a \cdot d}{n}\right)}{\sum\left(\frac{b \cdot c}{n}\right)} = \frac{7.343}{2.666} = 2.754$$

If one is interested in comparing more than two survival curves, multiple pair-wise comparisons can be performed. Also, since survival data does not follow any particular probability distribution it is appropriate to consider this test a nonparametric procedure (see Chapter 21).

Hazard Ratios

Another way to assess survival is to evaluate the hazard risk to the patients in a study. The **hazard function** is an estimate of the probability that a subject who has survived to the beginning of a specific study interval (actuarial or product-limit methods) will experience the definable event during that particular period. It is calculated as the negative natural log of the survival function:

$$\hat{h}(t_i) = -ln(\hat{S}_i) \qquad \text{Eq. 20.22}$$

Table 20.10 Determination of Strata and Log-Rank Test for Example Data

Interval	a_i	b_i	c_i	d_i	n_i	$(a_i \cdot d_i)/n_i$	$(b_i \cdot c_i)/n_i$
0-2.3	1	0	14	15	30	0.5000	0.0000
2.4-5.3	1	0	13	15	29	0.5172	0.0000
5.4-6.8	1	0	12	15	28	0.5357	0.0000
6.9-10.5	1	0	11	15	27	0.5556	0.0000
10.6-14.0	0	1	10	14	25	0.0000	0.4000
14.1-15.1	0	1	10	13	24	0.0000	0.4167
15.2-19.2	0	1	10	12	23	0.0000	0.4348
19.3-20.6	1	0	9	12	22	0.5455	0.0000
20.7-23.2	1	0	8	12	21	0.5714	0.0000
23.3-25.9	0	1	8	10	19	0.0000	0.4211
25.6-28.5	0	1	6	9	16	0.0000	0.3750
29.2-29.4	2	0	4	9	15	1.2000	0.0000
29.5-30.1	0	1	4	8	13	0.0000	0.3077
30.2-30.3	1	0	3	8	12	0.6667	0.0000
30.4-31.8	1	0	2	8	11	0.7273	0.0000
31.9-35.9	0	1	2	7	10	0.0000	0.2000
36.3-36.8	1	1	1	6	9	0.6667	0.1111
36.9-37.6	1	0	0	6	7	0.8571	0.0000
37.7-39.4	0	1	0	5	6	0.0000	0.0000
39.5-40.5	0	1	0	3	4	0.0000	0.0000
40.6-42.8	0	1	0	2	3	0.0000	0.0000
42.9-44.7	0	1	0	1	2	0.0000	0.0000
44.8-46.0	0	1	0	0	1	0.0000	0.0000
					$\Sigma =$	7.3431	2.6663

The hazard function is also referred to as the **hazard rate**, the **instantaneous failure rate**, the **force of mortality**, or the **life-table mortality rate**. In the case where death is the endpoint, the hazard rate is the proportion of patients dying in an interval per unit of time. The hazard function must be greater than zero and can be any positive value. An error term and confidence intervals can be calculated using the following equations:

$$SE(\hat{h}_i) = \frac{SE(\hat{S}_i)}{\hat{S}_i} \qquad \text{Eq. 20.23}$$

$$h_i = \hat{h}_i \pm z_{1-\alpha/2} \cdot SE(\hat{h}_i) \qquad \text{Eq. 20.24}$$

The hazard functions and error terms for all the intervals in our Kaplan-Meier example are presented in Table 20.11.

Table 20.11 Determination of Hazard Function from Table 20.6

Event (Months)	n_i	d_i	$\hat{S}_i$	SE($\hat{S}_i$)	h_i	SE(h_i)
2.3	30	1	0.9667	0.0328	0.0339	0.0339
5.3	29	1	0.9333	0.0455	0.0690	0.0488
6.8	28	1	0.9000	0.0548	0.1054	0.0609
10.5	27	1	0.8667	0.0621	0.1431	0.0716
14.0	25	1	0.8320	0.0686	0.1839	0.0824
15.1	24	1	0.7973	0.0740	0.2265	0.0928
19.2	23	1	0.7627	0.0785	0.2709	0.1029
20.6	22	1	0.7280	0.0822	0.3175	0.1129
23.2	21	1	0.6933	0.0853	0.3662	0.1230
25.9	19	1	0.6568	0.0883	0.4203	0.1344
28.5	16	1	0.6158	0.0918	0.4849	0.1491
29.4	15	2	0.5337	0.0962	0.6280	0.1802
30.1	13	1	0.4926	0.0971	0.7080	0.1972
30.3	12	1	0.4516	0.0973	0.7950	0.2155
31.8	11	1	0.4105	0.0968	0.8903	0.2357
35.9	10	1	0.3695	0.0954	0.9957	0.2582
36.8	9	2	0.2874	0.0901	1.2470	0.3137
37.6	7	1	0.2463	0.0861	1.4011	0.3496
39.4	6	1	0.2053	0.0810	1.5835	0.3944
40.5	4	1	0.1539	0.0752	1.8711	0.4888
42.8	3	1	0.1026	0.0654	2.2766	0.6368
44.7	2	1	0.0513	0.0488	2.9698	0.9516
46.0	1	1	…	…	…	…

One can think of a hazard ratio the same as relative risk. If the ratio is 0.25, then the relative risk of an event in one group is one-quarter the risk of that event in the second group. The **cumulative hazard function** is the difference between the observed death rate and the expected rate of death, for all time periods, if there was no significant difference between the two treatment groups.

$$h_i = \frac{\sum O_i}{\sum E_i} = \frac{\sum a_i}{\sum e_i} \qquad \text{Eq. 20.25}$$

These two values have already been reported or calculated for the first treatment level (a_i and e_i) in order to determine the Cochran-Mantel-Haenszel chi square test. Using the same method it is possible to calculate the expected values for the second treatment level (b_i):

$$e_i(b_i) = \frac{(a_i + b_i)(b_i + d_i)}{n_i} \qquad \text{Eq. 20.26}$$

Using our example data in Table 20.9, we have already reported the observed outcomes for both the experimental and control groups (the second and third columns, respectively). In the previous section we calculated the expected outcome for the experimental group (e_i in column seven). Using Eq. 20.26 the expected values are reported in the tenth column. The sums are reported for the observed and expected results at the bottom of Table 20.9. The hazard function for the experimental group is:

$$h_E = \frac{\sum a_i}{\sum e_i} = \frac{13}{17.677} = 0.735$$

and for the controlled group is:

$$h_C = \frac{\sum b_i}{\sum e_i(b_i)} = \frac{12}{7.323} = 1.639$$

The larger the hazard rate, the lower the chance of survival. Thus, it appears that chance of survival in the experimental group is greater than that of the control group. This is visually supported in Figure 20.6.

The **hazard ratio** is the relative risk of reaching the defined endpoint at any given time interval. The hazard ratio is a useful descriptive statistic when used in the context of the log-rank statistic, for comparing two groups. Another way to think of hazard, with respect to the survival curves created in the previous sections, is that hazard represents the slopes of the survival curves. It measures how rapidly subjects are dying or reaching some other endpoint. In the comparison of two survival curves, the hazard ratio compares two treatment levels. The results are interpreted similar to the relative risk or the odds ratio described in the previous chapter. A hazard ratio of 1.0 indicates that the two groups compared are identical. If the hazard ratio is 6.0, the group in the numerator has a six times greater risk compared to the group in the denominator. To compare two groups the hazard ratio can be estimated by

$$Hazard\ ratio = \frac{h_C}{h_E} \qquad \text{Eq. 20.27}$$

For illustration, using our example of treatment alternatives for Stage IV melanoma, the hazard ratio of control group to the experimental groups would be:

$$Hazard\ ratio = \frac{1.639}{0.735} = 2.220$$

Based on our study of 30 patients, the risk of dying is more than twice as great for the control group compared to the experimental groups receiving the additional RAF kinase inhibitor. One limitation with the hazard ratio is that it is assumed that the risk of death or other endpoint is constant throughout the period of time studied. If it is assumed that there are proportional hazard results (the ratio of hazard functions in deaths per time) are the same at each time point; in this case the log-rank test is more powerful. The CMS method is nearly identical to the log-rank method.

Multiple Regression with Survival Data: Proportional Hazards Regression

Up to this point we have looked at survival analysis with only one independent variable, in our example, two treatment levels. However, we may wish to control for additional characteristics, or covariates, for patients volunteering for a study (e.g., age, gender, ethnicity). One of the most commonly used methods is the Cox regression model, or **Cox proportional hazard regression model** (also referred to as **Cox's proportion hazards model**, **Cox PHM**) which accounts for the effects of predictor variables (continuous or discrete) on the dependent variable, which can include censored time-until-event data. The method is named after D.R. Cox who first proposed applying regression methodology to survival studies and it involves a proportional hazard regression model (Daniel). This model is a multivariate analysis used to identify a combination of variables that best predicts the outcomes in the group of patients. It may also independently test the effects of individual variables. It is a hazards model commonly used for survival analyses. The detailed description of this regression goes beyond the scope of this book, but excellent discussions of this method can be found in Kleinbaum (1996, pp. 86-112) or Klein and Moeschnerger (2003, pp. 243-287). A brief overview is presented below.

As discussed in the previous section, the hazard function [$h(t_i)$] describes the conditional probability that an event will occur, given survival up to that point in time. Similar to the multiple linear regression model discussed in Chapter 14, the model to measure k covariates can be described as:

$$h(t_i) = h_0(t_i)\, exp(\beta_1 x_1 + \beta_2 x_2 + \ .... + \beta_k x_k) \qquad \text{Eq. 20.28}$$

where β_i represents the beta coefficients (or weights) for each x_i covariate. Modifying the equation, the results can be interpreted as hazard ratios.

$$\frac{h(t_i)}{h_0(t_i)} = exp(\beta_1 x_1 + \beta_2 x_2 + ... + \beta_k x_k) \qquad \text{Eq. 20.29}$$

These regression coefficients represent the amount of change in the hazard resulting from the risk factors. This rearranged equation indicates that the exponential coefficient is the ratio of the conditional probabilities or the hazard ratio. It serves as an estimate of the odds ratio from the coefficient, similar to logistic regression from Chapter 18.

Using this method is it possible to compare survival in two or more levels of an independent variable adjusting for multiple covariables. Unfortunately the calculations for these tests are so cumbersome that they require computer programs to determine the best fit and calculate proportional hazards for each of the risk factors, or covariate) as hazard ratios. Most will also calculate 95% confidence intervals around each estimated proportional hazard. These are interpreted similarly to the ratios discussed in Chapter 19, the location of the value 1 with respect to the interval.

Wilcoxon Test

As mentioned earlier, the traditional comparison of two independent groups (e.g., t-test) is not appropriate, since survival times are usually not normally distributed and tend to be positively skewed. Also, censored data cannot be used if all the patients would need to reach the endpoint before the data can be analyzed. However, there are additional procedures for testing the null hypothesis that two survival curves are identical. One of the most common is the Wilcoxon test which appears in Minitab reports. It is reported with multiple names and minor modifications (including the **Breslow test, Gehan test, Gehan's generalized Wilcoxon test** and the **Gehan-Breslow-Wilcoxon test**). All are generalized Wilcoxon tests or generalized Kruskal-Wallis tests. These tests involve early weighting of the results which could be misleading when a large proportion of individuals in the study are censored at early time points in the study. The log-rank test gives equal weight to all time points, whereas Wilcoxon weights the early failures or deaths more heavily. Therefore, the preferred test results would be the CMH or log-rank test and results for the Wilcoxon results should be reported only if there is a strong reason to believe that the hazard results are not consistent at each time point.

Other Tests and Measures of Survival

Other tests to compare different levels of the independent variable include the **Tarone-Ware test,** which like the Wilcoxon test, involves weighted differences between actual and expected numbers of deaths at the observed time intervals. The **Peto log-rank test or Peto's Generalized Wilcoxon test** give more weight to the initial interval of the study where there are the largest numbers of patients at risk. If the rate of deaths is similar over time, the Peto log-rank test and the log-rank test will produce similar results. In situations where there are more than two levels of the independent variable either the Gehan's generalized Wilcoxon test, Peto and Peto's generalized Wilcoxon test or the log-rank test are recommended. If there are only two levels to the independent variable, the log-rank test will give results equivalent to Gehan's Generalized Wilcoxon test. If there is no censored data, all the patients die and there are no withdrawals, there are nonparametric alternatives such as the Mann-Whitney U test for two levels of an independent variable or the Kruskal-Wallis test for more than three levels. These tests will be discussed in the next chapter. Information about these tests can be found in the following references: Breslow test (Breslow; Gehan; Lee and Wang, pp. 107-109; Glantz, pp. 396, 397); Tarone-Ware test (Tarone and Ware; Miller, pp. 104-118); and Peto (Lee and Wang, 116, 117; Kleinbaum, pp. 65, 66).

Based on visual examination of survival curves, it is possible to estimate the **time of survival percentiles**. Most commonly these would be the 25th and 75th percentiles, in addition to the previously identified 50th percentile (median survival time).

Mortality rates (e.g., three- or five-year survival rates) are popular ways to deal with survival data. These are commonly used in oncology, but unfortunately the mortality rate cannot be used for all patients until the end of the specific length of time.

Another measure of survival is **person-years of observation**. Sometime used in

epidemiology it is the number of deaths per each 100 person-years of observations. It may be useful in comparing the results during one specific period of time compared to another period or compared to the results of another investigation. However, a problem with this measurement is that no statistical methods exist for comparing different intervals. A second problem is that the person-year of observation measure assumes that the risk of the definable event is constant through the study.

Manufacturing Related Survival Measurements

As mentioned earlier, survival statistics may also be applied to the stability or endurance of a manufactured product. Several terms may be reported when using computer software for survival data that may apply more appropriately to product reliability measures. For example, the sample **mean-time-to-failure** (MTTF) is commonly associated with the Kaplan-Meier method, whereas the median-time-to-failure is associated with actuarial calculations. The MTTF is the expected average time until the first failure of a piece of equipment or product. It is a statistical value and based on the mean for results expected for a large number of units. The standard error and confidence interval may be reported in computer printouts evaluating survival statistics. When there is censored data the mean of the data does not provide the best measure of the center because not all the failure times are known. The MTTF is an estimate of the theoretical center of the distribution that takes into account censored observations. Various algorithms are used to calculate the mean, median and confidence interval for Kaplan-Meier procedures, depending on the software used (Barker). These different approaches can result in differing reported values.

Other related terms include MTBF (mean-time-between-failures) and MTTR (mean-time-to-repair). Both terms are used in reference to a repairable item, whereas MTTF is used for non-repairable products. However, MTBF may be used for both repairable and non-repairable items. MTBF is a measure of reliability and used to estimate the amount of failures per million hours of use for a product. It refers to the life span of a product. Another way to report the MTBF is failure-in-time (FIT) which reports the expected failures per one billion hours of operation for a product.

Survival Statistics using Minitab®

Calculations for survival statistics using either the actuarial or Kaplan-Meier procedures are available under the "Stat" option:

Stat ➤ Reliability/Survival ➤ Distribution Analysis (Right Censoring) ➤ Nonparametric Distribution Analysis

Two major option panels appear in Figure 20.9. The top one allows the selection of the dependent variable (Variables:) and if comparing multiple levels of an independent variable this can be selected under "By Variable:". If there were no independent variable the lower "By Variable:" should be blank. If the *Estimate*... option is selected the lower panel in Figure 20.9 will appear. Kaplan-Meier or actuarial survival statistics can be selected. If "Actuarial" is selected there must be a

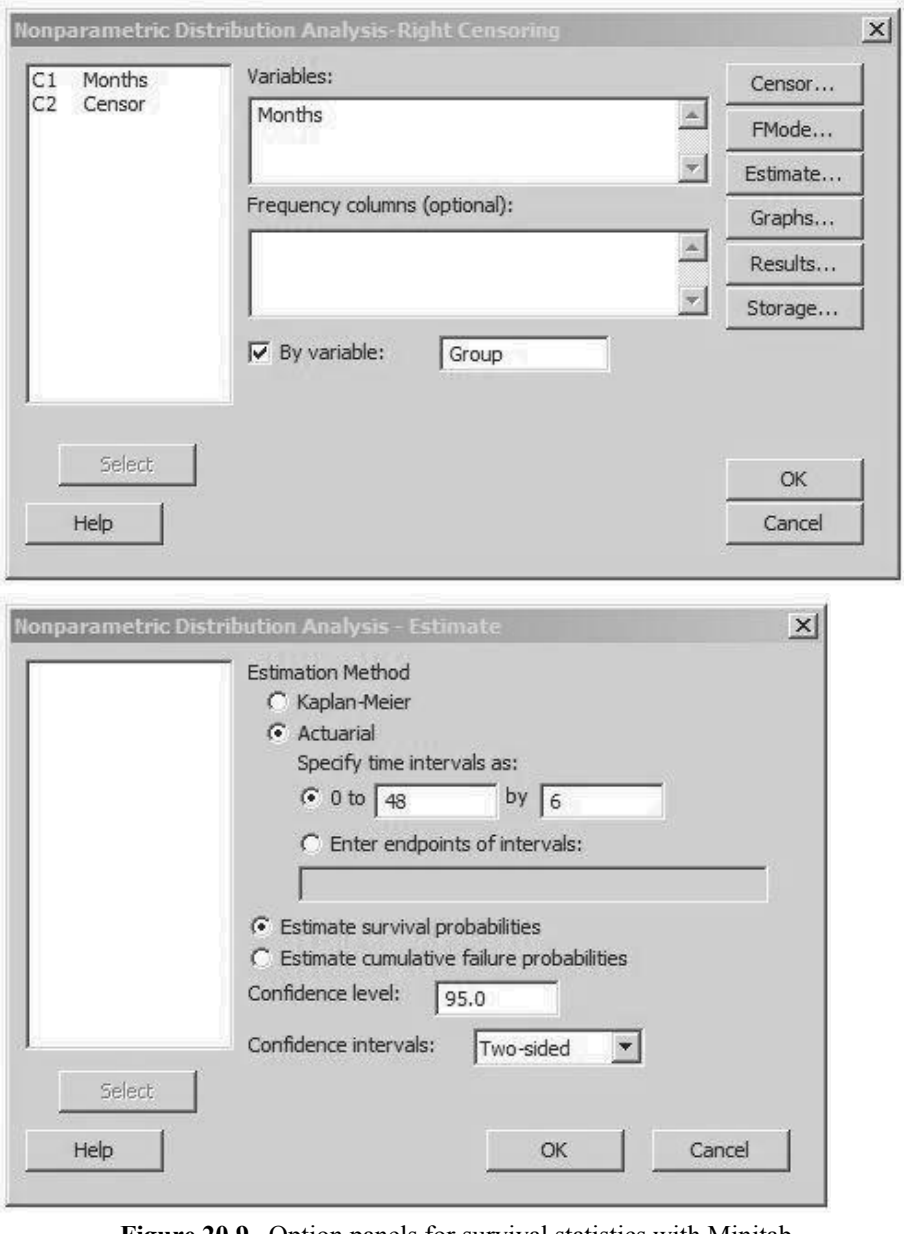


Figure 20.9 Option panels for survival statistics with Minitab.

maximum time period elected and "by" equal time periods. In the example the actuarial choices are made for the data in Table 20.1. Two additional option panels are also important and illustrated in Figure 20.10. The first appears when *Censor*... is selected on the top panel in Figure 20.9. If there is a column that indicates whether or

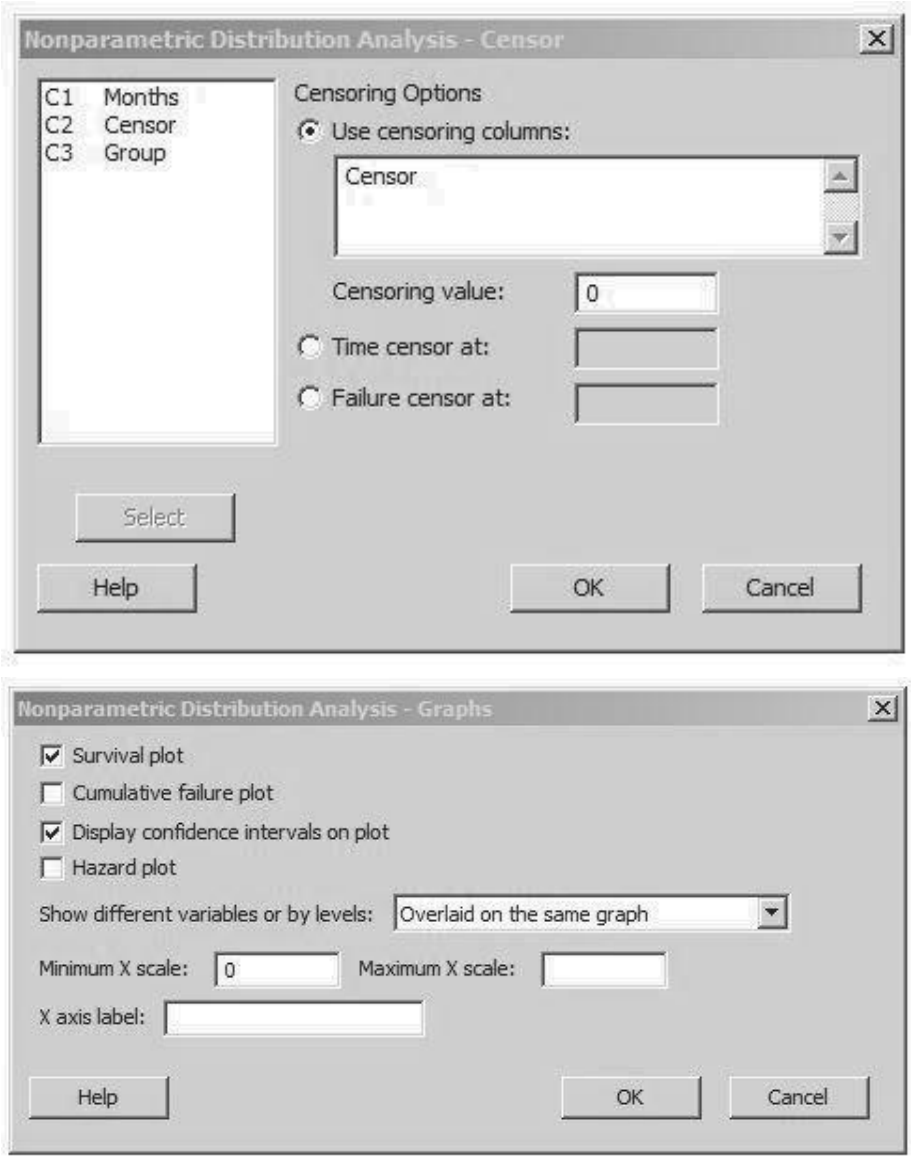


Figure 20.10 Additional option panels for survival statistics with Minitab.

not some of the data is censored, it is selected and entered into the space "Use censoring columns:". The appropriate numeric value (such as 0 or 1) is entered into "Censoring value:" indicates those rows with censored data. The lower panel in Figure 20.10 results from selecting the *Graphs…* option in the top panel in Figure

```
Censoring Information  Count
Uncensored value          25
Right censored value       5

Censoring value: Censor = 0

Nonparametric Estimates

Characteristics of Variable

          Standard   95.0% Normal CI
  Median     Error    Lower    Upper
31.5887   3.27014  25.1794  37.9981
```

Interval Lower	Interval Upper	Number Entering	Number Failed	Number Censored	Conditional Probability of Failure	Standard Error
0	6	30	2	0	0.06667	0.045542
6	12	28	2	0	0.07143	0.048670
12	18	26	2	1	0.07843	0.053240
18	24	23	3	0	0.13043	0.070224
24	30	20	4	3	0.21622	0.095710
30	36	13	4	0	0.30769	0.128008
36	42	9	5	1	0.58824	0.168807
42	48	3	3	0	1.00000	0.000000

Time	Survival Probability	Standard Error	95.0% Normal CI Lower	Upper
6	0.933333	0.0455420	0.844073	1.00000
12	0.866667	0.0620633	0.745025	0.98831
18	0.798693	0.0734872	0.654661	0.94273
24	0.694515	0.0850248	0.527870	0.86116
30	0.544350	0.0941252	0.359868	0.72883
36	0.376858	0.0954030	0.189871	0.56384
42	0.155177	0.0747678	0.008634	0.30172

Figure 20.11 Partial numeric output for actuarial survival statistics with Minitab.

20.9 and offers a variety of graphic options. The results for the selections in Figures 20.9 and Figure 20.10 are presented in Figure 20.11. Notice the results are similar to those reported in Table 20.3, where the "survival probability" is the label for $\hat{S}_i$ and the "standard error" is the label for $SE(\hat{S}_i)$. The graphic representation is presented in Figure 20.12 and looks similar to Figure 20.4. One minor limitation of Minitab is that you cannot change the weights or style for the individual plots (e.g., center line versus confidence bands). If possible the middle line would be heavier and the two outside

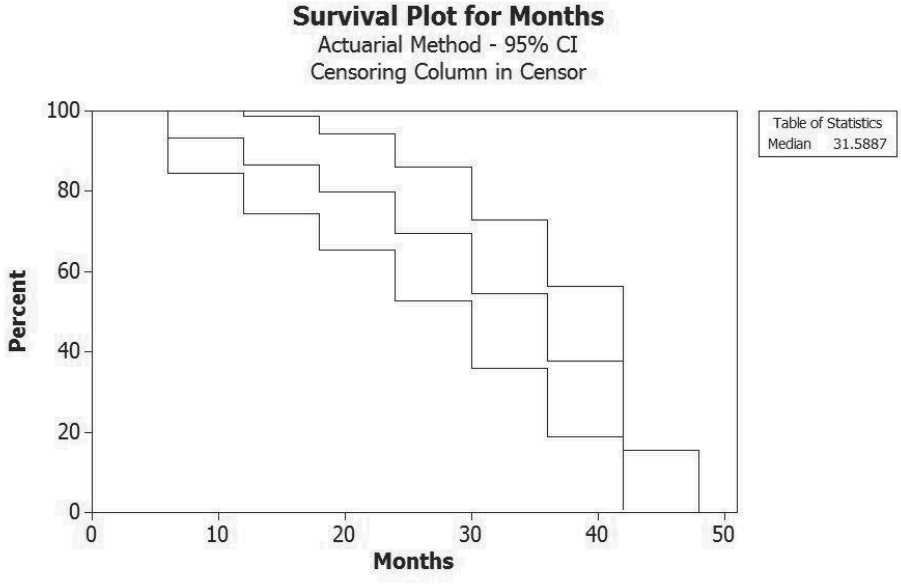


Figure 20.12 Graphic output for actuarial survival statistics with Minitab.

Table 20.12 Data to Illustrate Kaplan-Meier Using Minitab

Time until Hospital Admission					
Treatment A		Treatment B		Treatment C	
Days	Censored	Days	Censored	Days	Censored
6	0	3	0	12	0
9	0	12	0	18	0
14	0	15	0	30	0
27	1	21	0	41	0
35	0	25	0	57	0
50	0	32	0	74	0
81	0	39	1	83	1
85	0	51	0	90	1
90	1	60	0	90	1
90	1	75	0	90	1

lines would be dotted to match those in Figure 20.4. If there are multiple levels of the independent variable (indicated "By variable:"), Minitab compares the survival curves and reports both log-rank and Wilcoxon results, converts them to chi square values and reports the *p*-values.

Variable: Days
Treatment = A

Censoring Information	Count
Uncensored value	7
Right censored value	3

Censoring value: Censored = 1

Nonparametric Estimates

Characteristics of Variable

	Standard	95.0% Normal CI	
Mean(MTTF)	Error	Lower	Upper
53.1833	11.6583	30.3334	76.0332

Median = 50
IQR = 71 Q1 = 14 Q3 = 85

Kaplan-Meier Estimates

	Number	Number	Survival	Standard	95.0% Normal CI	
Time	at Risk	Failed	Probability	Error	Lower	Upper
6	10	1	0.900000	0.094868	0.714061	1.00000
9	9	1	0.800000	0.126491	0.552082	1.00000
14	8	1	0.700000	0.144914	0.415974	0.98403
35	6	1	0.583333	0.161015	0.267749	0.89892
50	5	1	0.466667	0.165775	0.141753	0.79158
81	4	1	0.350000	0.160208	0.035998	0.66400
85	3	1	0.233333	0.143114	0.000000	0.51383

Figure 20.13 Partial output for Kaplan-Meier statistics with Minitab.

Distribution Analysis: Days by Treatment

Comparison of Survival Curves

Test Statistics

Method	Chi-Square	DF	P-Value
Log-Rank	4.20208	2	0.122
Wilcoxon	2.58599	2	0.274

Figure 20.14 Summary output for Kaplan-Meier statistics with Minitab.

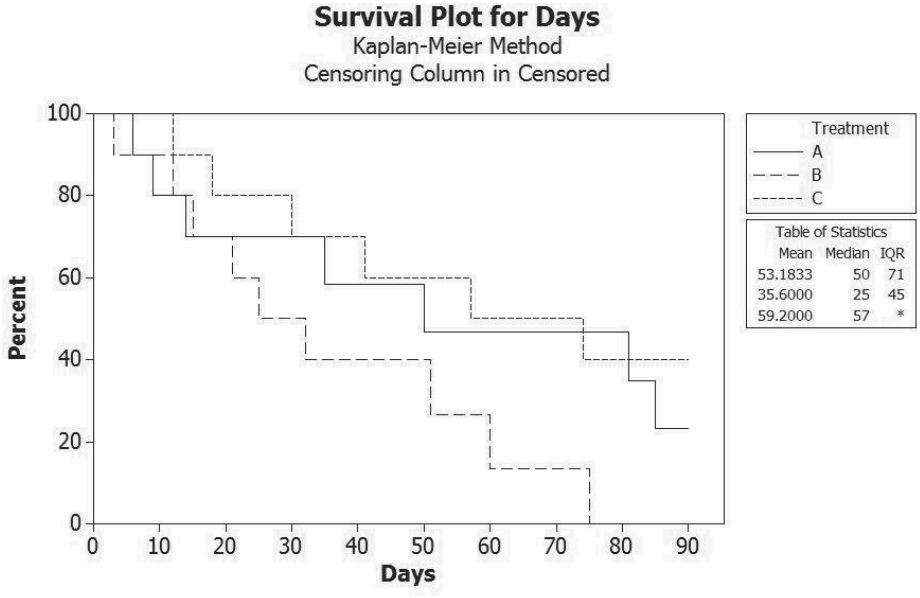


Figure 20.15 Graphic output for Kaplan-Meier statistics with Minitab.

To illustrate the use of the Kaplan-Meier statistic with Minitab a new set of data will be used (Table 20.12) comparing three factious treatments on the time required until hospitalization for a certain condition. To calculate the Kaplan-Meier statistic the option would be chosen in the lower panel in Figure 20.9. Output in Figure 20.13 represents the results for the first level of treatment and similar results would be presented for the other two treatments following the information in this figure. For each level of independent variable are reported there is a summary table with statistics and significance for log-rank and Wilcoxon tests (Figure 20.14). In this case there was a finding of no significant difference among the three treatments. The Minitab graphing option is presented in Figure 20.15.

References

Barker, C. (2009). "The mean, median, and confidence intervals of the Kaplan-Meier survival estimate: computations and applications," *American Statistician* 63:78-80.

Breslow, N.E. (1970). "A generalized Kruskal-Wallis test for comparing K samples subject to unequal patterns of censorship," *Biometrika* 57:579-594.

Brookmeyer, R. and Crowley, J. (1982). "A confidence interval for the median survival time," *Biometrics* 38:29-41.

Cutler, S. and Ederer, F. (1958). "Maximum utilization of the lifetable method in analyzing survival," *Journal of Chronic Diseases* 8:699-712.

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Eighth edition, John Wiley and Sons, New York, p. 658.

Gehan, E.A. (1965). "A generalized Wilcoxon test for comparing arbitrarily singly censored samples," *Biometrika* 52:203-223.

Glantz, S.A. (2002). *Primer of Biostatistics*, McGraw-Hill, New York, pp. 396,397.

Kaplan, E.L. and Meier, P. (1958). "Nonparametric estimation from incomplete observations," *Journal of the American Statistical Association* 53:457-81.

Klein, J.P. and Moeschberger, M.L. (2003). *Survival Analysis: Techniques for Censored and Truncated Data*, Springer-Verlag, New York.

Kleinbaum, D.G. (1996). *Survival Analysis: A Self-learning Text*, Springer-Verlag, New York.

Lee, E.T. and Wang, J.W. (2003). *Statistical Methods for Survival Data Analysis*, John Wiley and Sons, Hoboken, NJ.

Miller, R.G., Jr. (1981). *Survival Analysis*, John Wiley and Sons, New York, pp.104-118.

Tarone, R.E. and Ware, J.H. (1977). "On distribution-free tests for equality for survival distributions," *Biometrika* 64:156-160.

Suggested Supplemental Readings

Altman, D.G. (1991). *Practical Statistics for Medical Research*, Chapman and Hall, London, pp. 365-394.

Kleinbaum, D.G. (2011). *Survival Analysis: A Self-Learning Text*, Third edition, Springer, New York.

Lee, E.T. and Wang, J.W. (2003). *Statistical Methods for Survival Data Analysis*, John Wiley and Sons, Hoboken, NJ, pp. 116,117.

Peto R. and Peto, J. (1972). "Asymptotically efficient rank invariant test procedures," *Journal of the Royal Statistical Society A* 135:185-206.

Example Problems (Answers are provided in Appendix D)

1. A container manufacturer has developed a new safety closure system for prescription vials. The company tests these closures by asking 30 volunteers to open and close a vial 200 times or until there is a physical failure in the closure system. Each volunteer is asked to repeat this process with three vials. Failure is

Table 20.13 Number of Closure Openings until Failure

36	150	174	186	195	200*
65	154	175	187	195	200*
81	154	178	187	196	200*
97	156	179	189	197	200*
107	157	180	190	198	200*
115	159	180	190	198	200*
121	159	181	190	198	200*
128	162	182	191	200	200*
132	162	182	191	200*	200*
134	163	182	192	200*	200*
136	165	184	193	200*	200*
139	166	185	193	200*	200*
142	169	185	194	200*	200*
146	172	185	194	200*	200*
148	172	186	194	200*	200*

* Censored data.

clearly defined in the study protocol and the number of repetitions assumes that a maximum requirement for such a vial would be 120 (four openings per day for a 30-day supply of medication). The results are presented in Table 20.13. Using both the actuarial and Kaplan-Meier methods for estimating survival, calculate the survival function (with confidence intervals) and median number of closures before failure.

2. Infection is a common problem associated with a specific surgical procedure. The P&T Committee, based on a review of the literature, wanted to evaluate new Antibiotic B compared to the current Antibiotic A that they were using to prevent infection following this procedure. Forty patients were randomly divided into two treatment groups, receiving either Antibiotic A or Antibiotic B. They were followed to determine the number of hours (following survey) before they were discharged, infection free, from the hospital. The results are presented below (* indicates censored data):

Antibiotic A:	42, 57, 63, 98, 104* 105, 132, 132, 132, 133, 133, 133, 139, 140, 161, 180, 180, 195, 195, 233*
Antibiotic B:	43, 65, 88, 88, 90, 92, 106, 108, 112, 116, 116*, 120, 127, 130, 133, 135, 144*, 146, 165, 203

Was there a significant difference between the time-to-event (discharge) based on the type of antibiotic received?

3. Patients are randomly assigned to two different treatments for Stage III prostate cancer. The first is the current gold standard therapy, the second in a combination of products believed to produce better results. Was there a significant difference in the survival rates between the two treatment approaches based on the following results?

Survival Rates (Patients)

Gold Standard				Experimental Treatment	
Weeks	Censored	Weeks	Censored	Weeks	Censored
2	Y	15	N	3	Y
2	N	15	Y	3	Y
2	Y	15	N	4	Y
2	N	15	N	5	Y
4	N	15	Y	7	Y
4	N	15	N	9	Y
5	Y	15	N	10	N
5	Y	16	Y	11	Y
6	N	17	Y	13	Y
6	N	18	N	14	Y
6	Y	19	N	14	Y
7	Y	19	Y	16	Y
8	N	20	N	18	N
8	N	20	N	18	Y
9	Y	21	N	19	Y
10	N	22	Y	20	Y
10	Y	23	N	21	N
10	N	23	N	21	N
11	Y	23	N	21	Y
11	N	24	N	22	Y
11	N	24	Y	23	N
11	Y	25	N	23	Y
12	Y	25	Y	23	Y
12	N	25	N	24	Y
13	N	27	N	24	N
13	Y	28	N	25	Y
14	Y	29	N	27	Y
14	Y	30	N	29	N
14	Y	30	N	30	N
15	N	32	N	32	N

21

Nonparametric Tests

Nonparametric statistical tests can be useful when dealing with small sample sizes or when the requirement of a normally distributed population cannot be met or assumed. These tests are simple to calculate, but are traditionally less powerful and the researcher needs to evaluate the risk of a Type II error. Often referred to as **distribution-free statistics**, nonparametric statistical tests do not make any assumptions about the population distribution. One does not need to meet the requirements of normality or homogeneity of variance associated with the parametric procedures (z-test, t-tests, F-tests, correlation and regression). Chi square tests are often cited as distribution-free and have been covered in a previous chapter.

These distribution-free tests have been slow to gain favor in the pharmaceutical community, but are currently being seen with greater frequency in the literature, often in parallel with their parametric counterparts. This is seen in the following example of a 1989 clinical trial protocol:

> If the variables to be analyzed are normally distributed and homogeneous with respect to variance, a parametric analysis of variance that models the cross-over design will be applied. If these criteria are not fulfilled, suitable nonparametric tests will be used.

Nonparametric tests are relatively simple to calculate. Their speed and convenience offers a distinct advantage over the parametric alternatives discussed in the previous chapters. Therefore, as investigators we can use these procedures as a quick method for evaluating data.

Use of Nonparametric Tests

Nonparametric tests usually involve ranking or categorizing the data and by doing so we decrease the accuracy of our information (changing from the raw data to a relative ranking). We may obscure the true differences and make it difficult to identify differences that are significant. In other words, nonparametric tests require differences to be larger if they are to be found significant. We increase the risk that we will accept a false null hypothesis (Type II error). It may be to the researcher's advantage to tolerate minor doubts about normality and homogeneity associated with

a given parametric test, rather than to risk the greater error possible with a nonparametric procedure.

A **robust statistic** refers to test-based populations with assumed normality distributions and similar variances even when the underlying population may not be normal. Some of the parametric tests discussed previously (notably the t-tests) are known to be robust against the assumption of normality, especially if there are large sample sizes. However, other authors (e.g., Conover, 1999) would argue that nonparametric tests are preferable and even more powerful than parametric tests if the assumptions (normality and homogeneity) are false. Thus, results showing extremely different variances should be tested using the appropriate nonparametric procedure.

When dealing with ordinal dependent variable results, the nonparametric tests become the tests of choice. As discussed in Chapter 1, units on an ordinal scale may not be equidistant and violate assumptions required for parametric procedures. For example, consider the following commonly used scale for investigators to assess the cognitive functioning of Alzheimer's patients:

Cognitive Performance Scale Description

Score	Assessment
0	Intact
1	Borderline Intact
2	Mild Impairment
3	Moderate Impairment
4	Moderate to Severe Impairment
5	Severe Impairment
6	Very Severe Impairment

Is the difference between mild and moderate to severe impairment twice the difference between mild and moderate impairment? The answer is probably not. Therefore the conversion from the initial ordinal scale to the relative positioning of a rank order scale would be the more appropriate statistical test.

Nonparametric tests are particularly useful when there are potential outliers (to be discussed in Chapter 23). Because of the ranking involved, an extremely large or small observation will receive the rank of 1 or *N*. For example, assume the following numbers: 2, 3, 3, 4, 4, 5, 6, 7, and 15. In this case 15 would seem different from the other eight observations. However, when ranking the data the value 15 would be converted to rank 9 and its difference from the other observations would be minimized. What if the last value was 150 or even 15,000? The same rank of 9 would be assigned. Thus, nonparametric statistics are generally not affected by a single outlier.

This chapter will explore some of the most commonly used nonparametric tests that can be used in place of the previously discussed methods (i.e., t-tests, F-tests, correlation). In many nonparametric statistics, the median is used instead of the mean as a measure of central tendency. Nonparametric tests for creating confidence intervals around the median or comparing sample data to a hypothesized population include the: 1) one-sample sign test and 2) the Wilcoxon signed-ranks test. To analyze differences between two discrete levels of the independent variable, tests include the:

1) Mann-Whitney U and 2) median tests. For comparing how paired groups of data relate to each other, appropriate tests include: 1) Wilcoxon's matched-pairs test and 2) sign test. The analyses of variance models can be evaluated using: 1) the Kruskal-Wallis test or 2) Friedman two-way analysis of variance. Lastly, for correlation problems, the Spearman *rho* test may be substituted. These nonparametric procedures are extremely valuable and in many cases more appropriate when testing small sample sizes.

Ranking of Information

Most nonparametric tests require that the data be ranked on an ordinal scale. Ranking involves assigning the value 1 to the smallest observation, 2 to the second smallest, and continuing this process until N is assigned to the largest observation. For example:

Data	Rank	
12	1	
18	5	
16	3	$N = 5$
15	2	
17	4	

In the case of ties, the average of the rank values is assigned to each tied observation.

Data	Rank	
12	1	
18	9	
16	6	
15	4	
17	7.5	$N = 10$
14	2	
15	4	
15	4	
17	7.5	
20	10	

In this example there were three 15s (ranks 3, 4, and 5) with an average rank of 4 shared by the three observations. There are two 17s (ranks 7 and 8) with these observations sharing the average rank of 7.5.

When comparing sets of data from different groups or different treatment levels (levels of the independent variable), ranking involves all of the observations regardless of the discrete level in which the observation occurs:

Group A (n = 5)		Group B (n = 7)		Group C (n = 8)		Total (N = 20)
Data	Rank	Data	Rank	Data	Rank	
12	3	11	2	15	8.5	
18	17.5	13	4.5	15	8.5	
16	12	19	19.5	17	15	
15	8.5	17	15	19	19.5	
17	15	16	12	18	17.5	
		15	8.5	16	12	
		14	6	13	4.5	
				10	1	
∑ =	56.0	∑ =	67.5	∑ =	86.5	∑∑ = 210

Accuracy of the ranking process may be checked in two ways. First, the last rank assigned should be equal to the total N (in this example the largest rank was a tie between two observations (ranks 19 and 20), the average of which was 19.5. The second way to check the accuracy of the ranking procedure is the fact that the sum of all the summed ranks should equal $N(N + 1)/2$, where N equals the total number of observations:

$$Sum\ of\ Summed\ Ranks = \sum\sum R_i = \frac{N(N+1)}{2} \qquad \text{Eq. 21.1}$$

For the above example this check for accuracy in the ranking would be:

$$56.0 + 67.5 + 86.5 = 210 = \frac{20(21)}{2} = \frac{N(N+1)}{2}$$

Estimating Median Based on Walsh Averages

In Chapter 4 the median was defined as the center value for odd numbers of samples or the average of the middle two values for an even number of observations. Another way to estimate the median to account for the distribution of the data is an estimated median using what are called the **Walsh averages**. This involves averaging every possible pair of observations and the median for all those averages is reported as the estimated median. If data is symmetrical, the simple median and the estimate based on Walsh averages will be the same. But if the data is skewed, the estimated median will be weighted in the direction of that skew. For example, consider the six data points in Table 21.1, which appear to be skewed in the positive direction. The median for just the six data points would be 6.5 (the average of the two center points, (5 + 8)/2). All possible pair-wise averages are presented in the matrix in Table 21.1. In this case there are 21 pair-wise averages and the middle value (11th average in rank order) would be 7 which is the estimated median using Walsh averages. Thus the estimated median is slightly larger due to the skew in the data. Some computations and computer software packages use Walsh averages as the method for estimating the median.

Table 21.1 Example of Walsh Averages

	n_1					
n_2	1	2	5	8	12	18
1	1	1.5	3	4.5	6.5	9.5
2		2	3.5	5	7	10
5			5	6.5	8.5	11.5
8				8	10	13
12					12	15
18						18

One-Sample Sign Test

The one-sample sign test is a nonparametric procedure that can be used to estimate the population median based on sample data, create a confidence interval around the median and compare sample results with a predetermined hypothesized population median. It is called the sign test because data are converted to plus (+) and minus (–) signs depending on where they are larger or smaller than the hypothesized median. It is one of the oldest nonparametric procedures, reported as early as 1710 by British physician John Arbuthnott (Hollander and Wolfe). This test can be used as an alternative to the one-sample t-test (Chapter 9) and makes no assumptions about population symmetry (normal distribution). Observation must be on at least an ordinal scale and cannot be used for nominal data.

Based on sample data, the null hypothesis is that the population median from which the sample was selected is equal to some hypothesized median. The alternative hypothesis is that they are not equal:

$$H_0: \text{median } (M_i) = \text{hypothesized median } (M_0)$$
$$H_1: \text{median } (M_i) \neq \text{hypothesized median } (M_0)$$

This is an example of a two-tailed test based on using the binomial distribution to determine the probability of very small number of positive or negative signs. Samples are compared to the hypothesized median by subtracting that value from each sample value and recording the sign.

$$x_i - M_0$$

Observations greater than M_0 receive (+)s and those less than M_0 are assigned (–) signs. Once assigned their appropriate signs the number of (+) and (–) signs are counted. Those observations that are equal to the hypothesized median have no sign and are removed from consideration, with the number of observations (n) reduced accordingly. In the two-tailed test, the smallest number of signs (– or +) is used. If a

Table 21.2 Sample Data for Percent Label Claim

Sample (x_i)	x_i-M_0	Sign
96.7	-3.3	-
99.3	-0.7	-
100	0	-
98.2	-1.8	-
95.6	-4.4	-
102.3	+2.3	+
99.6	-0.4	-
94.7	-5.3	-
92.5	-7.5	-
92.3	-7.7	-
94.5	-5.5	-
97.8	-2.2	-

sufficiently small number of results exist (either (+)s or (–)s) the null hypothesis is rejected. In this case the probability such an occurrence would need to be less than $\alpha/2$.

If the researcher is interested in determining if the sample median is greater or less than the anticipated population, one-tailed hypotheses can be created:

$$H_0: M_i \geq M_0$$
$$H_1: M_i < M_0$$

$$H_0: M_i \leq M_0$$
$$H_1: M_i > M_0$$

If performing the one-tailed test there would need to be a sufficiently small number of (+)s to reject H_0: $M_i \leq M_0$, or a sufficiently small number of (–)s to reject $M_i \geq M_0$. In either case the probability would need to be less than α.

To illustrated the use of the one-sample sign test, consider the data presented in Table 21.2 which represents a sample taken from a batch of a product compared to the hypothesized median of 100% of the label claim. Here there are an even number of observations (12), so the observed median is the average of the center two numbers (M_i = 97.25 = (96.7 + 97.8)/2). In this case there are 10 (–) signs, 1 (+) signs and one response with no sign (equal to the hypothesized median). This last data point would be removed from the calculations since it equals the hypothesized median and n reduced to 11. What is the probability associated with such an occurrence by chance alone? To determine the answer, the binomial equation (Eq. 2.12) is used to determine the probability of 1 (+) sign out of 11 results.

$$p(x) = \binom{n}{x} p^x q^{n-x} = p(1) = \binom{11}{1} (0.5)^1 (0.5)^{10}$$

$$p(1) = \frac{11!}{1!10!}(0.5)^1(0.5)^{10} = (11)(0.50)(0.0009766) = 0.00537$$

However, we also need to add the probability of more extreme outcomes, which in this example would be no positive numbers ($p = 0$):

$$p(0) = \frac{11!}{0!11!}(0.5)^0(0.5)^{11} = (1)(1)(0.000488) = 0.00048$$

The sum of the two results would be the probability of one or less positive signs:

$$p(\leq 1) = 0.00537 + 0.00048 = 0.00585$$

Since this is a two-tailed test, to determine whether or not there is a difference to the positive or negative direction, the result would be multiplied by two, giving a probability of the results occurring by chance alone at $p = 0.0117$, which is less than $\alpha = 0.05$. Therefore, we would reject the null hypothesis and conclude the sample represented data that did not have the same median as same the hypothesized median of 100%. If it was originally planned as a one-tailed test to determine if the sample was less than the hypothesized median, the null hypothesis also would have been rejected because the *p*-value would have been only 0.0059.

To save calculating all the various combinations, Table B16 in Appendix B provides the results for binomial equations for smaller sample sizes with $p = 0.50$. In this example, the *p*-values would be read from the table for columns $x = 0, 1$ in the row where $n = 11$.

n = 11, x = 0	0.0005
n = 11, x = 1	0.0054
	0.0059

For larger sample sizes (usually 30 or more) an approximation can be made by calculating a *z*-value and referring to Table B2 in Appendix B to determine the associated *p*-value.

$$z = \frac{(x \pm 0.5) - 0.5n}{0.5\sqrt{n}} \qquad \text{Eq. 21.2}$$

The 0.5 is added because this is an approximation of a normal distribution based on discrete results and serves as a continuity correction. In the numerator +0.5 is used if x is less than $n/2$ and −0.5 would be used if x is greater than $n/2$. In this example the approximation would be:

$$z = \frac{(1 + 0.5) - 0.5(11)}{0.5\sqrt{11}} = \frac{-4}{1.66} = -2.41$$

Table 21.3 Probabilities Associated with Extreme Values in Table 21.1

Rank	Probability	Cumulative Probability	Corresponding Sorted Data
1	0.0005	0.0002	92.3%
2	0.0054	0.0031	92.5%
3	0.0269	0.0192	94.5%
4	0.0806	0.0998	94.7%
5	0.1611		95.6%
6	0.2256		96.7%
7	0.2256		97.8%
8	0.1611		98.2%
9	0.0806	0.0998	99.3%
10	0.0269	0.0192	99.6%
11	0.0054	0.0031	100%
12	0.0005	0.0002	102.3%

The z-value of 2.41 in Table B2 is associated with a $p = 0.4920$ from 0 to –2.41. To fall to the extreme of z = –2.41, the probability would be 0.500 – 0.4920 = 0.0080, which for smaller sample sizes is not a very good approximation of the binomial equation (0.0059).

Confidence intervals can be created around the median. Because these are distribution free statistics and lack the assumption of normality for parametric tests, it may not be possible to calculate the exact 95% confidence interval. Standard errors cannot be calculated for distribution-free statistics, but instead estimated locations within the ranked sample data are determined. These confidence intervals may not be symmetrical around the median. For the lower limit of the 95% confidence interval the rank is calculated as follows:

$$lower\ rank = \frac{n}{2} - \frac{1.96\sqrt{n}}{2} \qquad \text{Eq. 21.3}$$

For the upper limit, the calculation is:

$$upper\ rank = 1 + \frac{n}{2} + \frac{1.96\sqrt{n}}{2} \qquad \text{Eq. 21.4}$$

These formulas can be modified for 90% or 99% confidence intervals by substituting 1.64 or 2.58 for 1.96, respectively. Using the example previous example we can create Table 21.3 with the observations in rank order and their cumulative probability noted in the second column. Using all 12 data points the ranks approximating a 95% confidence interval would be

$$lower\ rank = \frac{12}{2} - \frac{1.96\sqrt{12}}{2} = 6 - 3.39 = 2.61 \approx 3$$

$$upper\ rank = 1 + \frac{12}{2} + \frac{1.96\sqrt{12}}{2} = 1 + 6 + 3.39 = 10.39 \approx 10$$

Thus, the approximate 95% confidence interval would be between ranks 3 and 10, or 94.5 and 99.6. This is not exactly a 95% confidence interval because the probability of being at these points or to their extreme would be 0.0392 (0.0192 + 0.0192) or a 96.1% confidence interval. The hypothesized population median of 100% does not fall within the interval of 94.5 to 99.6%; therefore the null hypothesis is rejected.

Wilcoxon Signed-Ranks Test

The second nonparametric procedure that can be used to estimate if data from a sample is significantly different from a hypothesized median and for creating a confidence interval is the Wilcoxon signed-ranks test. Frank Wilcoxon was a chemist with American Cyanamid and Lederle Laboratories and developed several nonparametric procedures during the 1940s (Salsburg, p. 161). Whereas the sign tests dealt with only the sign (plus or minus compared to the hypothesized median), Wilcoxon evaluated the magnitude of the differences from that median. The null hypotheses are identical to those used for the sign test, where the median for sample data is compared to a hypothesized median.

H_0: median (M_i) = hypothesized median (M_0)
H_1: median (M_i) ≠ hypothesized median (M_0)

In this case it is a two-tailed test, but one-tailed tests can also be performed to determine if the sample median is greater than (H_1: $M_i < M_0$) or less than ($M_i > M_0$) the anticipated population. As with the sign test, this Wilcoxon test can serve as an alternative to the one-sample z-test (Chapter 7) or the one-sample t-test (Chapter 9).

The magnitude of the difference for each sample from the hypothesized median is determined and then ranked from the smallest to the largest difference. Table 21.4 presents the data previously used for the sign test. Zero differences are eliminated from the calculations and the number of observations (n) reduced to correct for the removed data. In this case the single observed result of 100 is eliminated, n is reduced to 11 and the smallest absolute difference (99.6%) is 0.4 which received the first rank. The second smallest difference (99.3%) is ranked number 2. This process continues as previously described until the largest difference (−7.5 for 92.5%) which received the last rank of 11. If there were tied differences, regardless of the sign, they would share the same rank. Once the ranking is completed for a two-tailed test, those ranks associated with the least frequent sign (in this case the plus sign) are moved to the last column in Table 21.4. The ranks in the last column are summed and labeled T which will be used for determining whether or not to reject the null hypothesis. To determine the significance for smaller data sets (30 or less), the significance of the T-

Table 21.4 Sample Data Differences in Rank Order

Observed	Hypothesized	d	Rank |d|	Rank associated with least frequent sign
96.7	100	–3.3	6	
99.3	100	–0.7	2	
100	100	0		
98.2	100	–1.8	3	
95.6	100	–4.4	7	
102.3	100	+2.3	5	5
99.6	100	–0.4	1	
94.7	100	–5.3	8	
92.5	100	–7.5	11	
92.3	100	–7.7	10	
94.5	100	–5.5	9	
97.8	100	–2.2	4	
			T = ∑ =	5

value can be determined from Table B17 in Appendix B. In this case the probability of a $T = 5$ where $n = 11$ would be 0.01 or less ($\alpha/2$). This result is similar to those for the sign test.

For one-tailed tests, the ranks carried over to the last column (illustrated in Table 21.4) would depend on the direction of the tail of interest. If the alternative hypothesis is $M_i < M_0$) then the ranks associated with the positive signs are summed. If the alternative hypothesis is $M_i > M_0$ then the ranks associated with the negative signs are summed to create the T statistic. Using Table B17, the values for the α column would be used instead of the $\alpha/2$ column.

For larger sample sizes an approximation of the probability of rejecting the null hypothesis can be calculating the following two equations. If the sample median equals the hypothesized median, the expected total for the ranks is $E(Total) = n(n + 1)/2$. If H_0 is true then the total for each sign rank (+ or –) should be equal to half the total ranks (Eq. 21.1). Thus:

$$E(T) = \frac{n(n+1)}{2} \cdot \frac{1}{2} \qquad or \qquad E(T) = \frac{n(n+1)}{4} \qquad \text{Eq. 21.5}$$

Similar to previous statistics, a comparison is made looking at the difference between what is observed (T) and what is expected with the null hypothesis.

$$z = \frac{T - E(T)}{\sqrt{\frac{n(n+1)(2n+1)}{24}}} \qquad \text{Eq. 21.6}$$

As with previous equations, if the observed and expected values are identical the

numerator would be zero and the z-value would be zero. As the difference increases the z-value increases until it reaches a point of statistical significance with a given Type I error rate. In this procedure the decision rule is with a predetermined α, to reject H_0 if z is greater than $z(\alpha/2)$ from the normal standardized distribution (Table B2, Appendix B). For the example presented in Table 21.4 (even though it is small sample and we could use Table B17) it can be approximated and the decision rule would be, with $\alpha = 0.05$, reject H_0 if $z > 1.96$ and the computations would be as follows:

$$E(T) = \frac{(11)(12)}{4} = 33$$

$$z = \frac{5 - 33}{\sqrt{\frac{11(12)(2(11)+1)}{24}}} = \frac{-28}{\sqrt{126.5}} = -2.49$$

The decision is with $z < 1.96$, we cannot reject H_0 and we are unable to find a significant difference between median for the sample and the hypothesized median. Using table B2 the probability of a $z = -2.49$ would be 0.0064 (0.5000 – 0.4936). This approximation is similar to the results using Table B17.

If there are numerous ties in the rankings, an adjustment in the denominator can be made in the formula to account for these ties:

$$z = \frac{T - E(T)}{\sqrt{\frac{n(n+1)(2n+1)}{24} - \frac{\sum t^3 - \sum t}{48}}} \qquad \text{Eq. 21.7}$$

In the previous example, there were no ties so such an adjustment would not be required.

If the population distribution is assumed to be symmetrical a confidence interval can be created around the median. For smaller sample sizes a triangular matrix can be created with Walsh averages. This method is described by Daniel (1978, pp. 40-44). For larger samples a confidence interval is approximated by modifying Eq. 21.6:

$$Interval = [T - E(T)] \pm z_{\alpha/2}\sqrt{\frac{n(n+1)(2n+1)}{24}} \qquad \text{Eq. 21.8}$$

In the previous example the estimated 95% confidence interval would be:

$$Interval = 33 \pm 1.96\sqrt{\frac{11(12)(23)}{24}} = 33 \pm 22.04$$

$$+10.96 < T < +55.04$$

Similar to interpreting the t-test, since zero does not fall within the interval, it is impossible for 100% to fall within the interval; therefore, like the sign test the null hypothesis is rejected with 95% confidence.

Some computer software (including Minitab) will estimate the confidence interval in the original units of measure. The Wilcoxon signed rank test is slightly less powerful than the one-sample t-test when the population is normally distributed. Also with a normal distributed population, the confidence interval for the Wilcoxon test will be slightly wider. For distributions that are not normally distributed, Wilcoxon will be more powerful and produce narrower confidence intervals than the one-sample t-test. Comparing these two nonparametric procedures to their parametric counterpart, the one-sample t-test; using Minitab we see similar, but slightly different results:

Test	Center	Lower Limit	Upper Limit
One-sample t-test	96.96	94.97	98.94
One-sample sign test	97.25	94.55	99.52
Wilcoxon signed-ranks test	97.03	95.05	98.95

Note that the Wilcoxon test uses the Walsh averages to estimate the median which gives a slightly smaller center point than the sign test, reflecting a negative skew to the sample data.

Mann-Whitney Test

The Mann-Whitney test has numerous synonyms, including the **Mann-Whitney U**, **two-sample Wilcoxon rank sum test** and the **Wilcoxon-Mann-Whitney** (WMW) test. It is a procedure for the situation where the independent variable has two discrete levels and there is a continuous dependent variable (similar to the two-sample t-test described in Chapter 9). Data are ranked and a formula is applied. Note that the hypotheses below are not concerned with the means of the populations. The parameter of normality is not considered, where the t-test evaluated the null hypothesis the $\mu_1 = \mu_2$. The hypotheses for this nonparametric procedure are:

H_0: Samples are from the same population
H_1: Samples are drawn from different populations

There are two assumptions for performing the Mann-Whitney test: 1) the populations from which the samples are taken have the same shape; 2) observations are independent of each other. If the populations are normally distributed this test is slightly less powerful than the two-sample t-test and the confidence interval will be wider.

For this test the data are ranked and the sum for these ranks for one of the levels of the dependent variables will be used in the calculations.

Data Level 1	Rank	Data Level 2	Rank
d_{11}	R_{11}	d_{21}	R_{21}
d_{12}	R_{12}	d_{22}	R_{22}
d_{13}	R_{13}	d_{23}	R_{23}
...	...	...	...
d_{1j}	R_{1j}	d_{2j}	R_{2j}
	$\Sigma R_{1j} = W$		

We will label the sum associated with the first level W to be consistent with Minitab output. If the sum of the ranks for level 2 were selected we would get the exact same results for the z-value defined below, only the sign would be the opposite (e.g., minus instead of plus). The statistical values are calculated using the following two formulas where W is associated with n_1 (the number of observations associated with the sum W).

$$U = n_1 n_2 + \frac{n_1(n_1+1)}{2} - W \qquad \text{Eq. 21.9}$$

Here n_2 is the number of observations in the level of independent variable not summed. If the null hypothesis is true we would expect the two rank sums for each level to be about equal. The larger the difference between the two scores the greater the likelihood that there is a significant difference. If both levels were exactly the same we would expect the following for the expected U:

$$E(U) = \frac{n_1 n_2}{2} \qquad \text{Eq. 21.10}$$

We evaluate the difference between the U-value and $E(U)$ using the following formula to convert the results to a z-value:

$$z = \frac{U - \frac{n_1 n_2}{2}}{\sqrt{\frac{n_1 n_2 \cdot [n_1 + (n_2 + 1)]}{12}}} \qquad \text{Eq. 21.11}$$

The calculated z-value is then compared to values in the normalized standard distribution (Table B2, Appendix B). If the calculated z-value is to the extreme of the critical z-value (positive or negative) then H_0 is rejected. In the case of 95% confidence, the critical z-values would be either −1.96 or +1.96. The numerator of the equation is similar to the z-test of proportions; we are comparing an observed U-value to an expected value that is the average of the ranks ($n_1 n_2/2$).

As an example of the Mann-Whitney U test, a pharmacology experiment was conducted to determine the effect of atropine on the release of acetylcholine (ACh) from rat neostriata brain slices. The measure of ACh release through stimulation was

Table 21.5 Sample Data for the Mann-Whitney U Test

Control	Rank	Received Atropine	Rank
0.7974	3	1.7695	13
0.8762	4	1.6022	12
0.6067	1	1.0632	7
1.1268	9	2.7831	14
0.7184	2	1.0475	6
1.0422	5	1.4411	11
1.3590	10	1.0990	8
$\sum =$	34	$\sum =$	71

measured twice. Half of the sample received atropine before the second measurement. The ratios (stimulation 2 divided by stimulation 1) are presented in the first and third columns of Table 21.5. Is there a difference in the ratios between the control group and those administered the atropine? The hypotheses are:

H_0: Samples are from the same population
(i.e., no difference in response)
H_1: Samples are drawn from different populations
(i.e., difference in response)

The decision rule is, with $\alpha = 0.05$, reject H_0, if $|z|$ > critical $z_{(0.975)} = 1.96$. The rankings of the data are presented in the second and fourth columns of Table 21.5. A quick computational check for accuracy of the ranking shows that the ranking was done correctly:

$$\frac{N(N+1)}{2} = \frac{14(15)}{2} = 105 = 34 + 71$$

The calculation of the Mann-Whitney test statistics would be:

$$U = n_1 n_2 + \frac{n_1(n_1+1)}{2} - W = (7)(7) + \frac{(7)(8)}{2} - 34 = 43$$

$$z = \frac{U - \frac{n_1 n_2}{2}}{\sqrt{\frac{n_1 n_2 \cdot [n_1 + (n_2+1)]}{12}}} = \frac{43 - \frac{(7)(7)}{2}}{\sqrt{\frac{(7)(7)(7+8)}{12}}} = \frac{43 - 24.5}{7.83} = 2.36$$

Note that reversing Level 1 and Level 2 would produce identical results. In the above case the W is 71 and n_1 is 7:

$$U = (7)(7) + \frac{(7)(8)}{2} - 71 = 6$$

$$z = \frac{6 - \frac{(7)(7)}{2}}{\sqrt{\frac{(7)(7)\cdot[7+8)]}{12}}} = \frac{6 - 24.5}{7.83} = -2.36$$

The decision, either way, would be with $z > z_{critical} = 1.96$, reject H_0 and conclude that the samples are drawn from different populations and the response of the rat's neostriata release of ACh is affected by atropine. Using Table B2 in Appendix B, for a *z*-value of 2.36, the probability of being greater than a z-value of 2.36 would be 0.0091 (0.5000 − 0.4909). Being a two-tailed test the *p*-value would be 0.0182 (0.0091 × 2).

A confidence interval can be created but it is somewhat labor intense where the a point estimate is created for the difference between the two medians and an interval close to 95% confidence is created. Computer programs can create such intervals and will not be discussed at this time, but the interpretation is similar to the two-sample t-test. If zero falls within the interval there is no significant difference between the populations. If the confidence interval does not include zero then the null hypothesis is rejected.

Two-Sample Median Test

The two-sample median test is an alternative to the Mann-Whitney test when the independent variable has only two discrete levels. This test utilizes the median for all of the data points observed. The hypotheses are the same as the Mann-Whitney test.

H_0: Samples are from the same population
H_1: Samples are drawn from different populations

The first step is to create a 2 × 2 table using the *grand median* for all of the observations in both levels of the independent variable. As discussed previously, one valuable property of the median is that it is not affected by a single outlier (extreme value).

	Group 1	Group 2	
Above the median	a	b	n = total observations
Below the median	c	d	

The calculated *p*-value is determined using a formula that incorporates a numerator of all the margin values ($a + b$, $c + d$, $a + c$ and $b + d$) and a denominator involving each cell:

$$p = \frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!}{n!\,a!\,b!\,c!\,d!} \qquad \text{Eq. 21.12}$$

The decision rule is to reject H_0, if the calculated p-value is less than the critical p(α) in a normal standardized distribution, for example, $\alpha = 0.05$. Note that this formula is exactly the same as the Fisher exact test presented in Chapter 16. The difference between the two tests is that the median is created based on an ordinal or higher scaled data and the results are based solely on the observed results and not any more extreme scenarios as seen with the Fisher exact test.

As an example of the median test, the same data used for the Mann-Whitney test will be considered. In this case the grand median is between data points 1.0632 and 1.0990 (ranks 7 and 8 for all the data). The data for each level of the independent variable is classified as above or below the median and the results are presented in the following table:

	Control	Atropine	
Above the median	2	5	N = 14
Below the median	5	2	

In this example, all of the margin values (e.g., a+b) are seven and the computation of the probability of the occurrence is:

$$p = \frac{(2+5)!\,(2+5)!\,(5+2)!\,(2+5)!}{14!\,2!\,5!\,5!\,2!}$$

$$p = \frac{6.45 \times 10^{14}}{5.02 \times 10^{15}} = 0.128$$

With the calculated p = 0.128, there is a probability of this occurring 12.8% of the time by chance alone. We cannot reject H_0. The researcher cannot find a significant difference and must assume that the results are from the same population and there is no treatment effect.

Note that when using the Mann-Whitney test H_0 at the 0.05 level of significance, H_0 was rejected, but could not be rejected with the median test. If the same data is run using a t-test, the results are identical to the Mann-Whitney test:

Significance Level	0.1	0.05	0.01
Mann-Whitney test	Reject H_0	Reject H_0	Accept H_0
Two-sample median test	Reject H_0	Accept H_0	Accept H_0
t-test	Reject H_0	Reject H_0	Accept H_0

It appears that the median test is a slightly more conservative test than either the Mann-Whitney or t-tests, and more likely to result in a Type II error. This is due in

part to the small amount of information available from the median test results that are dichotomized into above and below the median, and only two outcomes are possible.

Wilcoxon Matched-Pairs Test

The Wilcoxon matched-pairs test offers a parallel to the matched-pair t-test discussed in Chapter 9. To accomplish this test, a traditional pre- and posttest (before-after) table is constructed and the differences are calculated similar to the matched-pair t-test. For example:

Subject	Before	After	d
1	67	71	+4
2	70	73	+3
3	85	81	–4
4	80	82	+2
5	72	75	+3
6	78	76	–2

The *absolute* differences (regardless of sign, positive or negative) are then ranked from smallest to largest.

Subject	Before	After	d	Rank |d|
1	67	71	+4	5.5
2	70	73	+3	3.5
3	85	81	–4	5.5
4	80	82	+2	1.5
5	72	75	+3	3.5
6	78	76	–2	1.5

Notice that the fourth and sixth subjects have identical differences (even though the signs are different): therefore, they share the average rank of 1.5 (ranks 1 and 2). Thus, the ranking process measures the magnitude of the difference regardless of the direction (positive or negative). A *T*-value is calculated for the sum of the ranks associated with the *least frequent* sign (+ or –).

Sub.	Before	After	d	Rank |d|	Rank Associated with Least Frequent Sign
1	67	71	+4	5.5	
2	70	73	+3	3.5	
3	85	81	–4	5.5	5.5
4	80	82	+2	1.5	
5	72	75	+3	3.5	
6	78	76	–2	1.5	1.5
				$T = \sum =$	7.0

Table 21.6 Example of Data for a Wilcoxon Matched-Pairs Test

Before	After	d	Rank |d|	Rank Associated with Least Frequent Sign
81	86	+5	6.5	
81	93	+12	8	
78	74	–4	4.5	4.5
80	80	0	-	
74	76	+2	3	
78	83	+5	6.5	
90	91	+1	1.5	
95	95	0	-	
68	72	+4	4.5	
75	74	–1	1.5	1.5
n = 8	∑ =	0		T = ∑ = 6

Note in the above example that the third and sixth subjects were the only two with negative differences (the least frequent sign); therefore, their associated ranks were the only ones carried over to the last column and summed to produce the *T*-value. If all the signs are positive or negative then the *T*-value would be zero and no ranks would be associated with the least frequent sign and $T = 0$.

Like the Wilcoxon signed-rank test, a unique aspect of this test is that a certain amount of data may be ignored. If a difference is zero, there is no measurable difference in either the positive or negative direction; therefore, a sign cannot be assigned. Thus, data associated with no differences are eliminated and the number of pairs (n) is reduced appropriately. To illustrate this point, note the example in Table 21.6. In this case n is reduced from 10 pairs to $n = 8$ pairs, because two of the results have zero differences. Also note that the least frequent sign was a negative, thus the *T*-value is calculated by summing only those rank scores with negative differences. The hypotheses for the Wilcoxon matched-pairs test are not concerned with mean differences, as seen with the t-test (where the null hypothesis was $\mu_d = 0$) and stated as follows:

H_0: No difference between pre- and post-measurements
H_1: Difference between pre- and post-measurements

The same calculations use for the Wilcoxon Signed-rank test (Eqs. 21.5 and 21.6) are used for this pair-wise comparison test. The expected *T*-value or *E(T)* is the anticipated result if there was no difference between the pre- and post-measurements.

$$E(T) = \frac{n(n+1)}{2} \cdot \frac{1}{2} \qquad or \qquad E(T) = \frac{n(n+1)}{4}$$

The test statistic once again involves a numerator that compares the difference between an expected value and an observed result, in this case the *T*-value:

$$z = \frac{T - E(T)}{\sqrt{\frac{n(n+1)(2n+1)}{24}}}$$

As with previous equations, if the observed and expected values are identical the numerator would be zero and the z-value would be zero. As the difference increases the z-value increases until it reaches a point of statistical significance with a given Type I error rate. In this procedure the decision rule is with a predetermined α, to reject H_0 if z is greater than $z(\alpha/2)$ from the normal standardized distribution (Table B2, Appendix B). For the example presented in Table 21.6 the decision rule would be, with $\alpha = 0.05$, reject H_0 if $z > 1.96$ and the computations would be as follows:

$$E(T) = \frac{(8)(9)}{4} = 18$$

$$z = \frac{6 - 18}{\sqrt{\frac{8(9)(2(8)+1)}{24}}} = \frac{-12}{\sqrt{51}} = -1.68$$

The decision is with $z < 1.96$, we cannot reject H_0 and we are unable to find a significant difference between pre- and post-measurements. Note that if we used the ranks associated "most frequent sign", in this case the negative signs, we would get the same z-value, only positive (+1.68). Using Table B2 in Appendix B, the p-value to the extreme of 1.68 would be 0.093 ($2 \times 0.5000 - 0.4535$).

Sign Test for Paired Data

The sign test is a second method for determining significant differences between paired observations and like the previous one-sample sign test is based on the binomial distribution. It is among the simplest of all nonparametric procedures. Similar to the Wilcoxon test, differences are considered and any pairs with zero differences are dropped, and the n of the sample is reduced. A table for the pairs is constructed and only the sign (+ or −) is considered. Using the same example presented for the Wilcoxon test we find signs listed in Table 21.7. If there are no significant differences between the before and after measurements we would expect half the numbers to be positive (+) and half to be negative (−). Thus $p(+) = 0.50$ and $p(-) = 0.50$. If there was no significant difference between the before-and-after measurements, the null hypotheses would be that the proportion of positive and negative signs would be equal.

H_0: No difference between measurement	or	H_0: $p(+) = 0.50$
H_1: Difference between measurements exists		H_1: $p(+) \neq 0.50$

The more the proportion of (+)s or (−)s differ from 0.50, the more likely that there is a

Table 21.7 Sample Data for a Sign Test

Before	After	d	Sign
81	86	+5	+
81	93	+12	+
79	74	–4	–
80	80	0	0
74	76	+2	+
78	83	+5	+
90	91	+1	+
95	95	0	0
68	72	+4	+
75	74	–1	–

significant difference and that the difference is not due to random error alone.

For sample sizes less than 10 the binomial distribution can be used and Eq. 2.12 would be used to define the probabilities associated with the distribution.

$$p(x) = \binom{n}{x} p^x q^{n-x}$$

Dropping the two zero differences the final number of paired observations is eight. What is the probability of six or more positive values out of eight differences, given that the probability of a positive value equals 0.50? Probabilities can be calculated using the binomial equation as below or taken from Table B16 in Appendix B.

$$p(6\ positives) = \binom{8}{6}(0.50)^6(0.50)^2 = 0.1092$$

$$p(7\ positives) = \binom{8}{7}(0.50)^7(0.50)^1 = 0.0313$$

$$p(8\ positives) = \binom{8}{8}(0.50)^8(0.50)^0 = 0.0039$$

Adding the three probabilities together

$$p(>5\ positives) = \Sigma = 0.1444$$

Thus, there is almost a 15% chance that there will be six or more positive differences out of the eight pairs by chance alone. Thus, we cannot reject H_0.

For 10 or more pairs of observations, we can employ Yates' correction for continuity for the one-sample z-test for proportions (modified from Eq. 15.2):

$$z = \frac{|p - P_0| - \frac{1}{n}}{\sqrt{\frac{(P_0)(1-P_0)}{n}}} \qquad \text{Eq. 21.13}$$

where p is the number of positive outcomes divided by the total number of pairs. In this particular case $p = 6/8 = 0.75$:

$$z = \frac{|0.75 - 0.50| - \frac{1}{8}}{\sqrt{\frac{(0.50)(0.50)}{8}}} = \frac{0.25 - 0.125}{\sqrt{0.0313}} = 0.71$$

In a normal standardized distribution table (Table B2, Appendix B) the area below the point where $z = 0.71$ is 0.7611 (0.5000 + 0.2611). Thus, the probability of being above $z = 0.71$ is 0.2389 and therefore not significant.

Kruskal-Wallis Test

Much as the F-test is an extension of the t-test, Kruskal-Wallis is an equivalent nonparametric extension or generalization of the Mann-Whitney test for more than two levels of an independent discrete variable. This test is an alternative to the one-way ANOVA (Chapter 10) and looks for differences among population medians. It is particularly useful when the dependent variable is on an ordinal scale or when the assumption of homogeneity of variance is violated. The null hypothesis is actually stating that all of the population medians are equal (H_0: $\eta_1 = \eta_2 = \eta_3 \ldots = \eta_k$). The alternative hypothesis is that a difference exists somewhere among the population medians (not all η's are equal) or more simply stated

H_0: Samples are from the same population
H_1: Samples are drawn from different populations

Like the Mann-Whitney test, data are ranked and rank sums calculated, then a new statistical formula is applied to the summed ranks.

Level 1	Rank	Level 2	Rank	...	Level k	Rank
d_{11}	R_{11}	d_{21}	R_{21}	...	d_{k1}	R_{k1}
d_{12}	R_{12}	d_{22}	R_{22}	...	d_{k2}	R_{k2}
...	...	...	...	...	...	...
d_{1j}	$\underline{R}_{1j}$	d_{2j}	$\underline{R}_{2j}$	...	d_{kj}	$\underline{R}_{kj}$
	$\sum R_{1j}$		$\sum R_{2j}$			$\sum R_{kj}$

Table 21.8 Data for a Kruskal-Wallis Example

Instrument A		Instrument B		Instrument C	
Assay	Rank	Assay	Rank	Assay	Rank
12.12	8	12.47	14	12.20	10
13.03	18	13.95	21	11.23	1
11.97	7	12.75	16	11.28	2
11.53	3	12.21	11	12.89	17
11.82	6	13.32	19	12.46	13
11.75	5	13.60	20	12.56	15
12.25	12			11.69	4
12.16	9				
∑ =	68		101		62

For the Kruskal-Wallis test the formula for the test statistic is:

$$H = \frac{12}{N(N+1)}\left[\sum \frac{(\sum R_{ij})^2}{n_j}\right] - 3(N+1) \qquad \text{Eq. 21.14}$$

The middle section of the equation involves the squaring of the individual sum of ranks for each of the k levels of the independent variable, dividing those by their respective number of observations and then summing these k results. The decision rule in this test is to compare the calculated Kruskal-Wallis H-statistic with a χ^2-critical value from Table B15 in Appendix B. The decision rule is:

$$\text{with } \alpha = 0.05, \text{ reject } H_0, \text{ if } H > \chi^2_{k-1}\,(0.95).$$

The degrees of freedom is based on the number of levels of the discrete independent variable minus one for bias ($K - 1$).

For an example of the Kruskal-Wallis test, assume that three instruments located in different laboratories were compared to determine if all three instruments could be used for the same assay (Table 21.8). Is there a significant difference based on the results (mg/tablet) based on the sample results presented in Table 21.8? The hypotheses are:

H_0: Samples are from the same population $(\eta_A = \eta_B = \eta_C)$
H_1: Samples are drawn from different populations

With three discrete levels in our independent variable, the number of degrees of freedom is two and χ^2_2 equals 5.99. The calculations are as follows:

$$H = \frac{12}{N(N+1)}\left[\sum \frac{(\sum R_{ij})^2}{n_j}\right] - 3(N+1)$$

$$H = \frac{12}{21(22)}\left[\frac{(68)^2}{8} + \frac{(101)^2}{6} + \frac{(62)^2}{7}\right] - 3(22)$$

$$H = 0.026(578.5 + 1700.2 + 549.1) - 66 = 7.52$$

The decision in this case, with $H > 5.99$, is to reject H_0 and conclude that there is a significant difference among the three pieces of equipment and they are not equal in their assay results. The *p*-value could be determined using Excel function (**CHISQ.DIST.RT**). In this case a probability of a chi square statistic of 7.52 with two degrees of freedom would be 0.023, well less than the acceptable Type I error of 0.05.

Some statisticians (e.g., Zar, p. 215) recommend a correction for ties (sharing of the same ranks) in the data, especially when there are a large number of such ties. This correction factor is:

$$C = 1 - \left[\frac{\sum(t^3 - t)}{N^3 - N}\right] \qquad \text{Eq. 21.15}$$

For example, assume there were four sets of pair ties, and three sets of triplicate ties are:

$$4\left[(2)^3 - 2\right] + 3\left[(3)^3 - 3\right]$$

N equals the total number of observations. In this particular example, the correction would be as follows:

$$C = 1 - \left[\frac{4[(2)^3 - 2] + 3[(3)^3 - 3]}{(21)^3 - 21}\right]$$

$$C = 1 - \frac{96}{9240} = 1 - 0.0104 = 0.9896$$

The corrected H statistic (H') is:

$$H' = \frac{H}{C} \qquad \text{Eq. 21.16}$$

since the denominator will be less than 1, this correction will give a slightly higher value than the original H statistic. The decision rule is to reject H_0, if H' is greater than $\chi^2_{K-1}(1-\alpha)$, which is the chi square value from Table B15. In the example above there were no ties, but assume in another example the H-value was 5.00 for three levels of the independent variable and the above scenario of four sets of pair and three sets of triplicate ties actually occurred. In this case the H' would be:

$$H' = \frac{5.00}{0.9896} = 5.05$$

In most cases the adjustment is negligible. Unlike Yates corrections, which produce a more conservative test statistic, this correction for ties produced a larger number more likely to find a significant difference. Thus, the correction for ties leads to a less conservative result than would be expected with the original H-statistic.

Post Hoc Comparisons Using Kruskal-Wallis

The Kruskal-Wallis *post hoc* comparison is a parallel to the Tukey test (Chapter 11) and uses the q-statistic for pair-wise differences between ranked sums. In this test, the numerator is the difference between the two sums of ranks (ΣR_{ij}) and the denominator represents is a new standard error term based on the sample size and number of levels in the independent variable. For equal numbers of observations per k-levels of the independent variable, the formula is as follows:

$$q = \frac{R_A - R_B}{\sqrt{\frac{n(nk)(nk+1)}{12}}} \qquad \text{Eq. 21.17}$$

Since only pair-wise comparisons can be performed, R_A is the sum of the ranks for the first level and R_B is the sum of the ranks for the second level of the independent variable being compared. If the cell sizes are not equal the formula is adjusted as follows:

$$q = \frac{R_A - R_B}{\sqrt{\frac{N(N+1)}{12}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}} \qquad \text{Eq. 21.18}$$

It can be further modified to correct for ties

$$q = \frac{R_A - R_B}{\sqrt{\frac{N(N+1)}{12} - \frac{\sum T}{12(N-1)}\left(\frac{1}{n_A} + \frac{1}{n_B}\right)}} \qquad \text{Eq. 21.19}$$

where:

$$\sum T = \sum_{i=1}^{m} (t_i^3 - t_i) \qquad \text{Eq. 21.20}$$

To illustrate this test, consider the significant results identified with the previous Kruskal-Wallis test evaluating the results produced by three analytical instruments, where the sums of ranks and *n*'s were as follows:

Instrument	ΣR	n
A	68	8
B	101	6
C	62	7
	N =	21

Since the sample sizes differ per instrument and there were no tied ranks, Eq. 21.18 will be used for the three possible pair-wise comparisons. Comparing Instrument A and B, the result for this *post hoc* procedure is:

$$q = \frac{68 - 101}{\sqrt{\frac{21(21+1)}{12}\left(\frac{1}{8} + \frac{1}{6}\right)}} = \frac{-33}{3.351} = -9.85$$

The interpretation of significance uses the same procedure discussed for the *q*-statistic in Chapter 11. If $q > q_{\alpha,k,N-k}$ of $q < -q_{\alpha,k,N-k}$ from Table B10 (Appendix B), reject the hypothesis of no difference between the two levels being compared. The results of all three pair-wise comparisons are presented in Table 21.9.

Mood's Median Test

A second nonparametric alternative to the one-way analysis of variance would be Mood's median test, sometimes referred to as the **sign scores test** or simply the **median test**. It is similar to the Kruskal-Wallis test where the null hypothesis is that

Table 21.9 Results of Kruskal-Wallis *Post Hoc* Comparisons

Pairing	q-statistic	Critical Value	Results
$R_A - R_B$	–9.85	3.61	Significant
$R_A - R_C$	1.87	3.61	
$R_B - R_C$	11.30	3.61	Significant

population median are the same for each level of the independent variable (H_0: $\eta_1 = \eta_2 = \eta_3 \ldots = \eta_k$). The Mood's test is less powerful than the Kruskal-Wallis and will usually produce narrower confidence intervals. However it is more robust against outliers (Chapter 23).

Similar to the two-sample sign test, a contingency table is created with each level of the independent variable representing a column and the two rows (one for the number of results equal to or less than the grand median for all the samples and the second for the number of results greater than the grand median (*H*, Greek capital letter for *eta*, η).

	Level 1	Level 2	Level 3	…	Level k	Total (R)
Scores ≤ H	n_{11}	n_{12}	n_{13}	…	n_{1k}	R_1
Scores > H	n_{21}	n_{22}	n_{23}	…	n_{2k}	R_2
Total	n_1	n_2	n_3	..	n_k	N

Using the margins (R_i's and *ni*'s) expected values under independence are calculated the same way as discussed in Chapter 16, Eq. 16.6). For example, the expected value for the upper left corner of the contingency table would be:

$$E(n_{11}) = \frac{(R_1)(n_1)}{N}$$

Using the observed data, above and below the grand median, and the expected values under the assumption all levels of the independent variable, it is possible to evaluate using the chi square statistic (Eq. 16.2) and evaluated for $K - 1$ degrees of freedom:

$$\chi^2 = \Sigma \frac{(O-E)^2}{E}$$

For example, consider data once again in Table 21.8 comparing the three instruments. Using all 21 data points the grand median is 12.21 (*H*) and the contingency table would be:

	Inst. A	Inst. B	Inst. C	Total
Scores ≤ H	6	1	4	11
Scores > H	2	5	3	10
Total	8	6	7	21

The expected values would be:

	Inst. A	Inst. B	Inst. C	Total
Scores ≤ H	4.19	3.14	3.67	11
Scores > H	3.81	2.86	3.33	10
Total	8	6	7	21

The chi square statistic is calculated as:

$$\chi^2 = \frac{(6-4.19)^2}{4.19} + \frac{(1-3.14)^2}{3.14} + \ldots \frac{(3-3.33)^2}{3.33} = 4.77$$

Unfortunately the results are not the same as those with the Kruskal-Wallis test. With the Mood's median test we would fail to reject the null hypothesis because our test statistic did not exceed the critical value of 5.99. The corresponding *p*-value would be much greater at 0.092 (using Excel as above). The difference could be due to the small sample size and the fact that the calculation violates the chi square requirement that all expected values equal or exceed five.

Friedman Two-Way Analysis of Variance

The Friedman procedure can be employed for data meeting the design for a randomized block design (Chapter 10), but that fail to conform to the criteria for parametric procedures. Somewhat of a misnomer, unlike the two-way ANOVA discussed in Chapter 12, this test requires only one observation per treatment-block combination. This randomized block design can be considered a nonparametric extension of the Wilcoxon matched-pairs test to more than two treatment levels or times. The null hypothesis is that the treatment has no effect.

H_0: No difference in the treatment levels
H_1: A difference exists in the treatment levels

The summed ranks are used in the following test statistic:

$$\chi_r^2 = \frac{12}{nk(k+1)} \sum (R_j)^2 - 3n(k+1) \qquad \text{Eq. 21.21}$$

where k represents the number of levels of the independent variable (treatments) and n is the total number of rows (blocks). Critical values for small sample sizes (e.g., fewer than five blocks or rows) are available (Daniel, 2005). Larger sample sizes can be approximated from the standard chi square table for $k - 1$ degrees of freedom. If the calculated χ_r^2 is greater than the critical χ^2 value (Table B15, Appendix B), H_0 is rejected.

First, the treatment effect for the blocking variables is calculated by ranking each level of the column variable per row. For example if the column variable consisted of four levels, each row for the blocking variable would be ranked and assigned values 1, 2, 3, and 4 per row. Ties would be averages, similar to previous tests. The data is ranked separately for each row. Then the ranks associated with each column are summed (R_j) and applied to Eq. 21.21.

To illustrate this process, assume we are attempting to determine if there is any significant difference among the three formulas. To reduce intersubject variability we administer all three formulations to the same subjects (in a randomized order). The results are presented in Table 21.10. The hypothesis would be as follows:

Table 21.10 Results of Three Formulations Administered at Random to Twelve Volunteers

Subject	Formula A	Formula B	Formula C
1	125	149	126
2	128	132	126
3	131	142	117
4	119	136	119
5	130	151	140
6	121	141	121
7	129	130	126
8	133	138	136
9	135	130	135
10	123	129	127
11	120	122	122
12	125	140	141

H_0: No difference exists among the three formulations
H_1: A difference exists among the three formulations

In this case the decision rule is to reject H_0 if the calculated χ_r^2 is greater than $\chi_2^2(0.95)$, which equals 5.99 (note that n equals 12, which is large enough to use the critical value from the chi square table). The degrees of freedom for the chi square value based on $k - 1$ treatment levels. The ranking of the data is presented in Table 21.11 where the responses for each subject (block) are ranked independently of all other subjects. Finally the ranks are summed for each of the treatment levels (columns) and presented at the bottom of Table 21.11. The computation of the χ_r^2 is:

$$\chi_r^2 = \frac{12}{12(3)(4)}[(17.5)^2 + (32.5)^2 + (22)^2] - 3(12)(4)$$

$$\chi_r^2 = (0.0833)(1846.5) - 144 = 9.81$$

Therefore, with the calculated χ_r^2 greater than 5.99 we would reject H_0 and assume that there is a significant difference among formulations A, B, and C. Using the Excel function CHISQ.DIST.RT it is possible to determine the probability of a chi square statistic of 9.81 with two degrees of freedom as 0.007, well less than the acceptable Type I error of 0.05.

Spearman Rank-Order Correlation

A **rank correlation coefficient** is a special type of bivariate correlation coefficient for relating two ordinal scaled variables. The Spearman rank-order correlation (also referred to as **Spearman rho**) is an example of a rank correlation coefficient. Similar to

Table 21.11 Example of the Freidman ANOVA for Data in Table 21.10

	Formula A		Formula B		Formula C	
Subject	Data	Rank	Data	Rank	Data	Rank
1	125	1	149	3	126	2
2	128	2	132	3	126	1
3	131	2	142	3	117	1
4	119	1.5	136	3	119	1.5
5	130	1	151	3	140	2
6	121	1.5	141	3	121	1.5
7	129	2	130	3	126	1
8	133	1	138	3	136	2
9	135	2.5	130	1	135	2.5
10	123	1	129	3	127	2
11	120	1	122	2.5	122	2.5
12	125	1	140	2	141	3
$\sum$ =		17.5		32.5		22

other nonparametric tests, this procedure ranks the observations, but each variable (*x* and *y*) is ranked individually and then the difference between the two ranks becomes part of the test statistic. As seen in the following example, a table (similar to Table 21.12) is created and the sum of the differences squared is inserted into the following formula:

$$\rho = 1 - \frac{6(\sum d^2)}{n^3 - n} \qquad \text{Eq. 21.22}$$

Unlike the correlation coefficient, which is concerned with the means for both the *x* and *y* variables, here the investigator is interested in the correlation between the rankings.

To illustrate this process the previous data regarding volunteer heights and weights (Table 15.2) will once again be used. The results of the ranking process for each continuous variable are presented in Table 21.12. The computation for the Spearman *rho* is:

$$\rho = 1 - \frac{6(\sum d^2)}{n^3 - n} = 1 - \frac{6(4)}{6^3 - 6} = 1 - \frac{24}{210} = 0.886$$

A perfect positive or a perfect negative correlation will both produce a $\sum d^2 = 0$; therefore, the result will always be a positive number. Thus, this procedure does not indicate the direction of the relationship. However, because the Spearman *rho* is used for small data sets, information can be quickly plotted on graph paper and the resulting scatter plot will indicate if the correlation is positive or negative. If the two

Table 21.12 Sample Data for Spearman Correlation

	Observed		Ranked			
Subject	Wgt.	Hgt.	Wgt.	Hgt.	D	D^2
1	96.0	1.88	5	6	−1	1
2	77.7	1.80	2	3	−1	1
3	100.9	1.85	6	5	1	1
4	79.0	1.77	3	2	1	1
5	73.0	1.73	1	1	0	0
6	84.5	1.83	4	4	0	0
					$\sum d^2 =$	4

continuous variables are normally distributed, the Pearson's correlation coefficient is more powerful than the test for Spearman's rank correlation. Spearman's statistic is useful when one of the variables is not normally distributed, if ordinal scales are involved, or if the sample sizes are very small.

Kendall's Coefficient of Concordance

Kendall's coefficient of concordance is another nonparametric procedure for comparing two or more ordinal variables. Data is ranked for each variable and the strength of the agreement between variables is assessed based on a chi square distribution. The test statistic is**:**

$$W = \frac{\sum R_i^2 - \frac{(\sum R_i)^2}{n}}{\frac{M^2(n^3 - n)}{12}} \qquad \text{Eq. 21.23}$$

where M in the number of ranked variables and n is the number of observations for each variable. This coefficient can also be used to evaluation the agreement among two or more evaluators or raters (see interrated reliability in Chapter 17). **Kendall's tau** tests (Chapter 17) could also be used for rank-order correlations. However, it is felt that the Spearman correlation is a better procedure (Zar, p .398) and for a larger n, Spearman is easier to calculate. More information about these latter tests can be found in Bradley (1968).

Theil's Incomplete Method

As discussed in Chapter 14, linear regression models assume that the dependent variable is normally distributed. If the y-variable is not normally distributed, several nonparametric approaches can be used to fit a straight line through the set of data points. Possibly the simplest method is Theil's "incomplete" method.

As with most nonparametric procedures, the first step is to rank the points in ascending order for the values of x. If the number of points is odd, the middle point (the median) is deleted, thus creating an even number of data points that is required for the test. Data points are then paired based on their order (the smallest with the smallest above the median, the second smallest with second smallest above the median) until the last pairing represents the largest x-value below the median with the overall largest x-value.

For any pair of points, where $x_j > x_i$, the slope, b_{ij}, of a straight line joining the two points can be calculated as follows:

$$b_{ij} = \frac{(y_j - y_i)}{(x_j - x_i)} \qquad \text{Eq. 21.24}$$

These paired slope estimates are ranked in ascending order and the median value becomes the estimated slope of the straight line that best fits all the data points. This estimated value of b is inserted into the straight line equation ($y = a + bx$) for each data point and each corresponding intercept is calculated ($a = y - bx$) for each line. These intercepts are then arranged in ascending order and the median value is used as the best estimate of the intercept.

As an example, consider the following. During an early Phase I clinical trial of a new therapeutic agent the following AUCs (areas under the curve) were observed at different dosages of the formulation. The data is already rank ordered by the x-variable.

Dosage (mg)	AUC (hr·µg/ml)
100	1.07
300	5.82
600	15.85
900	25.18
1200	33.12

Because there are an odd number of measurements ($n = 5$) the median value is removed from the database:

Point	Dosage	AUC
1	100	1.07
2	300	5.82
	~~600~~	~~15.85~~
3	900	25.18
4	1200	33.12

The slopes of the two lines are then calculated by the pairings of points 1 and 3, and 2 and 4. These slopes are:

$$b_{13} = \frac{25.18 - 1.07}{900 - 100} = \frac{24.11}{800} = 0.0301$$

$$b_{24} = \frac{33.12 - 5.82}{1200 - 300} = \frac{27.3}{900} = 0.0303$$

The median slope (b) is the average of the two slopes (0.0302). This measure is then placed in the formula for a straight line and the intercept is calculated for all three pairings.

$$a = y - bx$$

$$a_1 = 1.07 - (0.0302)(100) = -1.95$$

$$a_2 = 5.82 - (0.0302)(300) = -3.24$$

$$a_3 = 25.18 - (0.0302)(900) = -2.00$$

$$a_4 = 33.12 - (0.0302)(1200) = -3.12$$

The new intercept is the median for these four calculations, which is the average of the third and fourth ranked values:

$$Median\ \ intercept\ (a) = \frac{(-2.00) + (-3.12)}{2} = -2.56$$

These results are slightly different from the slope (0.0299) and intercept (–2.33) if calculated using a traditional linear regression model.

Theil's method offers three advantages over traditional regression analysis: 1) it does not assume that errors are solely in the y-direction; 2) it does not assume that the populations for either the x- or y-variables are normally distributed; and 3) it is not affected by extreme values (outliers). With respect to the last point, in the traditional least-squares calculation, an outlier might carry more weight than the other points and this is avoided with Theil's incomplete method.

Kolmogorov-Smirnov Goodness-of-Fit Test

The Kolmogorov-Smirnov test (**K-S test**) is a nonparametric alternative to the chi square goodness-of-fit test (Chapter 16) when smaller sample sizes are involved. The test is named for two Russian mathematicians Andrei Nikolaevich Kolmogorov and N.V. Smirnov (Salsburg, pp. 163-164). These individuals created two similar tests during the 1930s. Smirnov's work focused on the two-sample case to determine if the distributions for two samples were taken from the same population. This is sometimes referred to as the **Kolmogorov-Smirnov two-sample test.** A.N. Kolmogorov's work

addressed the one-sample case and the determination if the sample data was distributed according to the expectations of the population distribution.

The test is referred to as the **Kolmogorov-Smirnov one-sample test** or **Kolmogorov goodness-of-fit test.** This one-sample K-S test can be used to decide if the distribution of sample data comes from a population with a specific distribution (i.e., normal or skewed distribution). Two cumulative distribution functions are compared to determine if there is a significant difference between them. Distribution functions are compared for given values of x on both distributions. The first is the distribution of interest where the probability of a random value being equal to or less than x is defined as $F_0(x)$. Sample data are collected and this second observed or **empirical distribution function**, $S(x)$, is the best estimate of the distribution $F(x)$ from which the sample was taken. The magnitude of the difference between these two functions is used to determine where H_0 should be rejected. The hypotheses for these would be:

H_0: $F(x) = F_0(x)$ for all values of x
(the data follow a specified distribution)
H_1: $F(x) \neq F_0(x)$ for at least one value of x
(the data do not follow the specified distribution)

The sample distribution function $S(x)$, being the best estimate of $F(x)$, is used to determine the cumulative probability function for any given value of x:

$$S(x) = \frac{number\ of\ sample\ observations \leq x}{n} \qquad \text{Eq. 21.25}$$

Similarly, the probabilities $F_0(x)$ are calculated for the same points (x) of the proposed distribution to which the sample is being compared. For a two-sided test, the test statistic is:

$$D = sup\left|S(x) - F_0(x)\right| \qquad \text{Eq. 21.26}$$

Where *sup* is the supremum value or that point where the absolute difference is greatest. If the two distributions are presented graphically, D is the greatest vertical difference between $S(x)$ and $F_0(x)$. The D-value is compared to the critical values presented in Table B18 in Appendix B. If D exceeds the critical value based on the number of sample observations, there is a significant difference between the two distributions and H_0 is rejected.

As an example, ten samples are taken from the batch or a particular pharmaceutical product and assayed. The results, reported as percent label claim, are 101, 93, 97, 100, 109,102, 100, 99, 91, and 105 percent. Based on historical data it is assumed that a batch of this product is normally distributed with a mean of 100% label claim and a standard deviation of 6%. Does the sample come from our expected population (have distribution characteristics of a batch of this drug)? If both the population batch and sample are expected to be normally distributed we can use Eq. 6.3 to estimate the z-value for any percent label claim and use Table B2 in Appendix

Table 21.13 Comparison of Sample Data to Expected Population Distribution

% LC	*z*-value	$F_0(x)$ $p \le$ %LC	$S(x)$ $P \le$ %LC	$\lvert S(x) - F_0(x) \rvert$
90	−1.667	0.048		
91	−1.500	0.067	0.100	0.033
92	−1.333	0.091		
93	−1.167	0.122	0.200	0.078
94	−1.000	0.159		
95	–0.833	0.202		
96	–0.667	0.252		
97	–0.500	0.309	0.300	0.009
98	–0.333	0.369		
99	–0.167	0.434	0.400	0.034
100	0.000	0.500	0.600	0.100
101	0.167	0.566	0.700	0.134
102	0.333	0.631	0.800	0.169
103	0.500	0.691		
104	0.667	0.748		
105	0.833	0.798	0.900	0.102
106	1.000	0.841		
107	1.167	0.878		
108	1.333	0.909		
109	1.500	0.933	1.000	0.067
110	1.667	0.952		

B to determine the proportion of the results that should be below or equal to that point on the curve. The *S(x)* is calculated using Eq. 21.25 for the results of the ten samples. These results are presented in Table 21.13. In this example the largest difference (the supremum) is:

$$D = sup\left|0.800 - 0.631\right| = 0.169$$

Using Table B18, *D* is less than the critical value of 0.409; therefore, we fail to reject the null hypothesis and assume our sample data comes from the same distribution we would expect for our batch of drug. This result can be graphically represented as seen in Figure 21.1.

The previous example assumed a normal distribution. One advantage of the K-S test is that it does not depend on the underlying cumulative distribution function being tested. The chi square goodness-of-fit test depends on an adequate sample size for the approximations to be valid and the K-S test does not. Often the K-S test is preferred to the chi square for interval data and may be more powerful. However, despite these advantages, the test has limitations: 1) it applies to continuous distributions only, including ordinal scales; 2) it appears to be more sensitive near the center of the

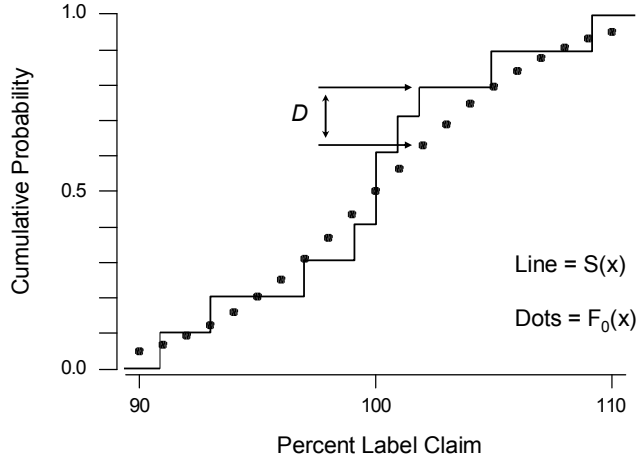


Figure 21.1 Illustration of a comparison between sample and predicted population distributions.

distribution and not at the tails; and 4) the entire distribution must be specified. The last limitation is the most serious and if the location, scale, and shape of the distribution are estimated based on the data, the critical region of the K-S test may no longer be valid. Due to this and less sensitivity at the tails of the distribution, some statisticians prefer using the Anderson-Darling test, which is discussed below.

As mentioned, the **Kolmogorov-Smirnov two-sample test** is available to test if two sample sets or two levels of a discrete independent variable come from the same distribution (same population). This test is based on the previously mentioned work of Smirnov. In this test we have two independent samples from ordinal or higher scales and we compare two empirical distributions, $F_1(x)$ and $F_2(x)$ and the hypotheses are:

H_0: $F_1(x) = F_2(x)$ for all values of x
H_1: $F_1(x) \neq F_2(x)$ for at least one value of x

The best estimates for the population distributions are the sample results, $S_1(x)$ and $S_2(x)$. Calculated similarly to the one-sample case, each $S(x)$ is based on the number of observations at a given point and the probability of being equal to or less than that value.

As an example, consider the Mann-Whitney test example previously presented comparing a control group to animals receiving atropine and the amount of ACH released (Table 21.5). In this particular example there are seven observations in each level for a total of 14 results. The data are presented in Table 21.14 where the fractions of results being equal to or below each given point are expressed for each level of the independent variable and the differences between these fractions are presented in the last column. Once again, the supremum (or largest difference) is identified and compared to the critical values on Table B19.

Table 21.14 Comparison of Two Levels of an Independent Variable Using the Kolmogorov-Smirnov Test

Control	Experimental	$p \le F_1(x)$	$P \le F_2(x)$	$\lvert F_1(x) - F_2(x) \rvert$
0.6067		1/7	0	1/7
0.7184		2/7	0	2/7
0.7974		3/7	0	3/7
0.8762		4/7	0	4/7
1.0422		5/7	0	5/7
	1.0475	5/7	1/7	4/7
	1.0632	5/7	2/7	3/7
	1.0990	5/7	3/7	2/7
1.1268		6/7	3/7	3/7
1.3590		7/7	3/7	4/7
	1.4411	7/7	4/7	3/7
	1.6022	7/7	5/7	2/7
	1.7695	7/7	6/7	1/7
	2.7831	7/7	7/7	0

$$D = sup\left|F_1(x) - F_2(x)\right| \qquad \text{Eq. 21.27}$$

In this case the D is 5/7 and equals the critical value of 5/7 on Table B19. Since D does not exceed the critical value, we would fail to reject the null hypotheses and, unlike the Mann-Whitney results, assume the two distributions are the same.

Anderson-Darling Test

The Anderson-Darling test is a modification of the Kolmogorov-Smirnov test to determine if sample data came from a population with a specific distribution. Where Kolmogorov-Smirnov is distribution-free, the Anderson-Darling test makes use of the specific distribution in calculating critical values and is a more sensitive test. The Anderson-Darling test can be used as an alternative to the either the chi square or Kolmogorov-Smirnov goodness-of-fit tests. The critical values for the Anderson-Darling test are dependent on the specific distribution that is being tested (e.g., normal, lognormal, or logistic distributions). The statistic for the Anderson-Darling is:

$$A_n^2 = -n - n^{-1}\sum(2i-1)\left[ln(P_i) + ln(1 - P_{n+1-i})\right] \qquad \text{Eq. 21.28}$$

where, in the case of a normal distribution, P_i is the probability that the standard normal distribution is less than $(x_i - \overline{X}/s)$. Even though it is possible to calculate the Anderson-Darling statistic, it is more convenient to use computer software designed to do the calculation. To interpret the results, the larger the A_n^2, the less likely the data comes from a normally distributed population.

Runs Tests

A runs test can be used to evaluate the randomness of sample data. As indicated in Chapter 8 random sampling is a requirement for all inferential statistics. If a sample fails the runs test, it indicates that there are unusual, non-random periods in the order with which the sample was collected. It is used in studies where measurements are made according to some well defined sequence (either in time or space). A **run** is defined as a sequence of identical events that is preceded and followed by an event of a different type, or by nothing at all (in a sequence of events this latter condition would apply to the first and last event). There are two different types of runs: 1) for continuous data a run refers to the values in a consecutively increasing or decreasing order; or 2) in the case of dichotomous results a run refers to consecutive data points with the same value. In the former case the test addresses whether the average value of the measurement is different at different points in the sequence and a run is defined dichotomously as a series of increasing values or decreasing values. The number of increasing values or decreasing values is defined as the length of the run. If the data set is random, the probability that the $(n + 1)$th value is larger or smaller than the nth value will follow a binomial distribution.

As an example of a dichotomous outcome and to illustrate defining a run as the number of consecutive identical results, we could record the results for a series of coin tosses. A run would be consecutive heads or consecutive tails. Assume the result of 20 tosses is as follows:

HHTTTHTHHHHTTHTTTHTH

In this case, the first run is two heads, followed by a second run of three tails, followed by a run of one head, etc. Using spacing we can see that our 20 tosses represent 11 runs:

HH TTT H T HHHH TT H TTT H T H

The statistical test will be a determination if the outcome of 11 runs is acceptable for a random set of data or if 11 runs are too few or too many runs for a random process. For a second example, assume ten volunteers in a clinical trial are assigned to either a control or experimental group. We would hope that the assignment is at random; however, if there are only two runs based on the sequence within which the volunteers were enrolled (CCCCC and EEEEE) one must question the randomization process since the number of runs is so small. Similarly randomization would be questionable if there were 10 runs (C E C E C E C E C E). Both scenarios appear to involve systematic assignment patterns, not random assignment.

Such runs tests could be used in quality control procedures (Chapter 7) where the sequential results are recorded as above or below the target value or in regression analysis (Chapter 14) where the residuals about the regression line would be expected to be above or below the line-of-best fit at random. In the latter case too few or too many runs might indicate that the relationship is not linear.

A **one-sample runs** test is illustrated by the previous coin-tossing experiment i.e., where we are considering whether a sequence of events is the result of a random process. In this case the hypotheses are:

H_0: The pattern of occurrence is determined by a random process
H_1: The pattern of occurrences is not random

To test the null hypothesis, observations are recorded for two mutually exclusive outcomes; N is the total sample size, n_1 is the number of observations for the first type, and n_2 is the number of observations for the second type. The test statistic is r, the total number of runs. Using our previous example of coin tosses the results would be: $N = 20$, $n_1 = 10$ (heads), $n_2 = 10$ (tails) and $r = 11$. There are several formulas for runs test in the literature; we will use the simple approach of referring to a table of critical values for the number of runs, based on the sample size. This table developed by Swed and Eisenbar at the University of Wisconsin is presented as Table B20 in Appendix B. Using this table, if the number of runs exceeds the number in the fourth column in each section or is less than the number in the third column in each section, the H_0 is rejected. Note that this is a two-tailed test and that modifications can be made to test for one-tailed tests (Daniel, 1978, pp. 54,55).

When either n_1 or n_2 exceeds 20 observations the following formula can be used for large samples:

$$z = \frac{r - \left(\frac{2n_1 n_2}{n_1 + n_2} + 1 \right)}{\sqrt{\frac{2n_1 n_2 (2n_1 n_2 - n_1 - n_2)}{(n_1 + n_2)^2 (n_1 + n_2 - 1)}}} \qquad \text{Eq. 21.29}$$

The distribution approximates the standard normal distribution in Table B2 in Appendix B, and interpretation can be made by rejecting the H_0 if the absolute z-value exceeds the critical value of $z_{1-\alpha/2}$.

For the **one-sample runs test for continuous data** for ordinal or higher order data, a run is a set of consecutive observations that are all either less than or greater than a specified value (e.g., mean, median). It involves no assumptions regarding the population distribution parameters. The runs test can be used to determine whether or not the data order as it was collected is random and therefore requires no assumptions about order. This test can be used to determine if the order of responses above or below a specified value, is random. For example, consider 30 sequential results on a five-point Likert scale, the mean of which is 3.87. Each time there is a switch between above or below scores there is a new run, in this case 12 runs.

(55) (3) (54) (23) (44455) (2233) (4554) (2) (4) (33) (55545) (3)

Minitab and other computer programs will generate the expected number of runs under complete randomness and determine if the difference between the observed and expected number of runs is significant.

The **Wald-Wolfowitz runs test** is a nonparametric procedure to test that two samples come from the same population (similar to the Mann-Whitney U test) with the same distribution. The test evaluates the number of runs to determine if the samples come from identical populations:

H_0: The two samples come from identically distributed populations
H_1: The two samples are not from identically distributed populations

If there are too few runs (runs in this case being consecutive observations from the same level) it suggests that the two samples come from different populations. It is assumed that the samples are independent and the dependent variable is measured on a continuous scale.

Once again the test statistic is *r* for the number of runs presented in both levels of the independent variable. The observations from the two samples (or levels of an independent variable) are ranked from smallest to largest regardless of level. However, it is important to keep track which level the sample represents. For this particular test we will use *A* to denote the first level and *B* for the second. As an example, let us use the data already used for the Mann-Whitney test and presented in Table 21.5. In this table the ranking has already been performed, but here we will count the runs associated with the control (*A*) and atropine (*B*) groups:

0.6067	0.7184	0.7974	0.8762	1.0422	1.0475	1.0632
A	A	A	A	A	B	B
1.0990	1.1268	1.3590	1.4411	1.6022	1.7695	2.7831
B	A	A	B	B	B	B

In this case the $r = 4$ runs (AAAAA BBB AA BBBB) and $n_1 = 7$ and $n_2 = 7$. Using Table B20 in Appendix B, it would require fewer than 4 or more than 12 runs to reject the null hypothesis that the two samples came from identical populations. Once again the values in Table B20 represent a two-tailed test.

Unfortunately, runs tests have very little power (Conover, 1999) and in both one-sample and two-sample cases can be replaced by more powerful nonparametric procedures, the K-S goodness-of-fit and Mann-Whitney test.

Range Tests

Although not really considered nonparametric tests, there are several quick and useful tests that can be performed using the range(s) for experimental data. In previous chapters, the standard deviation has been used as the most common measure of dispersion. For these procedures the **whole range** (*w*) of the sample is used (the difference between the largest and smallest observation). They are not considered nonparametric, because the sample means are involved in the calculations; therefore, the populations from which the samples are taken are assumed to be normally distributed.

The first test is a simple **range test**, which can be used instead of a one-sample t-test. In this case the results of a sample are evaluated to determine if it comes from a

given population.

$$H_0: \ \overline{X} = \mu_0$$
$$H_1: \ \overline{X} \neq \mu_0$$

As with previous tests, a ratio is established with the numerator representing the difference between the observed (sample mean, $\overline{X}$) and the expected (hypothesized population mean, μ_0). The denominator represents a measure of dispersion (the range, w).

$$T_I = \frac{|\overline{X} - \mu_0|}{w} \qquad \text{Eq. 21.30}$$

The calculated T_I is then compared to a critical value presented in Table B21 in Appendix B. If the calculated T_I is greater than the critical table value, H_O is rejected and a significant difference is assumed to exist between the sample and hypothesized population. As an example, assume that a dissolution test for a specific drug, under specific conditions (media, equipment, and paddle speed) is expected to be 75% at ten minutes. During one test the following values were observed: 73, 69, 73, 73, 67, and 76%. With the resultant sample mean of 71.8 and range of 9 (76 – 67). Do these results vary significantly from the expected dissolution result (μ_0) of 75%?

$$T_I = \frac{|71.8 - 75|}{9} = \frac{3.2}{9} = 0.356$$

The calculated T_I of 0.356 does not exceed the critical T_I-value of 0.399, therefore the null hypothesis cannot be rejected.

Similar to the one-sample t-test, a confidence interval can also be constructed using the same information and the critical value from Table B21, Appendix B.

$$\mu_0 = \overline{X} \pm T_{cv,n}(w) \qquad \text{Eq. 21.31}$$

This interval is equivalent to that previously described as Eq. 7.4:

$$\begin{matrix} \textit{Population} \\ \textit{mean} \end{matrix} = \begin{matrix} \textit{Estimated} \\ \textit{Sample mean} \end{matrix} \pm \begin{matrix} \textit{Reliability} \\ \textit{Coefficient} \end{matrix} \times \begin{matrix} \textit{Standard} \\ \textit{Error} \end{matrix}$$

Using the same dissolution sample data, we can create a confidence interval for the population from which our six tablets were sampled at 10 minutes:

$$\mu_0 = 71.8 \pm 0.399(9) = 71.8 \pm 3.59$$

$$68.21\% < \mu_0 < 75.39\%$$

The expected population value of 75% falls within the interval and produces the same result: failure to reject the null hypothesis.

A second range test, the **Lord's range test**, can be used as a parallel to the two-sample t-test. Here two sample means are compared to determine if they are equal:

$$H_0: \mu_A = \mu_B$$
$$H_1: \mu_A \neq \mu_B$$

Similar to the two-sample t-test a ratio is established with the difference between the means in the numerator and the degree of dispersion controlled in the denominator. In this case we substitute w_1 and w_2 for S_1 and S_2:

$$L = \frac{|\bar{X}_1 - \bar{X}_2|}{\frac{(w_1 + w_2)}{2}} \qquad \text{Eq. 21.32}$$

Here the calculated L-value is compared to the critical T_l-value in Table B21 in Appendix B. If the resultant value is greater than the critical value the null hypothesis is rejected. In this case it is also assumed that the dispersions are similar for the two samples.

To illustrate this, consider Problem 4 at the end of Chapter 9. Samples are taken from a specific batch of drug and randomly divided into two groups of tablets. One group is assayed by the manufacturer's own quality control laboratories. The second group of tablets is sent to a contract laboratory for identical analysis. Is there a significant difference between the results generated by the two labs? The means for manufacturer's lab and contact lab were 99.83 and 98.95, respectively. The range of observations for the manufacturer's data is 2.4 (101.1 − 98.7) and the contract lab range is 3.6 (101.1 − 97.5). Note first that the dispersions are fairly similar: 2.4 versus 3.6 and the sample sizes are equal $n_m = n_{cl}$. The critical value from Table B21 for n = 6 at $\alpha = 0.05$ is 0.399. The calculation of Lord's range test is as follows:

$$L = \frac{|99.83 - 98.95|}{(2.4 + 3.6)/2} = \frac{0.88}{3} = 0.293$$

The resultant 0.293 does not exceed the critical value of 0.399; therefore, we fail to reject H_O of equality and assume that the results are similar for both laboratories. These are the same results found in the answer to this problem at the end of Chapter 9.

Another quick test using the **ratio of ranges** is associated with a test for the homogeneity of variance. This can be used to replace the F_{max} or Cochran C test, discussed in Chapter 10, for comparisons of the spreads of two sets of data. For the range test F_R is computed using the following formula:

$$F_R = \frac{w_1}{w_2} \text{ or } \frac{w_2}{w_1} \qquad \text{Equ. 21.33}$$

whichever ratio is greater than 1 is compared to the critical value in Table B21, Appendix B. The sample size should be equal for both samples and if the computer F_R-value is greater than the critical table value then the hypothesis of equal dispersions is rejected. Using the previous example of the contract laboratory, we can test to see if ranges 2.4 and 3.6 represent similar dispersions.

$$F_R = \frac{w_2}{w_1} = \frac{3.6}{2.4} = 1.5$$

With n = 6 associated with both the numerator and denominator ranges, the critical value from Table B21 is 2.8 for a two-tailed test. Because the F_R is less than 2.8 we fail to reject the hypothesis of equal dispersion.

The last use of a range test is involved with outlier tests. This is discussed under the Dixon Q test in Chapter 23.

Nonparametric Tests using Minitab®

Several nonparametric tests are available with Minitab and are initiated from the Stats command on the initial menu bar. The first two options are for the one-sample sign tests and one-sample Wilcoxon rank test which compare sample data to a hypothetical population median and can create confidence intervals when there is only one level for an independent variable. The one-sample sign test involves the following:

Stat ➤ Nonparametrics ➤ 1-Sample Sign…

Options in the menu (Figure 21.2) allow you to create a confidence interval with corresponding point estimates (choose "Confidence interval" and set the level, with the default at 95%) or perform the one-sample sign test of the median (choose "Test median", set the hypothesized median in the box to the right and choose one-tailed or two-tailed test in the dropdown box below the hypothesized median)**.** Results for the data presented in Table 21.2 appear in Figure 21.3. The first results are for the sign test of the median (two-tailed). The second results represent the 95% confidence interval. For the first sign tests results, the reported median and confidence interval are based on all twelve data points. As noted in Figure 21.3, the output provides three CIs. What we are interested in is the center values, the NLI output (non-linear interpolation). In this example, the median is 97.3 with boundaries of the confidence interval at 97.6 and 99.8. The hypothesized median of 100% does not fall within these limits; therefore, we find the opposite results and would reject the null hypothesis with Type I error of 0.05.

The one-sample Wilcoxon test can be initiated using the following:

Stat ➤ Nonparametrics ➤ 1-Sample Wilcoxon…

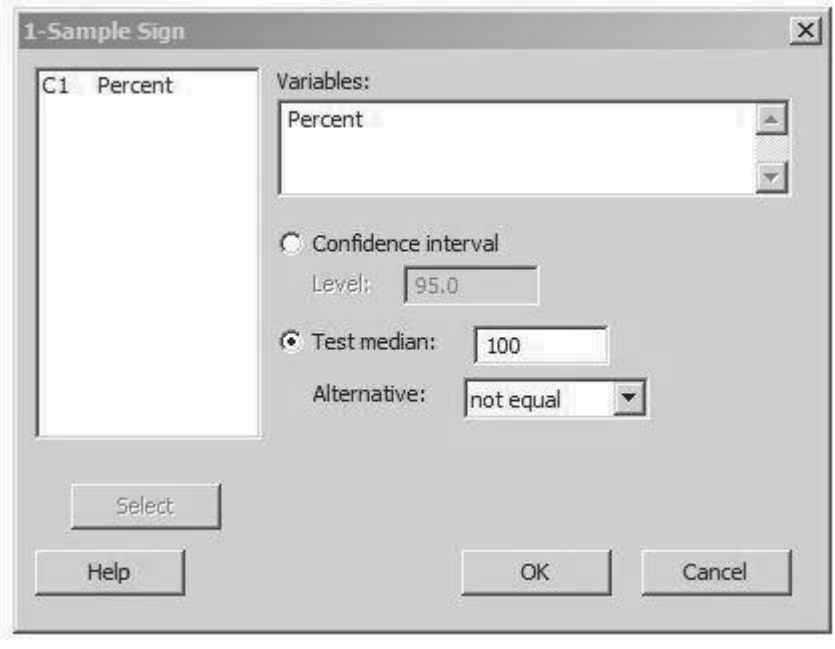


Figure 21.2 Options for one-sample sign test with Minitab.

Sign CI: Percent

```
Sign confidence interval for median

                                 Confidence
                      Achieved     Interval
          N  Median  Confidence  Lower  Upper  Position
Percent  12   97.25      0.8540  94.70  99.30         4
                        0.9500  94.55  99.52       NLI
                        0.9614  94.50  99.60         3
```

Sign Test for Median: Percent

```
Sign test of median =  100.0 versus not = 100.0

          N  Below  Equal  Above       P  Median
Percent  12     10      1      1  0.0117   97.25
```

Figure 21.3 Output for one-sample sign test with Minitab.

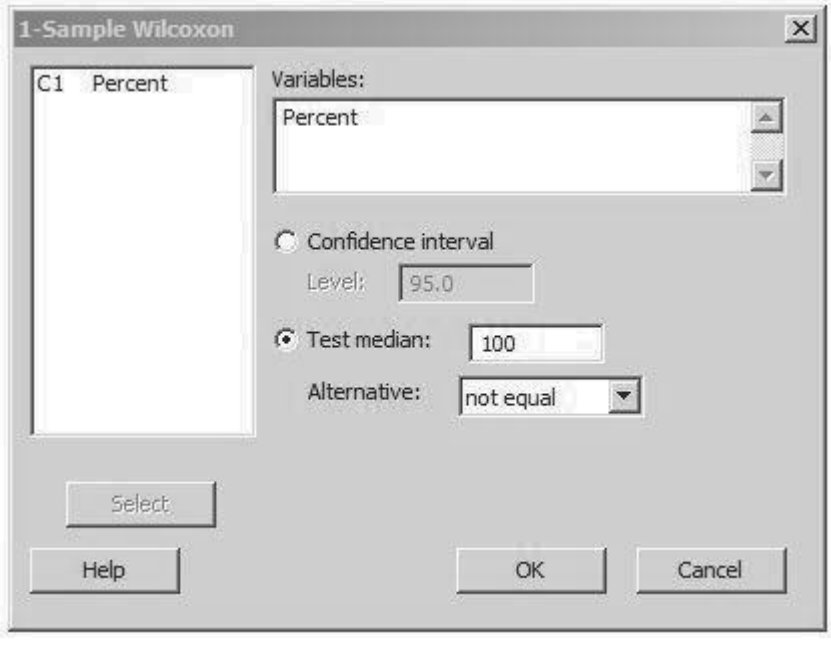


Figure 21.4 Options for one-sample Wilcoxon test with Minitab.

Wilcoxon Signed Rank Test: Percent

```
Test of median = 100.0 versus median not = 100.0

           N for   Wilcoxon          Estimated
         N   Test  Statistic      P     Median
Percent  12     11        5.0  0.014      97.03
```

Wilcoxon Signed Rank CI: Percent

```
                                    Confidence
           Estimated    Achieved      Interval
         N    Median  Confidence  Lower  Upper
Percent  12     97.03        94.5  95.05  98.95
```

Figure 21.5 Output for one-sample Wilcoxon test with Minitab.

The options menu (Figure 21.4) is identical to the one-sample sign test and the output looks similar (Figure 21.5).The input options allow you to create a confidence interval

Wilcoxon Signed Rank Test: Delta

Test of median = 0.000000 versus median not = 0.000000

	N	N for Test	Wilcoxon Statistic	P	Estimated Median
Delta	10	8	30.0	0.107	2.000

Figure 21.6 Output for Wilcoxon matched-pairs test with Minitab.

with corresponding point estimates by choosing the "Confidence interval" or perform the one-sample Wilxocon test by choosing the "Test median" and setting the hypothesized median in the box to the right and using the dropdown menu to choose a one-tailed or two-tailed test. The reported median is based on Walsh averages and the confidence intervals report data points closest to the requested levels.

The one-sample Wilcoxon test can also be used for paired data to calculate the Wilcoxon matched-pairs test. This involves some minor numerical manipulation using Calc ➤ Calculator; where the "Store results in variable:" will become the difference column. This is created in the "Expression:" box by simply subtracting one column variable from another. Using Table 21.6 as an example, the "Expression:" for column "Delta" is "after – before". The one-sample Wilcoxon test is then performed on the newly created difference column (Delta). An example of the results is presented in Figure 21.6 where the hypothesized median was zero difference, the Wilcoxon statistic is the sum of the positive signs and is an estimated *p*-value is reported.

For dealing with data on two levels of the independent variable there is the Mann-Whitney test:

Stat ➤ Nonparametrics ➤ Mann-Whitney…

Unlike previous tests, where each column is a variable, the options menu (Figure 21.7) requires data to be arranged in two columns. Each column represents one level of the independent variable ("First Sample" and "Second Sample") and the samples do not need to be the same lengths (or sizes). This is similar to data arrangement with Excel for the two-sample t-test. The default level of confidence is 95%, but can be changed and the dropdown menu allows for one-tailed or two-tailed testing. Minitab will calculate a confidence interval that is closest to the requested level of confidence. In the example output reported for data from Table 21.5 (Figure 21.8) the sample sizes and medians for both levels of the independent variable. Minitab reports a point estimate *eta* and a confidence interval. Sometimes symbol for the median is the Greek letter *eta* (η), and the *ETA* term is used in Minitab reports. The point estimate and confidence interval are calculated by a program algorithm. The point estimate is near the difference between the two medians (–0.5600). As discussed previously, if zero falls within the interval there is no significant difference between the populations. If the confidence interval does not include zero then the null hypothesis is rejected. The W is the sum for the ranks for the first median reported and the p-value is close to the

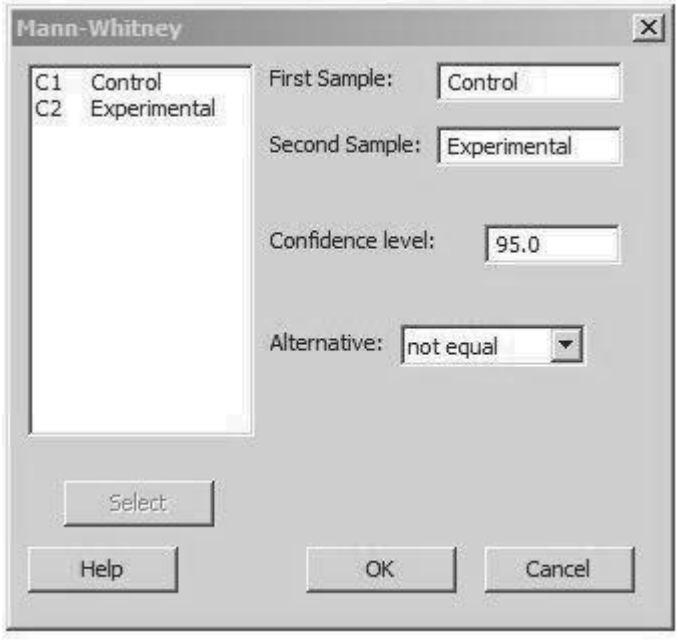


Figure 21.7 Option menu for Mann-Whitney in Minitab.

Mann-Whitney Test and CI: Control, Experimental

	N	Median
Control	7	0.8762
Experimental	7	1.4411

```
Point estimate for ETA1-ETA2 is -0.4565
95.9 Percent CI for ETA1-ETA2 is (-1.0509,-0.0569)
W = 34.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.0215
```

Figure 21.8 Output report for Mann-Whitney in Minitab.

one calculated previously (0.0182). If there are ties in the rankings, Minitab will make a correction similar to the one illustrated for the Wilcoxon test and report the adjusted *p*-value below the unadjusted results.

For the parallel to the one-way ANOVA, Minitab has available the Kruskal-Wallis test:

Stat ➤ Nonparametrics ➤ Kruskal-Wallis…

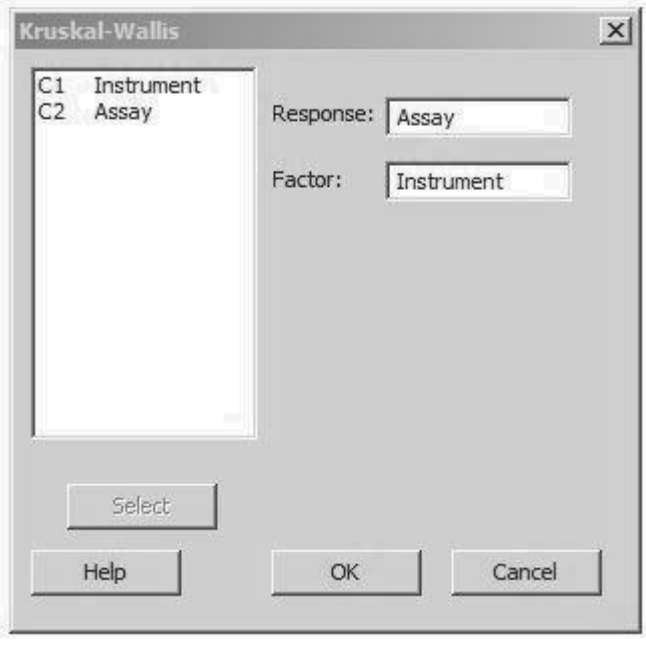


Figure 21.9 Option menu for Kruskal-Wallis in Minitab.

Kruskal-Wallis Test: Assay versus Instrument

Kruskal-Wallis Test on Assay

Instrument	N	Median	Ave Rank	Z
A	8	12.05	8.5	-1.45
B	6	13.04	16.8	2.72
C	7	12.20	8.9	-1.12
Overall	21		11.0	

H = 7.44 DF = 2 P = 0.024

Figure 21.10 Output report for Kruskal-Wallis in Minitab.

The input requires only the "Response:" which is the dependent variable and the "Factor:" identified as the independent variable (Figure 21.9). The output for Table 21.8 is presented in Figure 21.10, for each level of the independent variable and reports the median, the average rank and an associated *z*-value. Most important is the reporting of the Kruskal-Wallis *H*-statistic and associated *p*-value at the bottom. If there were tied rankings, the output report would include one additional line at the end with adjustments in *H*-statistic and *p*-value with the notation "(adjusted for ties)".

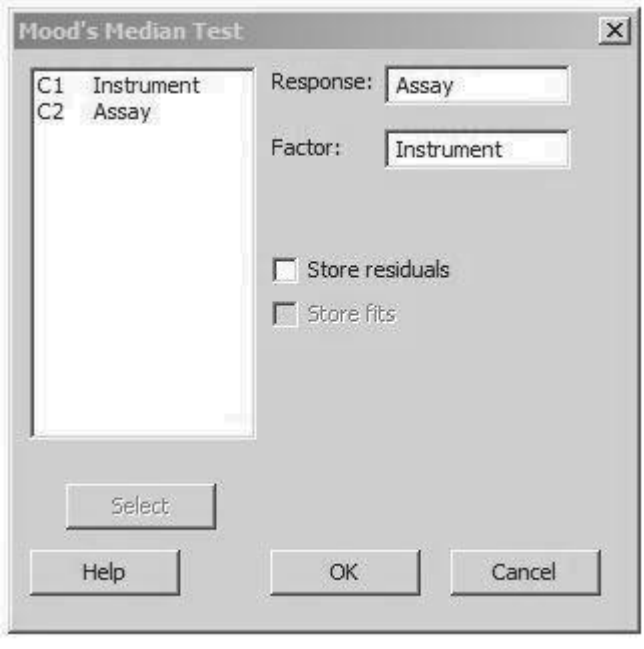


Figure 21.11 Option menu for Mood's median test in Minitab.

Mood Median Test: Assay versus Instrument

```
Mood median test for Assay
Chi-Square = 4.77    DF = 2    P = 0.092

                                        Individual 95.0% CIs
Instrument  N<=  N>  Median  Q3-Q1  ---------+---------+---------+-------
A              6   2   12.05   0.46         (---*---)
B              1   5   13.04   1.28                   (---------*---------)
C              4   3   12.20   1.28  (------------*------)
                                     ---------+---------+---------+-------
                                           11.90     12.60     13.30

Overall median = 12.21
```

Figure 21.12 Output report for Mood's median test in Minitab.

Mood's median test is another procedure for evaluating continuous data when there are two or more levels to the independent variable.

Stat ➤ Nonparametrics ➤ Mood's Median Test…

As seen in Figure 21.11, the input is similar to the Kruskal-Wallis, requiring only the location of the dependent variable (Response:) The output for Table 21.9 is presented

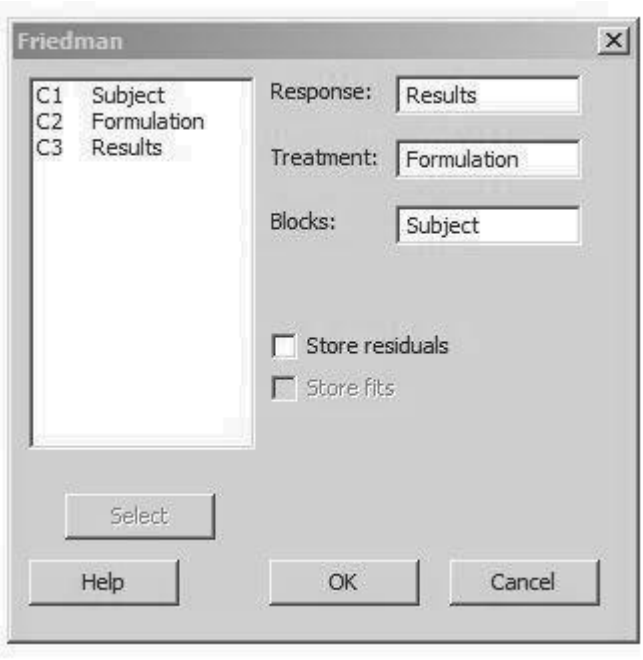


Figure 21.13 Option menu for the Friedman test in Minitab.

in Figure 21.12 for each level of the independent variable; it reports the median, number of observations less than or equal to the median, the number of observations greater than the median, the interquartile range (Q3-Q1) and a graphic of the 95% confidence interval around the median. The upper portion of the output provides the chi square statistic and associated *p*-value.

The Friedman test can be used as a nonparametric alternative to the complete randomized block design.

Stat ➤ Nonparametrics ➤ Friedman…

In the options menu the dependent variable is entered in the "Response:" variable, the independent variable as "Treatment:" and the blocking variable as "Blocks:" (Figure 21.13). The top of the report (Figure 21.14) gives both the Friedman statistics listed as *S* and the *p*-value. It also offers a correction factor for ties in the ranking lower in the report at the estimated medians and sum of ranks for each level of the independent variable.

For determining the correlation between ordinal sets of data, Minitab has a Spearman's *rho* option available:

Stat ➤ Tables ➤ Cross tabulation and Chi Square

Friedman Test: Results versus Formulation blocked by Subject

```
S = 9.88  DF = 2  P = 0.007
S = 10.77  DF = 2  P = 0.005 (adjusted for ties)

                                 Sum of
Formulation   N  Est Median      Ranks
A            12      124.75       17.5
B            12      132.25       32.5
C            12      126.25       22.0

Grand median = 127.75
```

Figure 21.14 Output report for the Friedman test in Minitab.

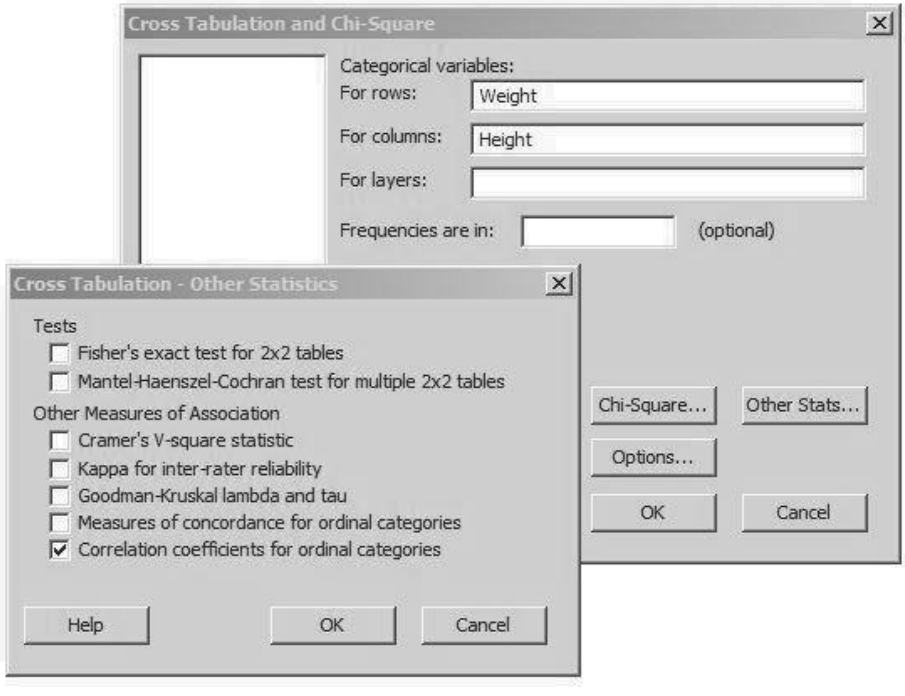


Figure 21.15 Option menus for Spearman's *rho* in Minitab.

Here one of the choices under the *Other Stats*... option is "Correlation coefficients for ordinal categories" (Figure 21.15). One dependent variable is moved to the "For rows:" variable and one to the "For columns:" variable. With "Correlation coefficient for ordinal categories" activated, the data in Table 21.12 would appear in the Minitab output in Figure 21.16 with the Spearman's *rho* result the same as that calculated by hand.

Tabulated statistics: Weight, Height

Rows: Weight Columns: Height

	1.73	1.77	1.80	1.83	1.85	1.88	All
73.0	1	0	0	0	0	0	1
77.7	0	0	1	0	0	0	1
79.0	0	1	0	0	0	0	1
84.5	0	0	0	1	0	0	1
96.0	0	0	0	0	0	1	1
100.9	0	0	0	0	1	0	1
All	1	1	1	1	1	1	6

Cell Contents: Count

Pearson's r 0.885714
Spearman's rho 0.885714

Figure 21.16 Output report for Spearman's *rho* in Minitab.

The last nonparametric test to be discussed for Minitab is the runs test:

Stat ➤ Nonparametrics ➤ Runs Test…

The options menu for this test (Figure 21.17) requests the column for the variable being tested for randomness. The default is above and below the mean, but any value could be entered as an alternative. The output is displayed in Figure 21.18 in which case the above and below the mean (labeled as K) was selected. The example was presented earlier with 30 observations creating 12 runs based on a mean of 3.87. In this program a run is consistent numbers above or below the mean (K=3.87). Based on a sample size of 30 the expected number of runs would be 14.93. The probability of observing 12 runs by chance alone is the *p*-value 0.239. Therefore we would fail to reject the null hypothesis of randomness.

References

Bradley, J.V. (1968). *Distribution-Free Statistical Tests*, Prentice-Hall, Englewood Cliffs, NJ, pp. 284-287.

Conover, W.J. (1999). *Practical Nonparametric Statistics*, Third edition, John Wiley and Sons, New York, p. 3.

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences, Eighth edition*, John Wiley and Sons, New York, pp. A-100-A-101.

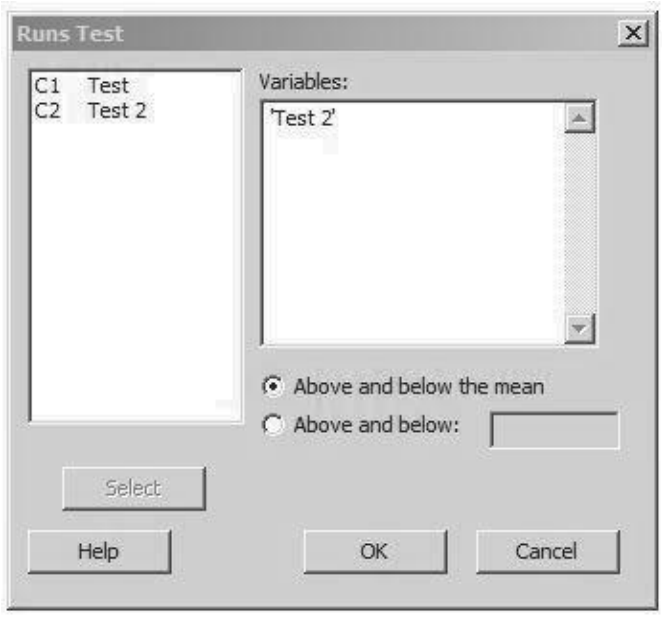


Figure 21.17 Option menus for runs test in Minitab.

Runs Test: Test 2

```
Runs test for Test 2

Runs above and below K = 3.86667

The observed number of runs = 12
The expected number of runs = 14.9333
19 observations above K, 11 below
P-value = 0.239
```

Figure 21.18 Output report for runs test in Minitab.

Daniel, W.W. (1978). *Applied Nonparametric Statistics*, Houghton Mifflin Company, Boston.

Hollander, M. and Wolfe, D.A.. (1999). *Nonparametric Statistical Methods*, 2nd edition,, John Wiley and Sons, New York, p. 28.

Salburg, D. (2001). T*he Lady Tasting Tea*, Henry Holt and Company, New York.

Zar, J.H. (2010). *Biostatistical Analysis*, Fifth edition, Prentice-Hall, Upper Saddle River, NJ, pp. 215 and 398.

Suggested Supplemental Readings

Conover, W.J. (1999). *Practical Nonparametric Statistics*, Third edition, John Wiley and Sons, New York.

Daniel, W.W. (2005). *Biostatistics: A Foundation for Analysis in the Health Sciences*, Eighth edition, John Wiley and Sons, New York, pp. 680-743.

Daniel, W.W. (2000). *Applied Nonparametric Statistics*, Second edition, Duxbury Classic Series, Pacific Grove, CA.

Sprent, P. and Smeeten, N.C. (2007). *Applied Nonparametric Statistical Methods*, Fourth edition, CRC Press, Boca Raton, FL.

Example Problems (Answers are provided in Appendix D)

Use the appropriate nonparametric test to answer all of the following questions.

1. Two groups of physical therapy patients were subjected to two different treatment regimens. At the end of the study period, patients were evaluated on specific criteria to measure percent of desired range of motion. Do the results listed below indicate a significant difference between the two therapies at the 95% confidence level? (Repeat of Problem 1, Chapter 9.)

Group 1			Group 2		
78	88	87	75	84	81
87	91	65	88	71	86
75	82	80	93	91	89
			86	79	

2. Following training on content uniformity testing, comparisons were made between the analytical results of the newly trained chemist with those of a senior chemist. Samples of four different drugs (compressed tablets) were selected from different batches and assayed by both individuals. The results are presented in Table 21.15. (Repeat of Problem 6, Chapter 9.)

3. The absorption of ultraviolet light is compared among three samples. Are there any significant differences among Samples A, B, and C (Table 21.16)?

4. Two scales are used to measure certain analytical outcome. Method A is an established test instrument, while Method B (which has been developed by the researchers) is quicker and easier to complete. Using Spearman's *rho*, is there a correlation between the two measures (Table 21.17)?

Table 21.15 Results of Content Uniformity Testing

Sample Drug, Batch	New Chemist	Senior Chemist
A,42	99.8	99.9
A,43	99.6	99.8
A,44	101.5	100.7
B,96	99.5	100.1
B,97	99.2	98.9
C,112	100.8	101.0
C,113	98.7	97.9
D,21	100.1	99.9
D,22	99.0	99.3
D,23	99.1	99.2

Table 21.16 Results from Ultraviolet Absorption

Sample A	Sample B	Sample C
7.256	7.227	7.287
7.237	7.240	7.288
7.229	7.257	7.271
7.245	7.241	7.269
7.223	7.267	7.282

Table 21.17 Results Analytical Outcomes for Two Methods

Sample	Method A	Method B
1	66	67
2	77	75
3	57	57
4	59	59
5	70	69
6	57	59
7	55	56
8	53	51
9	67	68
10	72	74

5. Six healthy male volunteers are randomly assigned to receive a single dose of an experimental anticoagulant at various dosages. Using Theil's incomplete method, define the line that best fits these six data points.

Subject	Dose (mg)	Prothrombin Time (seconds)
1	200	20
2	180	18
3	190	19
4	220	21
5	210	19
6	230	20

6. Thirty volunteers for a clinical trial are to be randomly divided into two groups of 15 subjects each. Using a random number table the assignments are presented below. Using the runs test, was the process successful?

Experimental Group			Control Group		
02	15	23	01	10	20
05	16	24	03	11	21
06	18	25	04	13	28
09	19	26	07	14	29
12	22	27	08	17	30

Numbers assigned in order of enrollment, 01 to the first volunteer and 30 to the last volunteer.

7. Repeat Problem 2, Chapter 9 (effectiveness of a bronchodilator) using an appropriate nonparametric alternative.

8. Repeat Problem 4, Chapter 9 (comparison of results between two laboratories) using an appropriate nonparametric alternative.

9. Repeat Problem 3, Chapter 10 (comparison of results from four different laboratories) using an appropriate nonparametric alternative.

10. Repeat Problem 3, Chapter 13 (comparison of two analytical methods) using the appropriate nonparametric alternative.

22

Statistical Tests for Equivalence

Up to this point, most of the statistical tests we have discussed are concerned with null hypotheses stating equality (e.g., H_0: $\mu_1 = \mu_2$). These tests were designed to identify significant differences and by rejecting the null hypothesis, prove inequality. As discussed in Chapter 8, when finding a result that is not statistically significant we do not accept the null hypothesis; we simply fail to reject it. The analogy was presented of jurisprudence where the jury will render a verdict of "not guilty," but never "innocent" if they failed to prove the accused guilty beyond a reasonable doubt. Similarly, if our data fails to show that a statistically significant difference exists, we do not prove equivalency. But what if we do want to show equality or at least similarity with a certain degree of confidence?

To address this topic several tests will be presented that are commonly used for bioequivalence testing in pharmacy along with an approach in clinical trials referred to as noninferiority studies. In the former case, if we produce a new generic product, is it the same as the originator's product? Are we producing the same product from batch to batch, or are there significant variations between batches of our drug product? In the latter case, the FDA and other agencies are asking manufacturers to prove that their new therapeutic agents are at least as good as existing agents and not inferior. The tests presented in this chapter will help answer these questions.

Bioequivalence Testing

In order for an oral or injectable product to be effective it must reach the site of action in a concentration large enough to exert its effect. Bioavailability indicates the rate and/or amount of active drug ingredient that is absorbed from the product and available at the site of action. *Remington: The Science and Practice of Pharmacy* (Malinowski, p. 995) defines bioequivalence as an indication "that a drug in two or more similar dosage forms reaches the general circulation at the same relative rate and the same relative extent." Thus, two drug products are bioequivalent if their bioavailabilities are the same and may be used interchangeably for the same therapeutic effect. In contrast to previous tests that attempted to prove differences, the objective of most bioequivalence statistics is to prove that two dosage forms are the same or at least close enough to be considered similar, beyond a reasonable doubt.

The measures of bioavailability are based upon measures of the concentration of the drug in the blood and we must assume that there is a direct relationship between the concentration of drug we detect in the blood and the concentration of the drug at the site of action. The criterion involve the evaluation of the peak plasma concentration (C_{max}), the time to reach the peak concentration (T_{max}), and/or the area under plasma concentration-time curve (AUC). The AUC measures the extent of absorption and the amount of drug that is absorbed by the body, and is the parameter most commonly evaluated in bioequivalence studies. Many excellent text books deal with the issues associated with measuring pharmacokinetic parameters: the extent of bioavailability and bioequivalence (Welling and Tse, 1995; Evans, Schentag, and Jusko, 1992; Winter, 2010). The purpose of this discussion is to focus solely on the statistical manipulation of bioequivalence data.

There are three situations requiring bioequivalence testing: a) when a proposed marketed dosage form differs significantly from that used in the major clinical trials for the product; b) when there are major changes in the manufacturing process for a marketed product; and c) when a new generic product is compared to the innovator's marketed product (Benet and Goyan, 1995). Regulatory agencies allow the assumption of safety and effectiveness if the pharmaceutical manufacturers can demonstrate bioequivalence with their product formulations.

Experimental Designs for Bioequivalence Studies

Before volunteers are recruited and the actual clinical trial is conducted, an insightful and organized study is developed by the principal investigator. As discussed in Chapter 1, the first two steps in the statistical process are to identify the questions to be answered and the hypotheses to be tested (defined in the study objectives). Then the appropriate research design is selected (to be discussed below) and the appropriate statistical tests are selected. For *in vivo* bioavailability studies, the FDA requires that the research design identifies the scientific questions to be answered, the drugs(s) and dosage form(s) to be tested, the analytical methods used to assess the outcomes of treatment, and benefit and risk considerations involving human testing (21 *Code of Federal Regulations*, 320.25(b)).

Study protocols should not only include the objectives of the study, the patient inclusion and exclusion criteria, the study design, dosing schedules, and physiological measures, but also a statistics section describing the sample size, power determinations, and the specific analyses that will be performed. These protocols are then reviewed by an institutional review board to evaluate the benefit and risk considerations for the volunteers. Two types of study designs are generally used for comparing the bioavailability parameters for drugs. Each of these designs employs statistics or modifications of statistics presented in previous chapters.

The first design is a **parallel group design**, which is illustrated in Figure 22.1. In this design, volunteers are assigned to one of two similar groups and each group receives only one treatment (either the test drug or the reference standard). In order to establish similar groups, volunteers are randomly assigned to one of the two groups using a random numbers table as discussed in Chapter 2. For example, assume that 30 healthy volunteers (15 per group) are required to compare two formulations of a particular product. Using a random numbers table, the volunteers (numbered 01 to 30)

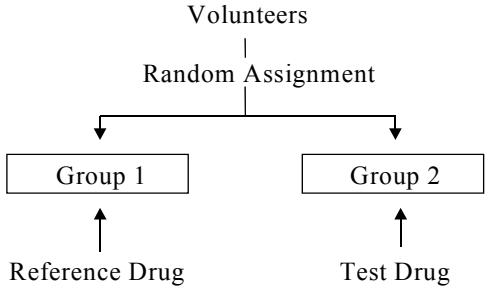


Figure 22.1 Parallel design involving two groups.

Table 22.1 Results of a Random Sample of 30 Volunteers for a Clinical Trial

Group 1			Group 2		
02	15	23	01	10	20
05	16	24	03	11	21
06	18	25	04	13	28
09	19	26	07	14	29
12	22	27	08	17	30

are assigned to one of the two groups (Table 22.1). Because of random assignment to the two treatment levels (groups), it is assumed that each set of volunteers is identical to the other (e.g., same average weight, average lean body mass, average physiological parameters). Therefore, any differences in the bioavailability measures are attributable to the drug formulation received. Results from this parallel design can be simply evaluated using a two sample t-test (Chapter 9). Also, if more than two formulations are involved, the volunteers can be randomly assigned to k treatment levels and the one-way analysis of variance can be employed (Chapter 10).

In the parallel group design each volunteer receives only one of the formulations of a drug. This design can be extremely useful for Phase II and Phase III clinical trials. It is easy to conduct and exposes volunteers to risk only once, but cannot control for intersubject variability. The design is appropriate when there is an anticipated small intersubject variability in response to the drug. To minimize patient risk, the parallel group design can be used for studies involving drugs with long elimination half-lives and potential toxicity. Also, the design can be employed with ill patients or long periods to determine therapeutic response. However, the parallel group design is not appropriate for most bioavailability or bioequivalence studies. With intersubject variability unaccounted for, this design provides a less precise method for determining bioavailability differences.

To overcome some of the disadvantages of the parallel design, a second more rigorous approach is the **crossover study design**. In this design, volunteers are once

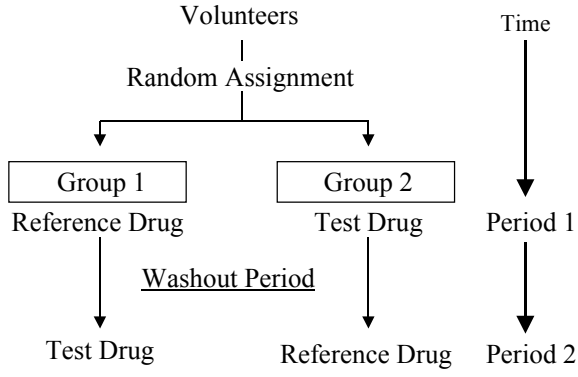


Figure 22.2 Two-period crossover design for two groups.

again randomly assigned to two groups, but each group receives all the treatments in the study. In the case of the two formulations described above, each volunteer would receive both treatments. The order in which the volunteers receive the formulations would depend on the group to which they were assigned (Figure 22.2). Using the same volunteers from our example in Table 22.1, if we employ a crossover study design, those subjects randomly assigned to Group 1 (volunteers 02, 05, 06, etc.) will first receive the reference drug (*R*). After an appropriate "washout" period, the same volunteers will receive the test drug (*T*). For those volunteers assigned to Group 2 the order of the drugs will be reversed, with the test drug first, followed by the reference standard. In this simple two-period crossover study design (referred to as a standard 2 × 2 crossover design). The subjects in Group 1 receive an *RT* sequence and those in Group 2 a *TR* sequence. Note that every volunteer will receive both the test and reference drug.

The washout mentioned above is a predetermined period of time between the two treatment periods. It is intended to prevent any carryover of effects from the first treatment to the second treatment period. In this type of design, the washout period should be long enough for the first treatment to wear off. This washout period could be based on the half-life of the drug being evaluated. After five half-lives the drug can be considered removed from the body, with approximately 96.9% of the drug eliminated. Obviously, if the washout period is not sufficiently long there is a **carryover effect** and the second bioavailability measures will not be independent of the first measurements and would violate statistical criteria. Using well designed studies it is assumed that the washout period is sufficiently long to prevent any carryover effects.

In clinical trials, individual volunteers can contribute a large amount of variability to pharmacokinetic measures. Thus the crossover design provides a method for removing intersubject variability by having individuals serve as their own controls. The FDA recommend the crossover design when evaluating pharmacokinetic parameters (21 *Code of Federal Regulations* 320.26(b) and

320.27(b)). In addition to having volunteers serving as their own controls and reducing intersubject variability, these study designs also require fewer subjects to provide the same statistical power because the same volunteers are assessed for each treatment level.

Results from the crossover design presented in Figure 22.2 could be evaluated using either the paired t-test (Chapter 9), a complete randomized block design (Chapter 10), or Latin square design (Chapter 12). If more than two formulations are involved the volunteers can be randomly assigned to k treatment levels and the complete randomized block or Latin square design can be used.

A third possible research design is a **balanced incomplete block design**. This method overcomes several disadvantages associated with the complete randomized block design used in crossover studies. When there are more than two treatment levels, the complete crossover design may not be practical since such a design would involve an extended period of time, with several washout periods and an increased likelihood of volunteers withdrawing from the study. Also, such designs involve a larger number of blood draws, which increases the risk to the volunteers. An incomplete block design is similar to a complete block design, except not all formulations are administered to each block. The design is incomplete if the number of treatments for each block is less than the total number of treatments being evaluated in the study. Each block, or volunteer, is randomly assigned to a treatment sequence and the design is "balanced" if the resulting number of subjects receiving each treatment is equal. A complete discussion of this design is presented by Kirk (1968).

Selection of the most appropriate study design (parallel, crossover, or balanced incomplete block design) depends on several factors. These include: 1) the objectives of the study; 2) the number of treatment levels being compared; 3) characteristics of the drug being evaluated; 4) availability of volunteers and anticipated withdrawals; 5) inter- and intrasubject variability; 6) duration of the study; and 7) financial resources (Chow and Liu, 2000).

Two-Sample t-Test Example

When pharmaceutical manufacturers and regulatory agencies began studying the bioequivalence of drug products, the general approach was to use a simple two-sample t-test or analysis of variance to evaluate plasma concentration-time curves (e.g., $C_{max,}$ $T_{max,}$ AUC). Since these traditional statistical tests were designed to demonstrate differences rather than similarities, they were incorrectly used to interpret the early bioequivalence studies. In the 1970s researchers began to note that traditional hypothesis tests were not appropriate for evaluating bioequivalence (Metzler, 1974).

Most of the statistical procedures involved with bioequivalence testing require that the data approximate a normality distribution. However, most of the bioavailability measures (AUC, t_{max}, and C_{max}) have a tendency to be positively skewed. Therefore, a transformation of the data may be required before analysis. The log transformation (Chapter 6) on AUC is usually performed to adjust for the skew to the distribution. This log-transformed data is then analyzed using the procedures discussed below.

Table 22.2 Data from Two Randomly Assigned Groups (AUC in ng·hr/ml)

Acme Chemical (New Product)		Innovator (Reference Standard)	
61.3	91.2	80.9	70.8
71.4	80.1	91.4	87.1
48.3	54.4	59.8	99.7
76.8	68.7	70.5	62.6
60.4	84.9	75.7	85.0
Mean =	69.75	Mean =	78.35
S =	13.76	S =	12.79

To illustrate the problems that exist when using some of our previous statistical tests, consider the example of a clinical trial comparing Acme Chemical's new generic antihypertensive agent to the innovator's original product. This would portray the third situation cited previously by Benet. We designed a very simple study to compare the two formulations of the same chemical entity, by administering them to two groups of randomly assigned volunteers. Only ten volunteers were assigned to each group. Our primary pharmacokinetic parameter of interest is the AUC (ng·hr/ml). The results of our *in vivo* tests are presented in Table 22.2.

If we use our traditional two-sample t-test as discussed in Chapter 9, the hypotheses would be:

$$H_0: \mu_1 = \mu_2$$
$$H_1: \mu_1 \neq \mu_2$$

The decision rule, based on α of 0.05 is to reject H_0 if $t > t_{18}(0.025) = +2.104$ or $t < -t_{18}(0.025) = -2.104$. The statistical analysis using Eq. 9.3 and Eq. 9.6 would be as follows:

$$S_p^2 = \frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2} = \frac{9(13.76)^2 + 9(12.79)^2}{18} = 176.46$$

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\frac{S_P^2}{n_1} + \frac{S_P^2}{n_2}}} = \frac{69.75 - 78.35}{\sqrt{\frac{176.46}{10} + \frac{176.46}{10}}} = \frac{-8.60}{5.94} = -1.45$$

The result is that we fail to reject H_0 because the *t*-value is not to the extreme of the critical value of −2.104. Therefore, with 95% confidence, we failed to prove a difference between the two formulations. However, at the same time we *did not* prove that the formulations were equal; we only failed to find a difference.

Since in most cases the sample sizes are the same, we can make the following substitution for the denominator in Eq. 9.6. However, if we do run into unequal sample sizes ($n_1 \neq n_2$) we can substitute the left side of equation for the standard error portion in any of the formulas discussed in this chapter.

$$\sqrt{\frac{S_P^2}{n_1} + \frac{S_P^2}{n_2}} = \sqrt{\frac{2S_p^2}{n}} \qquad \text{Eq. 22.1}$$

A potential problem exists with the Type II error in our statistical analysis. As discussed in Chapter 8, β is the error of failing to reject H_0 (equality) when there is a true difference between the formulations we are testing. As shown in Figure 8.5, with smaller sample sizes there is a greater likelihood of creating a Type II error. If an unethical entrepreneur wished to prove his product was equal to an innovator's drug, the easiest way to accomplish this would be to use very small sample sizes, apply traditional statistical methods, fail to reject H_0, and conclude that the two products were equivalent. To avoid such deceptions, the FDA developed guidelines to ensure adequate power in bioequivalence tests (e.g., the 80/20 rule discussed below).

Power in Bioequivalence Tests

For most bioequivalence studies, the sample size is usually 18 to 24 healthy normal volunteers. To detect a clinically important difference (20%), a power calculation is often performed prior to the study to determine the number of subjects needed to have the desired power (80%). For example, the following is a typical statement associated with a proposed protocol: "A sample size of 28 healthy males will be enrolled in this study to ensure study completion by at least 24 patients. Based on (a previous study cited) a sample size of 20 patients can provide at least 80% probability to show that the 90% confidence interval of the mean AUC value for the clinical lot of (test drug name) is within ±20% of the reference mean AUC value." Note that the investigators increased the sample size to ensure that there would be sufficient power once the data was collected. Also, more than the required number of subjects are recruited in anticipation of possible replacements for dropouts.

In the previous example we were unable to reject the null hypothesis that $\mu_1 = \mu_2$ based on 10 volunteers for each product. However, we might ask ourselves, if there was a difference between the two formulations, was our sample size large enough to detect a difference? In other words, was our statistical test powerful enough to detect a desired difference? Let us assume that we want to be able to detect a 10% difference from our reference standard ($78.35 \times 0.10 = 7.84 = \delta$). Using a formula extracted from Zar (2010), the power determination formula would be:

$$t_\beta \geq \frac{\delta}{\sqrt{\frac{2\,S_p^2}{n}}} - t_{\alpha/2} \qquad \text{Eq. 22.2}$$

where $t_{\alpha/2}$ is the critical *t*-value for $\alpha = 0.05$, n is our sample size per level of our discrete independent variable, and the resultant t_β is the *t*-value associated with our Type II error. To determine the power we will need to find the complement $(1 - \beta)$ of Type II error. Using our data we find the following:

$$t_\beta \geq \frac{7.84}{\sqrt{\frac{2(176.46)}{10}}} - 1.96$$

$$t_\beta \geq \frac{7.84}{5.89} - 1.96 = 1.32 - 1.96 = -0.64$$

If we used a full table of critical t-values instead of the abbreviated version presented in Table B3 in Appendix B (for example, *Geigy Scientific Tables*, 7th ed., Ciba-Geigy Corp., Ardsley, NY, 1974, pp. 32-35) or used an Excel function [TDIST(t_β,df,1*tailed*) in Excel 97-2003 or T.DIST.RT(t_β,df) in Excel 2010], we would find the table probability associated with t-values with 18 degrees of freedom at $p = 0.25$ for $t = -0.6884$ and $p = 0.30$ for $t = -0.5338$. Through interpolation, a calculated *t*-value of –0.64 has a probability of 0.27. Using Excel software the *p*-value would be 0.2651, or approximately 0.27. This represents the Type II error. The complement, 0.73 (1 – 0.27), is the power associated with rejecting H_0 (bioequivalence) when in truth H_0 is false.

Let us further assume that we want to have at least 80% power to be able to detect a 10% difference between our two sets of tablets. We can modify the above formula to identify the appropriate sample size:

$$n \geq \frac{2S_p^2}{\delta^2}(t_\beta + t_{\alpha/2})^2 \qquad \text{Eq. 22.3}$$

If we look at the first column of Table B3 in Appendix B, the values listed for the various degrees of freedom represent our *t*-value for a one-tailed test with $\beta = 0.20$. In this case we would interpolate the *t*-value to be 0.862 for 18 degrees of freedom. The $t(1 - \alpha/2)$ for 18 degrees of freedom is 2.10. Applied to our example:

$$n \geq \frac{2(173.46)}{(7.84)^2}(0.862 + 2.10)^2 \geq (5.64)(8.77) \geq 49.48$$

In this case, the sample size we should have used to ensure a power of at least 80%, to detect a difference as small as 10%, would have been a minimum of 50 volunteers per group.

Rules for Bioequivalence

To control the quality of bioequivalence studies the FDA has considered three possible standards: 1) the 75/75 rule; 2) the 80/20 rule; and 3) the ± 20 rule. The **75/75 rule** for bioequivalence requires that bioavailability measures for the test product be within 25% of those for the reference product (greater than 75% and less than 125%) in at least 75% of the subjects involved in the clinical trials (*Federal Register*, 1978). This rule was easy to apply and compared the relative bioavailability by individual subject, removing intersubject variability. The rule was very sensitive when the size of the sample was relatively small, but was not valuable as a scientifically based decision rule. This 1977 rule was criticized for its poor statistical nature, was never finalized, and was finally abandoned in 1980.

A more acceptable FDA criterion has focused on preventing too much Type II error and requires that manufacturer performs a retrospective assessment of the power associated with their bioequivalence studies. In any study, there must be at least an 80% power to detect a 20% difference. In other words, this **80/20 rule** states that if the null hypothesis cannot be rejected at the 95% confidence level ($1 - \alpha$), the sample size must be sufficiently large to have a power of at least 80% to detect a 20% difference to be detected between the test product and reference standard. (*Federal Register*, 1977). This 20% difference appears to have been an arbitrary selection to represent the minimum difference that can be regarded as clinically significant. Once again using the previous example, based on a pooled variance of 173.46, a desired difference of 20% (in this case 15.67 ng·hr/ml, $78.35 \times 0.20 = \delta$), a Type I error rate of 0.05, and a Type II error rate of 0.20, the required sample size would be at least 12 volunteers per group.

$$n \geq \frac{2(173.46)}{(15.67)^2}(0.862 + 2.10)^2 \geq (1.41)(8.77) \geq 12.37$$

This seems like a dramatic drop in the number of subjects required (at least 50 for a 10% difference and only 13 for a 20% difference), but it demonstrates how important it is to define the difference the researcher considers to be important (Table 22.3).

In this case, even though we have enough power to detect a significant difference we still have failed to prove that the null hypothesis is true. Alternative tests are needed to work with the data presented. Similar to the approach used in Chapter 9 presenting the t-test, we will first use a confidence interval approach and then a hypothesis testing format to prove that even if there are differences between the new product and the reference standard, that difference falls within acceptable limits.

The last measure of bioequivalence, the **±20 rule**, concerns the average bioavailability and states that the test product must be within 20% of the reference drug (between 80% and 120%). The ±20 rule appears to be most acceptable to the FDA. As will be seen in the following sections the ±20 rule can be tested by use of either a confidence interval or two one-tailed t-tests. These two methods are briefly introduced for comparisons for one test product to a reference standard. For a more in-depth discussion of these tests and more complex bioequivalence tests, readers are referred to the excellent text by Chow and Liu (2000).

Table 22.3 Sample Size Required to Detect Various Differences with 80% Power Where the Reference Standard Mean is 78.35 (Table 21.2)

Difference (%)	Minimum Sample Size
5	180
10	45
15	20
20	12
25	8
30	5

Creating Confidence Intervals

Considering the earlier example of the comparison of our new generic product to the innovator's product, we could write our hypotheses as follows, where the innovator's drug is referred to as the reference standard:

$$H_0: \mu_T = \mu_R$$
$$H_1: \mu_T \neq \mu_R$$

Here μ_T represents our new or "test" product and μ_R the "reference" or innovator's product. An alternative method for writing these hypotheses was seen in Chapter 9 when we discussed confidence intervals:

$$H_0: \mu_T - \mu_R = 0$$
$$H_1: \mu_T - \mu_R \neq 0$$

But, as discussed, we cannot prove true equality ($\delta = 0$). Rather we will establish an acceptable range and if a confidence interval falls within those limits we can conclude that any difference is not therapeutically significant. Using this method for testing bioequivalence we create a confidence interval for the population difference, $\mu_T - \mu_R$, based on our sample results, $\overline{X}_T - \overline{X}_R$. The FDA has used a 90% confidence interval ($\alpha = 0.10$). If the 90% confidence interval falls completely between 0.80 and 1.20, the two products are considered bioequivalence (an absolute difference less than 20%). With respect to a comparison of a test product to a reference standard, we want the test product to fall between 0.80 and 1.20:

$$0.80 < \mu_T - \mu_R < 1.20$$

As noted earlier in this chapter, pharmacokinetic parameters, such as C_{max} and AUC often involve log transformations before the data is analyzed to ensure a normal distribution. The general formula for such a confidence interval would be:

$$\mu_T - \mu_R = (\overline{X}_T - \overline{X}_R) \pm t_{\upsilon(1-\alpha)}\sqrt{\frac{2S_p^2}{n}} \quad \text{Eq. 22.4}$$

This is almost identical to Eq. 9.4 for the two-sample t-test. Because of formulas discussed later in this chapter, we will simplify the formula to replacing the sample difference with *d* and our standard error term with *SE*:

$$d = \overline{X}_T - \overline{X}_R \quad \text{Eq. 22.5}$$

$$SE = t_\upsilon(1-\alpha)\sqrt{\frac{2S_p^2}{n}} \quad \text{Eq. 22.6}$$

If one thinks of this problem as an ANOVA with $\nu_1 = 1$ in Chapter 10, the MS_W (mean square within) from the ANOVA table can be substituted for the S_p^2 term. Also, note that we are performing two one-tailed tests with 5% error loaded on each tail ($1 - \alpha$). Also, if the sample sizes are not equal the standard error portion of the equation can be rewritten as:

$$SE = t_\upsilon(1-\alpha)\sqrt{\frac{S_p^2}{n_1} + \frac{S_p^2}{n_2}} \quad \text{Eq. 22.7}$$

Using Eqs. 22.4 through 22.7 we can create a confidence interval based on the same units of measure as the original data (e.g., AUC in ng·hr/ml). A better approach would be to calculate confidence intervals about the observed relative bioavailability between the test product and the reference standard; converting the information into percentages of the reference standard. With the FDA's recommendation of at least 80% bioavailability in order to claim bioequivalence, the ratios of the two products are more often statistically evaluated than the differences between the AUCs.

$$80\% < \frac{\mu_T}{\mu_R} < 120\%$$

This ratio of bioavailabilities between 80 and 120% is an acceptable standard by the FDA and pharmaceutical regulatory agencies in most countries. The last step is to create a ratio between the change and the reference standard so outcomes can be expressed as percent of the reference standard:

$$Lower\ Limit = \frac{(d - SE) + \overline{X}_R}{\overline{X}_R} \times 100\% \quad \text{Eq. 22.8}$$

$$Upper\ Limit = \frac{(d + SE) + \overline{X}_R}{\overline{X}_R} \times 100\% \qquad \text{Eq. 22.9}$$

Finally the resultant confidence interval is expressed as:

$$Lower\ Limit\ <\ \frac{\mu_T}{\mu_R}\ <\ Upper\ Limit \qquad \text{Eq. 22.10}$$

What we create is a confidence interval within which we can state with 95% confidence where the true population ratio falls based on our sample.

Applying these formulas to our previous example (Table 22.2) for Acme Chemical's generic and the innovator's product, we find the following results:

$$SE = t_v(1-\alpha)\sqrt{\frac{2S_p^2}{n}} = (1.734)\sqrt{\frac{2(176.46)}{10}} = 10.30$$

$$d = \overline{X}_T - \overline{X}_R = 69.75 - 78.35 = -8.6$$

$$Upper\ Limit = \frac{(-8.6 + 10.3) + 78.35}{78.35} \times 100\% = 102.17\%$$

$$Lower\ Limit = \frac{(-8.6 - 10.3) + 78.35}{78.35} \times 100\% = 75.88\%$$

Thus, in this case, with 95% confidence, the true population ratio is between:

$$75.88\% < \frac{\mu_T}{\mu_R} < 102.17\%$$

This fails to meet the FDA requirement of falling within the 80% to 120% range. Therefore, we would conclude that the two products are not equivalent.

Comparison Using Two One-Sided t-Tests

The last method involves hypothesis testing to determine if we can satisfy the requirements for bioequivalence. As discussed previously, the absolute difference between the two products should be less than 20% of the reference standard:

$$|\mu_T - \mu_R| < 20\%\ \mu_R$$

This method, proposed by Hauck and Anderson (1984), overcomes some of the negative aspects of the previous approaches by using two one-sided t-tests to evaluate

bioequivalence. In this case we deal with two null hypotheses, which indicate outcomes outside of the acceptable differences for bioequivalence:

$$H_{01}: \quad \mu_T - \mu_R \leq -20\%$$
$$H_{02}: \quad \mu_T - \mu_R \geq +20\%$$

The two alternate hypotheses represent outcomes that fall within the extremes:

$$H_{11}: \quad \mu_T - \mu_R > -20\%$$
$$H_{12}: \quad \mu_T - \mu_R < +20\%$$

Obviously, both of the null hypothesis must be rejected in order to prove:

$$80\% < \mu_T - \mu_R < 120\%$$

The equations for these two one-tailed tests (**TOST**) involve two *thetas* that define the "equivalence interval" where $\theta_1 < \theta_2$. In other words, θ_2 is always the upper equivalence limit and θ_1 the lower limit. In the case of equivalency less than 20%, each theta represents a 20% difference in the units from which the data was collected: $\theta_2 = +20\%$ value and $\theta_1 = -20\%$ value. Schuirmann's (1987) formulas for calculating the two one-sided t-tests are:

$$t_1 = \frac{(\overline{X}_T - \overline{X}_R) - \theta_1}{\sqrt{MS_E}\ \sqrt{2/n}} \qquad \text{Eq. 22.11}$$

$$t_2 = \frac{\theta_2 - (\overline{X}_T - \overline{X}_R)}{\sqrt{MS_E}\ \sqrt{2/n}} \qquad \text{Eq. 22.12}$$

These equations test H_{01} and H_{02}, respectively. As with past tests of hypotheses, we establish a decision rule based on the sample size and a 95% confidence in our decision. Our decision rule is with $\alpha = 0.05$, reject H_{01} or H_{02} if $t > t_{df}(1 - \alpha)$. Each hypothesis is tested with a Type I error of 0.05 (α). Traditionally we have tested the hypothesis with a total $\alpha = 0.05$; in the procedure we actually use $1 - 2\alpha$ rather than $1 - \alpha$ (Westlake, 1988). This corresponds to the 90% confidence intervals discussed in the previous section.

In this case theta represents our desired detectable difference (δ) and, as discussed previously, the MS_E or MS_W for only two levels of the discrete independent variable ($\nu_1 = 1$) is the same as S_p^2. Therefore, the equations can be rewritten as follows:

$$t_1 = \frac{(\overline{X}_T - \overline{X}_R) - \delta_1}{\sqrt{\frac{2S_p^2}{n}}} \qquad \text{Eq. 22.13}$$

$$t_2 = \frac{\delta_2 - (\overline{X}_T - \overline{X}_R)}{\sqrt{\frac{2S_p^2}{n}}} \qquad \text{Eq. 22.14}$$

Using our previous example (Table 22.2) and once again assuming we wish to be able to detect a 20% difference for an innovator's product:

$$\delta = 78.35 \times 0.20 = 15.67$$

Therefore, $\delta_1 = -15.67$; $\delta_2 = +15.67$, $S_p^2 = 173.46$ and our critical value through interpolation for $t_{18}(1 - \alpha)$ is 1.73. The decision rule, with $\alpha = 0.05$, is to reject H_{01} or H_{02} if either or both *t*-values exceed 1.73.

$$t_1 = \frac{(-8.6) - (-15.67)}{\sqrt{\frac{2(176.46)}{10}}} = \frac{7.07}{5.94} = 1.20$$

$$t_2 = \frac{15.67 - (-8.6)}{\sqrt{\frac{2(176.46)}{10}}} = \frac{24.27}{5.94} = 4.09$$

In this case we were able to reject H_{02} and prove that the difference was less than 120% ($\mu_{Test} - \mu_{Reference} < +20\%$), but failed to reject H_{01}. We were not able to prove that $\mu_{Test} - \mu_{Reference}$ was greater than 80%. Therefore, similar to our confidence interval in the previous section, we are unable to show bioequivalence between Acme's generic and the innovator's reference standard.

Clinical Equivalence

Superiority and noninferiority studies are similar to equivalence studies. Based on the researcher's objectives clinical trials used to compare a new product to an already approved agent could be designed to: 1) test the equivalence of the two products (previously discussed); 2) establish the superiority of the new product; or 3) show noninferiority of the new product compared to the already approved agent. This section will focus primarily on the last type of assessment.

Superiority Studies

For completeness, a **superiority trial** is designed to evaluate the response to an investigational agent and determine if it is superior to that of a comparative product. Superiority studies are the most effective way to establish efficacy, either by showing: 1) superiority to a placebo (placebo-controlled trial); 2) superiority to an active

control; or 3) a dose-response relationship. Thus, the comparator could be either an active or placebo control. However, using a placebo control raises serious ethical questions, especially if there is an alternative effective therapy available: "In cases where an available treatment is known to prevent serious harm, such as death or irreversible morbidity in the study population, it is generally inappropriate to use a placebo control" (ICH, 1999). In most cases superiority studies could be handled as one-tailed two-sample t-tests (Chapter 9) with the hypotheses:

$$H_0: \quad \mu_T \leq \mu_C$$
$$H_1: \quad \mu_T > \mu_C$$

where μ_T is the response to the new (test) product and μ_C is the active control (or comparator agent). If there is sufficient data to reject the null hypothesis with a certain degree of confidence (e.g., $1 - \alpha = 0.95$), then the new agent is proven to be superior to the comparator. The hypotheses also can be written as a one-tailed confidence interval:

$$H_0: \quad \mu_T - \mu_C \leq 0$$
$$H_1: \quad \mu_T - \mu_C > 0$$

If a confidence interval is created using Eq. 9.4 and if all the results are positive (zero does not fall within the confidence interval), we can reject the null hypothesis and with 95% confidence conclude that the test product is superior to the control. This is illustrated in Figure 22.3 as confidence interval *A*.

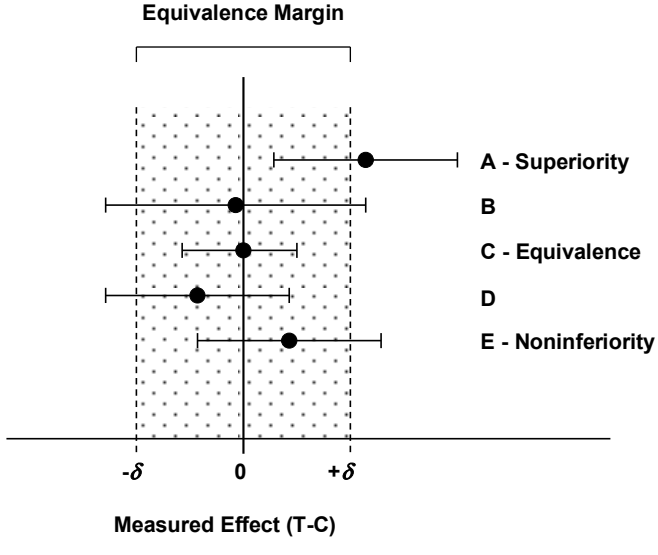


Figure 22.3 Illustrations of various confidence intervals.

Noninferiority Studies

Noninferiority is a relatively new term, dating back to the late 1970s. For a while in the 1990s the terms equivalence and noninferiority were sometimes used interchangeably and both referred to an equivalency test (Wang, 2003). As discussed in the first part of this chapter an **equivalence trial** (or bioequivalence test) is intended to show that the difference in the amount of response to two or more treatments is clinically unimportant. This difference (previously defined as the difference between sample means, Eqs. 22.9 and 22.10) represent equivalence if the results fall between the established upper and lower limits :

$$H_{01}: \quad \mu_T - \mu_C \leq -\delta$$
$$H_{02}: \quad \mu_T - \mu_C \geq +\delta$$

$$H_{11}: \quad \mu_T - \mu_C > -\delta$$
$$H_{12}: \quad \mu_T - \mu_C < +\delta$$

If both null hypotheses are rejected then the following would be proven using this approach:

$$-\delta < \mu_T - \mu_C < +\delta$$

It is virtually impossible to prove that the results from two treatments are exactly equivalent ($\delta = 0$). Therefore, as seen in the previous hypotheses, the goal was to show that the results differ by no more than a certain amount (e.g., $\delta < 10\%$). This acceptable difference is termed the **equivalence margin**. For equivalence testing, if the results from the two treatments differ by more than the equivalence margin in either direction, then the assumption of equivalence cannot be proven. The *deltas* can be thought of as the boundaries for an equivalence margin or as clinically acceptable differences. So equivalency trials are designed to show that two treatments do not differ by more than some predetermined equivalency margin (illustrated as confidence interval C in Figure 22.3). Note with confidence interval B in this same figure, both the lower and upper limits of the confidence interval extend beyond the boundaries of the equivalence margin. Another name for the area between the two boundaries is the **zone of indifference**. For equivalence testing the statistical analysis of the difference is based on the two-sided confidence interval. However, as seen previously, operationally this testing involves two simultaneous one-sided tests to test two null hypotheses that the treatment difference is outside an acceptable limit. In most cases, each hypothesis is tested with a 5% Type I error rate, with a resulting 90% confidence interval. Failure to reject either of the null hypotheses would be visually represented by one of the ends of the interval extending beyond its respective boundary.

In contrast to equivalence testing, a **noninferiority trial** is concerned only with the lower limits of the equivalency margin. A simple way to think of noninferiority trials is to view them as one-sided equivalency tests.

$$H_{01}: \quad \mu_T - \mu_C \leq -\delta$$
$$H_{11}: \quad \mu_T - \mu_C > -\delta$$

With a noninferiority trial the primary goal is to prove the alternative hypothesis that the investigational agent is not clinically inferior to the comparative agent. These studies are intended to show that the effect of a new treatment is not worse than that of an active control by more than a specified margin. A confidence interval approach can be used to test the outcome for a noninferiority trial. Similar to the previous bioequivalence studies, a single one-sided 95% confidence interval is created and if the estimated population differences between the two agents (test drug and comparator) fall entirely within the positive side of the noninferiority margin ($> -\delta$) the null hypothesis is rejected. Any improvement (a positive δ) meets the criteria of noninferiority. In Figure 22.3, confidence interval D would represent an unsuccessful test, whereas E would be a successful test of noninferiority. In many cases the established evidence of effectiveness for an experimental treatment through noninferiority studies will be a regulatory requirement for drug approval. So an important question is how large is the margin to be clinically insignificant?

Choosing the δ value is crucial. One possible approach to determine the equivalence margin is to base it on a clinical determination of what is considered a minimally important effect (Snapinn, 2000). According to the ICH, "this margin is the largest difference that can be judged as being clinically acceptable and should be smaller than differences observed in superiority trials of the active comparator" (ICH, 1998). The choice of δ will be based on the purpose for conducting the clinical trial and should be clearly stated in the protocol. The selection of δ should provide, at the minimum, assurance that difference ($\mu_T - \mu_C$) has a clinical effect greater than zero. Also, the choice of the margin should be independent of any power considerations. The sample sizes for these types of studies are very sensitive to the assumed effect of the new drug relative to the control. For a discussion of power and sample size see Chan (2002). The ICH provides some guidance (E-9 and E-10) on the design and analysis of such trials, but does not set specific limits for δ. These require decisions by the primary investigator based on sound clinical judgments. The decision on δ should always be made on both realistic clinical judgments and sound statistical grounds. The decision is made on a study-by-study basis and no rule of thumb applies to all clinical situations.

A potential problem is the choice of active comparator in these noninferiority studies. As mentioned previously the use of a placebo control raises ethical concerns. Thus, an active control is usually used. However, the assumption is made that the active control is effective. For that reason the comparator should be chosen with care. If the comparator is not effective, proving noninferiority will not result in the conclusion that the new treatment drug is effective. The ability to distinguish between an active and placebo control is **assay sensitivity**. If the assay sensitivity cannot be assumed, a noninferiority study cannot demonstrate the effectiveness of a new agent, because assay sensitivity is not measured in a noninferiority trial. Assay sensitivity is dependent on the size of the effect one is interested in detecting. Either a noninferiority or equivalence trial may have assay sensitivity for an effect of 20% but not an effect of 10%. Therefore, it is essential to know the effect of the control drug.

Assay sensitivity can be accomplished by using concurrent placebo control or through historical evidence. Therefore, sensitivity must be assumed based on historical experience with the comparator agent and requires evidence external to the study. The ICH guidelines list several factors that can reduce assay sensitivity, including: poor patient compliance; poor diagnostic criteria; concomitant medications; excessive variability in the measurements; and a biased end-point assessment (ICH 1999). For that reason, some studies involve three arms: new test drug, active control, and placebo control. Such a study would be optimal because it: 1) assesses assay sensitivity; 2) measures the effect of the new drug; and 3) compares the effects of the two active treatments (Temple and Ellenberg, 2000). A discussion of the evaluation of these three-way studies is presented by Pigeot et al. (2003).

As an example, consider the following fictitious clinical trial. One hundred and twenty newly diagnosed hyperlipidemic patients are randomly assigned to one of two legs in a clinical trial; the first group receives StatinA which is on the hospital formulary (the comparator agent). The second group receives a newly marketed agent (StatinB), which reportedly has a better safety profile. Patients are followed for six months. At the end of the study period the changes in total cholesterol levels are recorded for each group. The two possible scenarios and their summary statistics are presented in Table 22.4. As seen in the table, the average decrease in total cholesterol for the comparator product was –40 mg/dl. Prior to the study it was determined that the noninferiority for the new product would be based on a less than 10% difference compared to the comparator product. Since a negative result is desired, we would not want to see a positive result or increase in total cholesterol. Thus, the upper bounds of equivalency margin would be a change equal to +4 mg/dl.

$$H_0: \quad \mu_{StatinB} - \mu_{StatinA} \geq +4 \text{ mg/dl}$$
$$H_1: \quad \mu_{StatinB} - \mu_{StatinA} < +4 \text{ mg/dl}$$

To evaluate the null hypothesis we will use a one-tailed, two-sample confidence interval created by a Student t-test with $\alpha = 0.05$ (modified from Eq. 9.4).

Table 22.4 Two Potential Results Involving a Noninferiority Trial

	StatinA (C)	StatinB (T)	Difference (T – C)
Scenario A			
$\overline{X}_d =$	–40.0	–41.1	1.1 mg/dl lower
$S_d =$	15.4	20.3	
$n =$	60	60	
Scenario B			
$\overline{X}_d =$	–40.0	–39.8	0.2 mg/dl higher
$S_d =$	15.4	7.7	
$n =$	60	60	

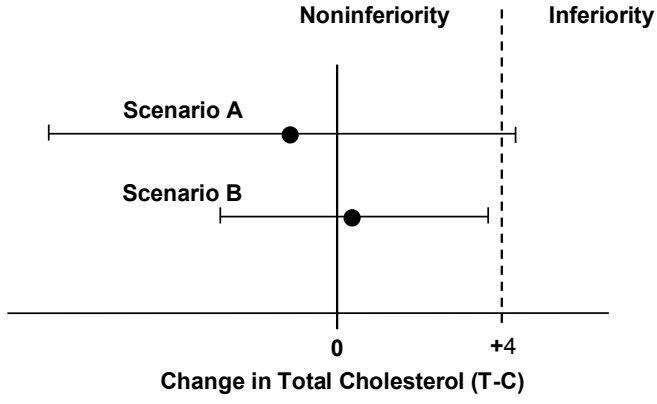


Figure 22.4 Confidence intervals created by data in Table 22.4.

$$\mu_B - \mu_A = (\overline{X_B} - \overline{X_A}) + t_{nB+nA-2}(1-\alpha)\sqrt{\frac{S_p^2}{n_B} + \frac{S_p^2}{n_A}} \qquad \text{Eq. 22.15}$$

In scenario A, even though the new StatinB performs slightly better than StatinA, it fails the test for noninferiority (where $S_p^2 = 324.63$) because the upper limit is greater than +4.

$$\mu_B - \mu_A = [-41.1 - (-40.0)] + 1.66\sqrt{\frac{324.63}{60} + \frac{3.24.63}{60}}$$

$$\mu_B - \mu_A = -1.1 + 1.66(3.29) = -1.1 + 5.46 = +4.36$$

However, in scenario B, StatinB on the average did not perform quite as well as the comparator StatinA, but it does pass the test for noninferiority (where $S_p^2 = 148.23$) because the upper limit is less than +4:

$$\mu_B - \mu_A = [(-39.8 - (-40.0)] + 1.66\sqrt{\frac{148.23}{60} + \frac{148.23}{60}}$$

$$\mu_B - \mu_A = +0.2 + 1.66(2.22) = +0.2 + 3.69 = +3.89$$

As seen in Figure 22.4 the primary contributing cause for these different results is the width of the interval caused by the much greater variance in the StatinB in scenario A. This greater variance results in an overall wider interval and failure to have an

interval less than +4 mg/dl. Displayed in Figure 22.4 are the 90% confidence intervals for both scenarios.

There are a number of other statistical tests involving rate ratios that can be used for the evaluation of differences between treatments (e.g., odds ratio, relative risk, hazard ratio). In these cases the same principles apply, except the assessment of "no difference" is represented by the value one, not zero. For example, using hazard ratio (Chapter 19), if the new treatment is evaluated to be noninferior compared to an active control, the object of the trial is to demonstrate the effectiveness of this new treatment by demonstrating noninferiority. In this case the hypotheses are:

$$H_0: \quad HR(T/C) \geq 1 + \delta$$
$$H_1: \quad HR(T/C) < 1 + \delta$$

where $\delta > 0$ is the noninferiority margin. The null hypothesis is rejected if $\tau_0(\delta)$ is less than -1.96 or -2.58 for 95% or 99% confidence, respectively. If the log of the hazard ratio is used the hypotheses would be:

$$H_0: \quad \log HR(T/C) \geq 1 + \delta$$
$$H_1: \quad \log HR(T/C) < 1 + \delta$$

and the null hypotheses rejected if $log\ HR(T/C) + 1.96SE[log\ hr(T/C)] < \delta$. Both these hypotheses are tested at the one-sided 2.5% significance level. In both cases, if H_0 is rejected, it is possible to conclude that the new treatment is noninferior and no worse than the active control within this fixed margin δ.

Dissolution Testing

Dissolution tests provide an *in vitro* method to determine if products produced by various manufacturers or various batches from the same manufacturer are in compliance with compendia or regulatory requirements. For example, the *United States Pharmacopeia* (2011) states that aspirin tablets ($C_9H_8O_4$) must have "not less than 90% and not more than 110% of labeled amount of $C_9H_8O_4$." In addition, the tolerance level for dissolution testing is that "not less than 80% of the labeled amount of $C_9H_8O_4$ is dissolved in 30 minutes."

Dissolution profiles can be used to compare multiple batches, different manufacturers, or different production sites to determine if the products are similar with respect to percent of drug dissolved over given periods of time. The assumption made is that the rate of dissolution and availability will correlate to absorption in the gut and eventually similar effects at the site of action. This assumption can be significantly enhanced if manufacturers can establish an *in vivo-in vitro* correlation between their dissolution measures and bioavailability outcomes (FDA, 1997, p. 7).

Using aspirin tablets as an example, consider the two sets of profiles seen in Figure 22.5. All batches meet the dissolution criteria of 80% in 30 minutes, but the profiles vary. Are they the same or different enough to consider the batches as not equivalent?

SUPAC-IR Guidance

To answer the question of equivalency in dissolution profiles the FDA has proposed a guidance for manufacturers issued as "Scale-Up and Post-Approval Changes for Immediate Release Solid Oral Dosage Forms" (SUPAC-IR). This guidance is designed to provide recommendations for manufacturers submitting new drug applications, abbreviated new drug applications and abbreviated antibiotic applications to change the process, equipment or production sites following approval of their previous drug submissions (*Federal Register*, 1995). Previous evaluations involved single-point dissolution tests (e.g., the previous aspirin monograph). The SUPAC-IR guidance can assist manufacturers with changes associated with: 1) scale-up procedures; 2) site changes in the manufacturing facilities; 3) equipment or process changes; and 4) changes in components or composition of the finished dosage form.

Under SUPAC-IR there are two factors that can be calculated: 1) a difference factor (f_1), and 2) a similarity factor (f_2). The published formulas are as follows:

$$f_1 = \{[\sum |R_t - T_t|] \ / \ [\sum R_t]\} \cdot 100 \qquad \text{Eq. 22.16}$$

$$f_2 = 50 \ Log \{ [1 + \frac{1}{n} \sum (R_t - T_t)^2]^{-0.5} \cdot 100 \} \qquad \text{Eq. 22.17}$$

where n is the number of time points in the dissolution profile, R_t is the percent dissolved for the reference standard at each time period, T_t is percent dissolved for the test product at the same time period, and *log* is the logarithm base 10. We will slightly rewrite these formulas to remove the negative fractional root terminology:

$$f_1 = \frac{\sum |R_t - T_t|}{\sum R_t} \times 100 \qquad \text{Eq. 22.18}$$

$$f_2 = 50 \ Log \left[\frac{1}{\sqrt{1 + \frac{1}{n} \sum (R_t - T_t)^2}} \times 100 \right] \qquad \text{Eq. 22.19}$$

The guidance for equivalency is that the f_1-value should be close to 0 (generally values less than 15) and the f_2-value should be close to 100, with values greater than 50 ensuring equivalency. If the two dissolution profiles are exactly the same (one laying exactly over the second) the f_2 value will be 100. As the f_2-value gets smaller there is a greater difference between the two profiles. An f_2 of 50 represents a 10% difference, thus the SUPAC-IR guidance requires a calculated f_2-value between 50 and 100 for equivalency. As an example, consider the data presented in Table 22.5 and Figure 22.5, which show the results of dissolution tests performed on two batches of the same drug, one produced with the original equipment used for the product NDA application, and the second with newer equipment. Are the following two

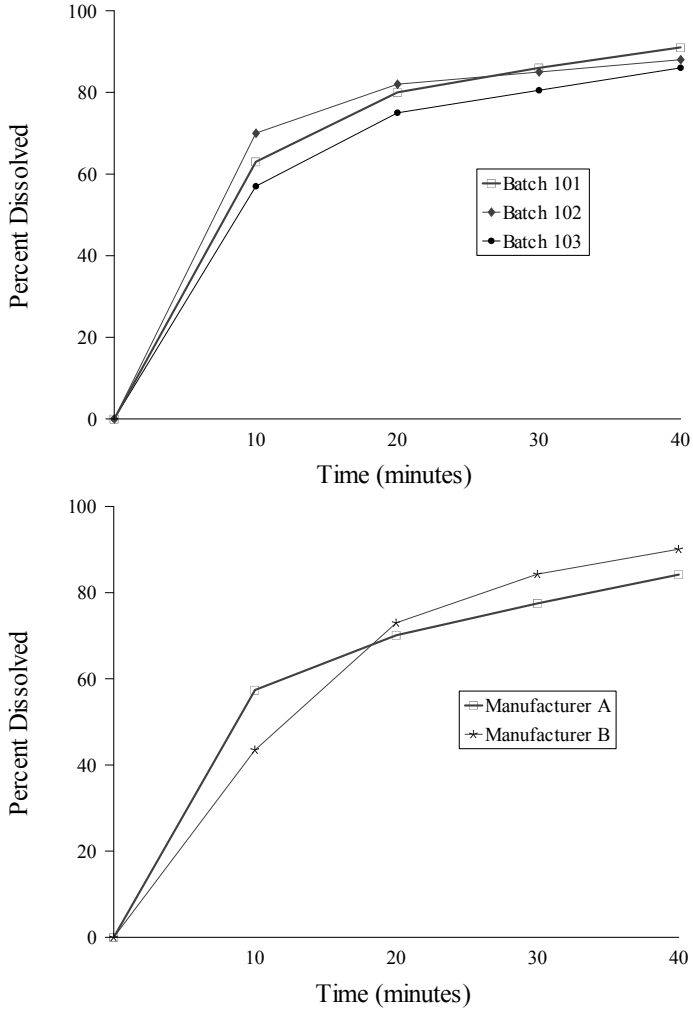


Figure 22.5 Examples of dissolution profiles.

profiles the same (less than a 10% difference) based on the f_1 and f_2 formulas proposed under SUPAC-IR?

Several criteria must be met in order to apply the f_1 and f_2 calculations (FDA, 1997): 1) test and reference batches should be tested under exactly the same conditions, including the same time points; 2) only one time point should be considered after 85% dissolution for both batches; and 3) the "percent coefficient of variation" (what we have called the relative standard deviation in Chapter 5) at the

Table 22.5 Example of Data from Dissolution Tests on Two Drug Batches

Time (minutes):	15	30	45	60
	Batch Produced Using Original Equipment			
	63.9	85.9	85.4	93.6
	42.9	75.8	74.5	87.4
	58.1	77.3	83.2	86.4
	62.4	79.3	76.2	79.2
	52.5	74.5	90.3	94.5
	59.1	65.1	87.5	86.1
Mean:	56.48	76.32	82.85	87.87
SD:	7.74	6.79	6.29	5.61
RSD:	13.71	8.91	7.59	6.38
	Batch Produced Using Newer Equipment			
	78.5	85.6	88.4	92.9
	67.2	72.1	80.2	86.8
	56.5	80.4	83.1	85.4
	78.9	85.2	89.8	91.4
	72.3	84.1	85.4	94.1
	84.9	72.1	79.0	85.9
Mean:	73.05	79.92	84.32	89.42
SD:	10.13	6.33	4.35	3.83
RSD:	13.87	7.91	5.16	4.28

earlier time points should be no more than 20% and at other time points should be no more than 10%. The rationale for these criteria is discussed by Shah and colleagues (1998).

The data presented in Table 22.5 fulfills all three criteria. Therefore, both the f_1 and f_2 statistics can be used to evaluate the equivalency of these two types of production equipment. To calculate certain values required by the statistics, we can create an intermediate table:

Time	R_t	T_t	$\lvert R_t - T_t \rvert$	$(R_t - T_t)^2$
15	56.48	73.05	16.57	274.56
30	76.32	79.92	3.60	12.96
45	82.85	84.32	1.47	2.16
60	87.87	89.42	1.55	2.40
∑=	303.52	326.71	23.19	292.08

Using these numbers produces the following results:

$$f_1 = \frac{\sum |R_t - T_t|}{\sum R_t} \times 100 = \frac{23.19}{303.52} \times 100 = 7.64$$

$$f_2 = 50 \cdot log\left[\frac{1}{\sqrt{1+\frac{1}{n}\Sigma(R_t - T_t)^2}} \times 100\right]$$

$$f_2 = 50 \cdot log\left[\frac{1}{\sqrt{1+\frac{1}{4}(292.08)}} \times 100\right]$$

$$f_2 = 50 \cdot log\,(11.62) = 50(1.06) = 53.3$$

In this example, f_1 is less than 15 and f_2 is greater than 50; therefore, we would conclude that the two dissolution profiles are not significantly different.

Equivalent Precision

One last assessment of equivalence is associated with measures of precision. This comes from an informational general chapter in the *United States Pharmacopeia*. The author was fortunate to chair the expert committee that prepared *<1010> Analytical Data – Interpretation and Treatment*. One section of this chapter provided guidance for assessing if two methods produced equivalent precision by assessing the variances of both methods. The procedure involves calculating a 90% confidence interval based on an *F*-ratio for comparing the precision of the two methods. The hypotheses involve comparison of a new analytical method to an existing standard:

H_0: $\sigma_{new} = \sigma_{standard}$
H_1: $\sigma_{new} \neq \sigma_{standard}$

The confidence interval is based on the ratio of the variances to create upper and lower limits.

$$Lower\ Limit < \frac{\sigma^2_{new}}{\sigma^2_{standard}} < Upper\ Limit \qquad \text{Eq. 22.20}$$

If the upper limit of the confidence interval for the ratios is less than four, the two methods can be considered equivalent.

The best estimate of the population variance ratios is the sample variance ratio. These ratios are calculated by using *F*-values from Table B7 in Appendix B. The lower limit is the sample variance ratio divided by the *F*-value fromTable B7 with n_1 - 1 and n_2 - 2 as the numerator and denominator degrees of freedom at $1-\alpha = 0.95$ at the right side of the distribution. The upper limit involves a similar determination, but uses the *F*-value where $\alpha = 0.05$ at the left side of the distribution. This can be

calculated by taking the reciprocal of the *F*-value with the degrees of freedom reversed from the right side

$$F_{0.05,n_1-1,n_2-1} = \frac{1}{F_{0.95,n_2-1,n_1-1}} \qquad \text{Eq. 22.21}$$

These *F*-values can also be determined using Excel (FINV in Excel 97-2003 or F.INV in Excel 2010, probability = 0.05). Thus, the two limits are calculated as follows:

$$Lower\ Limit = \frac{\frac{S^2_{new}}{S^2_{standard}}}{F_{0.95,n_1-1,n_2-1}} \qquad \text{Eq. 22.22}$$

$$Upper\ Limit = \frac{\frac{S^2_{new}}{S^2_{standard}}}{F_{0.05,n_1-1,n_2-1}} \qquad \text{Eq. 22.23}$$

As an example of this test for equivalent precision, assays using the current method of analysis produced a standard deviation of 0.79. A proposed new method analyzing a similar sample from the same batch of product produced a standard deviation of 0.84. These results are based on 12 samples using the new method and six samples with the current standard method. Do these two tests have similar precision? In tabular format the results are:

Method	SD	Variance	n	Degrees of Freedom
New	0.84	0.706	12	11
Standard	0.79	0.624	6	5

The *F*-values would be $F_{0.95,11,5} = 4.704$ and $F_{0.05,5,11} = 0.213$. The ratio for the two sample variances would be 1.131 (0.706/0.624). The results would be as follows:

$$Lower\ Limit = \frac{\frac{S^2_{new}}{S^2_{standard}}}{F_{0.95,n_1-1,n_2-1}} = \frac{1.131}{4.704} = 0.240$$

$$Upper\ Limit = \frac{\frac{S^2_{new}}{S^2_{standard}}}{F_{0.05,n_1-1,n_2-1}} = \frac{1.131}{0.213} = 5.310$$

$$0.240 < \frac{\sigma^2_{new}}{\sigma^2_{standard}} < 5.310$$

In this example the upper limit exceeds four and therefore the precision of the two methods is not equivalent. If the sample sizes were larger and the same variances observed, the difference might not be significant. For example, if there were 18 results for the new method and 12 results for the standard procedure, the lower limit and upper limit *F*-values would be 2.685 and 0.372, respectively. The resulting confidence interval would be:

$$0.421 < \frac{\sigma^2_{new}}{\sigma^2_{standard}} < 3.036$$

Thus, as seen with other statistics, sample size can influence the results of the evaluation. The larger the sample size, generally the smaller the interval.

References

Federal Register (1977). 42: 1648.

Federal Register (1978). 43: 6965-6969.

Federal Register (1995). 60: 61638-61643.

FDA (1997). "Guidance for industry: dissolution testing of immediate release solid oral dosage forms," (BP1), Center for Drug Evaluation and Research, Food and Drug Administration, Rockville, MD, p. 9.

ICH Steering Committee (1998). "ICH E9 – Statistical principles for clinical trials," International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. *Federal Register* 63:49583-49598.

ICH Steering Committee (1999). "E10 – Choice of control group and related issues in clinical trials," International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. *Federal Register* 64:51767-51780.

United States Pharmacopeia (2011). 34th revision, United States Pharmacopeial Convention, Rockville, MD, p. 1934.

Benet, L.Z. and Goyan, J.E. (1995). "Bioequivalence and narrow therapeutic index drugs," *Pharmacotherapy* 15:433-440.

Chan, I.S. (2002). "Power and sample size determination for noninferiority trials using an exact method," *Journal of Biopharmaceutical Statistics* 12:457-469.

Chow, S.C. and Liu, J.P. (2000). *Design and Analysis of Bioavailability and Bioequivalence Studies*, Second edition, Marcel Dekker, Inc., New York.

Evans, W.E., Schentag, J.J., and Jusko, W.J. (eds), (1992). *Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring*, Third edition, Applied Therapeutics, Vancouver, WA.

Hauck, W.W. and Anderson, S. (1984). "A new statistical procedure for testing equivalence in two-group comparative bioavailability trials," *Journal of Pharmacokinetics and Biopharmaceutics* 12:83-91.

Kirk, R.E. (1968). *Experimental Design: Procedures for the Behavioral Sciences*, Brooks/Cole Publishing, Belmont, CA, pp. 424-440.

Malinowski, H.J. (2000). "Bioavailability and bioequivalency testing," Chapter 53 in *Remington: The Science and Practice of Pharmacy*, Twentieth edition, Gennaro, A.R. (ed.), Lippincott, Williams and Wilkins, Baltimore, MD, pp. 995-1004.

Metzler, C.M. (1974). "Bioavailability: a problem in equivalence," *Biometrics* 30:309-317.

Pigeot, I., Schäfer, J., Röhmel, J., and Hauschke, D. (2003). "Assessing noninferiority of a new treatment in a three-arm clinical trial including a placebo," *Statistics in Medicine* 22:883-899.

Schuirmann, D.J. (1987). "Comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability," *Journal of Pharmacokinetics and Biopharmaceutics* 15:660.

Shah, V.P., et al. (1998). "*In Vitro* dissolution profile comparison: statistics and analysis of the similarity factor, f_2," *Pharmaceutical Research* 15:891-898.

Snapinn, S.M. (2000). "Noninferiority trials," *Current Controlled Trials in Cardiovascular Medicine* 1:19-21.

Temple, R. and Ellenberg, S.S. (2000). "Placebo-controlled trials and active-control trials in the evaluation of new treatments," *Annals of Internal Medicine* 133:455-463.

Wang, S.J., Hung, H.M.J., and Tsong, Y. (2003). "Noninferiority analysis in active controlled clinical trials," *Encyclopedia of Biopharmaceutical Statistics*, Chow, S.C. (ed.), Marcel Dekker, New York, p. 674.

Westlake, W.J. (1988). "Bioavailability and bioequivalence of pharmaceutical formulations," *Biopharmaceutical Statistics for Drug Development*. Peace, K.E., (ed.), Marcel Dekker, New York, p. 342.

Welling, P.G. and Tse, F.L.S. (1995). *Pharmacokinetics*, Second edition, Marcel Dekker, Inc., New York.

Winter, M.E. (2010). *Basic Clinical Pharmacokinetics*, Fifth edition, Lippincott, Williams and Wilkins, Baltimore, MD.

Zar, J.H. (2010). *Biostatistical Analysis*, Fifth edition, Prentice-Hall, Upper Saddle River, NJ, p. 150.

Suggested Supplemental readings:

Chan, I.S.F. (2004). "Noninferiority and equivalency trials," *Journal of Biopharmaceutical Statistics* 14:261,262.

Chan, I.S.F. (2003). "Statistical analysis of noninferiority trials with a rate ratio in small-sample match-pair designs," *Biometrics* 59:1170-1177.

Rodda, B.E. (1990). "Bioavailability: design and analysis," *Statistical Methodology in the Pharmaceutical Sciences*. Berry, D.A., (ed.), Marcel Dekker, New York, pp. 57-82.

Schuirmann, D.J. (1987). "Comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability," *Journal of Pharmacokinetics and Biopharmaceutics* 15:657-680.

Schuirmann, D.J. (1990). "Design of bioavailability/bioequivalence studies," *Drug Information Journa*l 15:315-323.

Shah, V.P., et al. (1998). "*In Vitro* dissolution profile comparison: statistics and analysis of the similarity factor, f_2," *Pharmeutical Research* 15:891-898.

Westlake, W.J. (1988). "Bioavailability and bioequivalence of pharmaceutical formulations," *Biopharmaceutical Statistics for Drug Development*. Peace, K.E. (ed.), Marcel Dekker, New York, p. 329-352.

Example Problems (Answers are provided in Appendix D)

1. In a clinical trial, data comparing Gigantic Drugs' new generic product was compared with the innovator's branded antipsychotic; both products contain the exact same chemical entity. One subject did not complete the study. The results were as follows:

	Innovator	Generic
Mean =	289.7	271.6
Standard Deviation =	18.1	20.4
n =	24	23

Use the confidence interval approach and the two one-tailed t-tests to check for bioequivalence, assuming there should be less than a 10% difference between the two products.

2. Production of a certain product in two different countries (A and B) were compared to the manufacturer's original production site (standard). Dissolution data is presented in Table 22.6 and Figure 22.6. Visually it appears that site B has a profile closer to the reference standard, but do both of the foreign facilities meet the SUPAC-IR guidelines for similarity?

Table 22.6 Dissolution Data (percent)

Time (minutes)	Country A	Country B	Standard
15	57.3	54.1	49.8
30	66.4	67.7	70.8
45	71.9	75.4	80.9
60	76.4	81.4	86.7
75	80.4	85.6	90.9
90	84.6	88.8	93.6

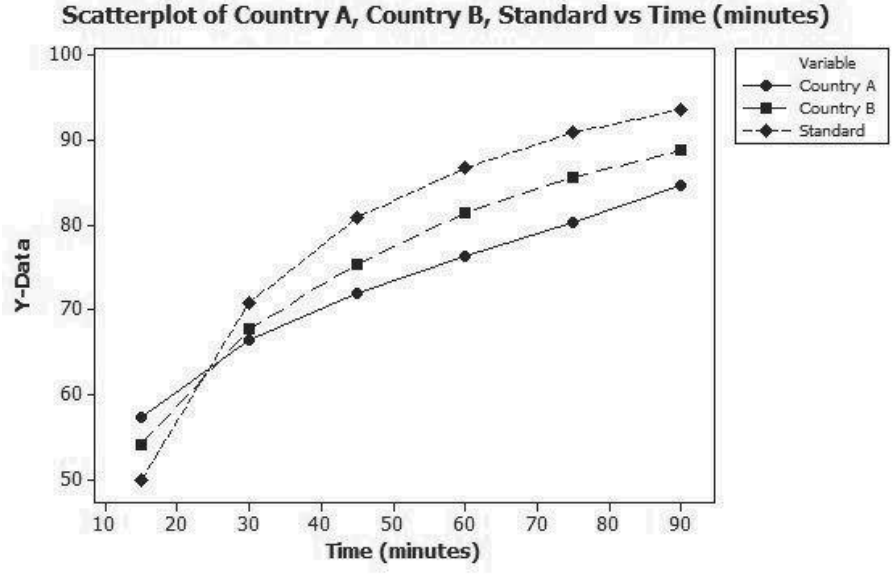


Figure 22.6 Dissolution profiles for two foreign countries.

23

Outlier Tests

An outlier is an extreme data point that is significantly different from the remaining values in a set of observations. Based on information, either investigational or statistical, an outlier value may be removed from the data set before performing an inferential test. However, removal of an outlier is discouraged unless the data point can be clearly demonstrated to be erroneous. Rodda (1990) provided an excellent description of outliers when he portrayed them as "… much like weeds; they are very difficult to define and are only called outliers because they are inconsistent with the environment in which they are observed." Outliers can dramatically affect the outcome of a statistical analysis. This is especially true if the sample size is small. However, we need to use care in our decision-making process to ensure that we remove the weed and not a budding piece of data.

Regulatory Considerations

Outliers are often referred to as **aberrant results** and have been the source of regulatory discussions and guidances. Prior to the introduction of USP Chapter <1010> in 2005, there were no compendia guidances on the treatment of outliers, except with respect to biological assays presented in USP <111>. This lack of guidance or "silence" on the part of USP, was noted in the 1993 case of *United States versus Barr Laboratories, Inc.* (812 F. Supp. 458, 1993). Judge Wolin's ruling in the Barr case pointed out the need for compendia guidance in this area of outliers, as well as other analytical measures. USP <1010> attempts to address many of these issues (USP, 2011). This USP chapter lists a litany of other synonyms for outlying results, including "anomalous, contaminated, discordant, spurious, suspicious or wild observations; and flyers, rogues, and mavericks."

In 1998 the FDA focused attention on a similar problem, out-of-specification (OOS) test results, and issued a draft guidance (FDA, 1998). In addition to outlier tests, the guidance attempts to address retesting, resampling, and averaging of test results. This guidance was updated in 2006, but it ignored <1010> and no changes were made in the outlier section (FDA, 2006).

Similar to other laboratory results, potential outliers must be documented and interpreted. Both USP <1010> and the FDA guidance propose a two-phase approach to identifying and dealing with outliers. When an outlier is suspected, the first phase

is a thorough and systematic laboratory investigation to determine if there is a possible assignable cause for the aberrant result. Potential assignable causes include "human error, instrumentation error, calculation error, and product or component deficiency" (USP, 2011). If one can identify an assignable cause in the first phase, then the outlier can be removed and retesting of the same sample ($n - 1$) or the addition of a new sample from the same population is permissible. However, if no assignable cause can be identified, then the second phase is to evaluate the potential aberrant value using statistical outlier tests as part of the overall outlier investigation. When used correctly, the outlier tests described below are valuable statistical tools; however, any judgment about the acceptability of data in which outliers are observed requires careful interpretation.

The term "outlier labeling" refers to an informal recognition of a potential aberrant value (often performed visually using graphing procedures discussed in Chapter 4). Use of statistical procedures to determine if any value is truly aberrant is termed "outlier identification." Determining the most appropriate outlier test will depend on the assumed population distribution and the sample size.

If, as the result of either thorough investigation or outlier test, a value is removed as an outlier, the step is termed an "outlier rejection." Both the FDA and USP note that using an outlier test cannot be the sole means for outlier rejection. Even though the outlier tests can be useful as part of the determination of the aberrant nature of a data point, the outlier test can never replace the value of a thorough laboratory investigation. All data, especially outliers, should be kept for future reference. Outliers are not used to calculate the final reportable values, but should be footnoted in tables or reports.

The author has a simple rule for dealing with potential outliers. Perform the intended statistical analysis both with and without the potential outlier(s), which can be easily accomplished with computer software. If the results of the analysis are the same (rejecting or failing to reject the null hypothesis), the question of whether a value is an outlier becomes a moot issue.

Outliers on a Single Continuum

With both descriptive and inferential statistics it is common to report the center and distribution for the sample data. An uncharacteristic observation could be either a valid data point that falls to one of the extreme tailing ends of our continuum or due to some error in data collection. In the latter case, the point would be considered an outlier. Many detectable and undetectable effects could cause such an extreme measurement, including: 1) a temporary equipment malfunction; 2) a technician or observer misreading the result; 3) an error in data entry; 4) a calculation error; 5) contamination; or 6) a very large or small measurement within the extremes of the distribution. With respect to the last point, an outlier does not necessarily imply that an error has occurred with the experiment, only that an extreme value has occurred. Vigilance is important with any data manipulation and an inspection of data for recording or transcribing errors is always warranted before the statistical analysis.

Another consideration is that a potential outlier could be a legitimate observation in a strongly skewed distribution and represent a value at the extreme end of the longer tail. Transforming data may be first required to create a normally distributed

sample before performing some of the outlier tests. Even an extremely high value in a strong positively skewed distribution may be a true value and not necessarily an outlier. As discussed in Chapter 6, common transformations include using the logarithms or square roots of the individual data points. Alternatively, for nonnormally distributed populations, there are robust measures for central tendency and spread (the median and median absolute deviation) and exploratory data analysis (EDA) methods. Use of a nonparametric procedure or other robust technique, is termed "outlier accommodation" and usually involves rank ordering the data that minimized the influence of outliers. Various transformations or ranking of the data can be used to minimize the effect of an outlier. This was pointed out in Chapter 21, when nonparametric statistics were described as being influenced less by outliers than are traditional parametric tests, whose calculations are affected by measures of dispersion (variance and standard deviation). In addition, outliers could represent data points accidentally sampled from a population that is different from the intended population. Also, care should be taken that computer programs do not handle missing data as real values (in most cases assigning a value of zero).

Extreme values can greatly influence the most common measures of central tendency; they can distort the mean and greatly inflate the variance. This is especially true with small sample sizes. In contrast, the median and quartile measures are relatively insulated from the effects of outliers. For example consider the following assay results (in percentages):

97, 98, 98, 95, 88, 99

Whether or not it is determined that 88% is a true outlier, it has an important effect on the mean and spread (range and variance) of the sample and can be termed an **influential observation**, which will be discussed later in this chapter. Table 23.1 shows the impact this one observation can have on various measures of central tendency. As seen in the table, this extreme value pulls the mean in the direction of that value, increases the standard deviation by a factor greater than two, and the range is increased almost threefold. However, the median is relatively unaffected. This would also be true even if the lowest value was 78 or even 68%. As mentioned, nonparametric tests rely on ranking of observations; in many cases they use the median as the center of the distribution, and are less affected by outliers. In fact, using the various statistical tests listed below, the value 88% would not be rejected as an outlier. It would be considered only an influential observation.

Table 23.1 Impact of a Potential Outlier on Measures of Central Tendency

	88% Included	88% Not Included
Mean	95.8	97.4
Standard Deviation	4.1	1.5
Range	11	4
Median	97.5	98

Table 23.2 Impact of a Potential Outlier on Measures of Central Tendency with Two Sample Sizes

	Case 1		
	86% Not Included	86% Included	Case 2
n	5	6	12
Mean	97.4	95.5	96.5
S.D.	1.5	4.8	3.5
Range	4	11	11
Median	98	97.5	98

A second example of assay results is presented below. In this case the more extreme value (86%) would be defined as an outlier, with 95% confidence using the test procedures discussed below. In this particular sample there are only six tablets:

97, 98, 98, 95, 86, 99

For illustrative purposes, assume in this second case that these results were part of a larger sample of twelve tablets.

97, 98, 98, 95, 86, 99
98, 98, 97, 99, 98, 95

Without the outlier, both the first case and second case have approximately the same mean and standard deviation. Notice in Table 23.2 that the greater sample size "softens" the effect of the outlier. In the second case, 86% would not be identified as an outlier using the tests described in this chapter. If possible, additional measurements should be made when a suspect outlier occurs, particularly if the sample size is very small.

To test for outliers we need at least three observations. Naturally the more information we have (the larger the sample size), the more obvious an outlier will become, either visually or statistically. For a sample size as small as three observations, there would need to be a wide discrepancy for one data point to be deemed an outlier. If an outlier is identified, it is important to try and identify a possible cause for this extreme value (e.g., miscalculation, data entry error, contamination). The identification of an outlier can lead to future corrective action in the process or research being conducted, but it can also serve as a potential source of new information about the population.

A simple technique to "soften" the influence of possible outliers is called **winsorizing** (Dixon and Masey, 1969). Using this process the two most extreme observations (the largest value and the smallest value) are changed to the value of their next closest neighbor ($x_1 \rightarrow x_2$; $x_n \rightarrow x_{n-1}$). For example, consider the following rank ordered set of observations, where 11 might be an outlier:

11,21,24,25,26,26,27,28,29,31

Our suspected outlier would be replaced with the second lowest number. Also we would replace the largest value with the second largest value:

21,21,24,25,26,26,27,28,29,29

For the first set of data the mean and standard deviation are 24.8 ± 5.6 and for the winsorized data they are 25.6 ± 2.9. For this set of data the potential outlier has little impact (+3% change) on our sample mean, but dramatically changes in the standard deviation (48% decrease). Although not a statistical test for outliers, winsorizing might provide a quick measure of the impact of extreme values on the measures of central tendency for our sample.

Plotting and the Number of Standard Deviations from the Center

By using various plotting methods to display the data, outliers may become readily visible. For example, box-and-whisker plots are specifically designed to identify possible outliers (Figure 23.1). As discussed in Chapter 4, each of the "whiskers" or t-bars extends from the box three semi-interquartile ranges (SIQR) above and below the median (the SIQR being the distance between the upper or lower quartile and the median, Eq. 4.1). Observations that fall above or below the whiskers may be identified as potential outliers. Potential outliers can also be observed using other graphic techniques including stem-and-leaf plots, histograms, line charts, or point plots. In addition, scatter plots can be useful in identifying potential outliers involving two or more continuous variables. An example of the box-and-whisker plots will be presented later when discussing residuals under the bivariate outliers section.

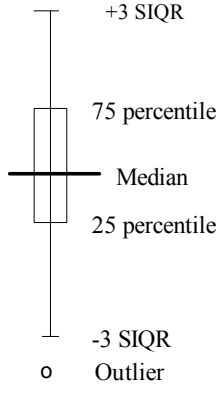


Figure 23.1 Box-and-whisker plot.

The "Huge" Rule

One method for detecting an aberrant value is to compare the potential outlier to the sample mean and standard deviation with the potential outlier removed from the calculations. This general rule of thumb is to consider the data point as an outlier if that point is located more than four standard deviations from the mean as calculated without the suspected outlier (Marascuilo, 1971). The rationale for this rule is that it is extremely unlikely ($p < 0.00005$) to find values more than four standard deviations from the expected center of a normal distribution. The distance, in standard deviations, is measured between the mean and the potential outlier:

$$M = \frac{|x_i - \overline{X}|}{S} \qquad \text{Eq. 23.1}$$

where $\overline{X}$ and S are calculated from the sample data, ignoring the outlier value (x_i). If M is greater than four, then the data point is considered to be an outlier.

To illustrate this rule of thumb test, consider the following observations:

99.3, 99.7, 98.6, 99.0, 99.1, 99.3, 99.5, 98.0,
98.9, 99.4, 99.0, 99.4, 99.2, 98.8, 99.2

Using this set of 15 observations, is data point 98.0 an outlier? For the huge rule, the mean and standard deviation are calculated without 98.0 and the number of standard deviations is calculated between this mean and 98.0. These sample results are $\overline{X}$ = 99.17 and S = 0.29 without 98.0. Note that if the potential outlier is included the results would be $\overline{X}$ = 99.09 and S = 0.41. The standard deviation increases by almost 50%. If not an outlier, 98.0 can certainly be considered an influential data point. The calculation of the number of standard deviations from the mean for our potential outlier is:

$$M = \frac{|x_i - \overline{X}|}{S} = \frac{|99.17 - 98.0|}{0.29} = \frac{1.17}{0.29} = 4.03$$

Since the data point 98.0 is more than 4.00 below the mean it is disregarded as an outlier. Several other procedures are available to statistically determine if observations are outliers or simply extremes of the population from which the sample is selected. The most commonly used statistics to detect univariate outliers (involving one discrete independent variable) are the Grubbs' test and the Dixon Q test and these will be discussed below. Also discussed in this chapter will be Hampel's rule. Other possible tests include: 1) Youden's test for outliers (Taylor, 1987); 2) Cochran's test for extreme values of variance (Taylor, 1987); and 3) studentized deleted residuals (Mason, 1989).

Grubbs' Test for Outlying Observations

Grubbs' procedure involves ranking the observations from smallest to largest ($x_1 < x_2 < x_3 < \ldots x_n$) and calculating the mean and standard deviation for all of the observations in the data set (Grubbs, 1969). This test is also referred to as **extreme studentized deviate test** or **ESD test**. One of the following two formulas is used, depending upon whether x_1 (the smallest value) or x_n (the largest value), is suspected of being a possible outlier.

$$T = \frac{\overline{X} - x_1}{S} \quad or \quad T = \frac{x_n - \overline{X}}{S} \qquad \text{Eq. 23.2}$$

These formulas are occasionally referred to as the **T procedure** or **T method**. This resultant T is compared to a critical value on Table B23 (Appendix B), based on the sample size (n) for a given allowable error (α). The error level for interpreting the result of the Grubbs' test is the same as our previous discussion of hypothesis testing. Once again α will represent the researcher-controlled error rate. Assuming we want to be 95% confident in our decision and use the 5% level (right column in Table B23), we may incorrectly reject an outlier 1 in 20 times. If T is greater than the critical value, the data point can be rejected as an outlier. Using the previous example, the information is first ranked in ascending order (Table 23.3). The mean and standard deviations are then calculated with the proposed outlier included. As noted above, the results are: $\overline{X} = 99.09$ and $S = 0.41$. Using Grubbs' test we first identify the critical value on Table B21; in this case it is 2.409 for $n = 15$ and $\alpha = 0.05$. The calculation of

Table 23.3 Sample Rank Ordered Data for Outlier Tests

	Value
x_1	98.0
x_2	98.6
x_3	98.8
...	98.9
	99.0
	99.0
	99.1
	99.2
	99.2
	99.3
	99.3
...	99.4
x_{n-2}	99.4
x_{n-1}	99.5
x_n	99.7

the Grubbs' test is

$$T = \frac{\overline{X} - x_1}{S} = \frac{99.09 - 98.0}{0.41} = \frac{1.09}{0.41} = 2.66$$

Since our calculated value of 2.66 exceeds the critical value of 2.409, once again 98.0 is rejected as an outlier.

Dixon Q Test

A third method to determine if a suspected value is an outlier is to measure the difference between that data point with the next closest value and compare that difference to the total range of observations (Dixon, 1953). Various ratios of this type (absolute ratios without regard to sign) make up the **Dixon test** for outlying observations, also referred to as the **Dixon Q test**. Both the Grubbs' test and Dixon Q test assume that the population from which the sample is taken is normally distributed. The advantage of this test is that it is not required to estimate the standard deviation. First the observations are rank ordered similar to the Grubbs' test (Table 23.3):

$$x_1 < x_2 < x_3 < \ldots x_{n-2} < x_{n-1} < x_n$$

Formulas for the Dixon test use ratios of ranges and subranges within the data. The ratios are listed in Table 23.4 where the choice of ratio is dependent on the sample size and whether x_1 or x_n is suspected to be an outlier. If the smallest observation is suspected of being an outlier, use the ratios are presented on the upper half of Table 23.4. However, if the largest value is evaluated as the outlier, use the ratios in the lower half of Table 23.4. The resultant ratio is compared to the critical values in Table B24 (Appendix B). If the calculated ratio is greater than the value in the table, the data point can be rejected as an outlier. Using the Dixon test for the data presented in Table 23.3, the critical value from Table B24 is $\tau = 0.525$, based on $n = 15$ and $\alpha = 0.05$. The calculated Dixon ratio would be:

$$\frac{(x_3 - x_1)}{(x_{n-2} - x_1)} = \frac{98.8 - 98.0}{99.4 - 98.0} = \frac{0.8}{1.4} = 0.57$$

Because this calculated value of 0.57 exceeds the critical value of 0.525, we once again reject 98.0 as an outlier.

The Grubbs' and Dixon's tests may not always agree regarding the rejection of the possible outlier, especially when the test statistic results are very close to the allowable error (e.g., 5% level). The simplicity of Dixon's test is of most benefit when small samples are involved and only one observation is suspected as an outlier. Grubbs' test requires more calculations (e.g., determining the sample mean and standard deviation), but is considered to be the more powerful of the two tests. Also, Grubbs' test can be used when there is more than one suspected outlier (Mason, p. 512). As with any statistical test that measures the same type of outcomes, the

Table 23.4 Ratios for Dixon's Test for Outliers

Sample Size	Ratio	If x_1 is suspected	
$3 \le n \le 7$	τ_{10}	$\frac{x_2 - x_1}{x_n - x_1}$	Eq. 23.3
$8 \le n \le 10$	τ_{11}	$\frac{x_2 - x_1}{x_{n-1} - x_1}$	Eq. 23.4
$11 \le n \le 13$	τ_{21}	$\frac{x_3 - x_1}{x_{n-1} - x_1}$	Eq. 23.5
$14 \le n \le 25$	τ_{22}	$\frac{x_3 - x_1}{x_{n-2} - x_1}$	Eq. 23.6
Sample Size	Ratio	If x_n is suspected	
$3 \le n \le 7$	τ_{10}	$\frac{x_n - x_{n-1}}{x_n - x_1}$	Eq. 23.7
$8 \le n \le 10$	τ_{11}	$\frac{x_n - x_{n-1}}{x_n - x_2}$	Eq. 23.8
$11 \le n \le 13$	τ_{21}	$\frac{x_n - x_{n-2}}{x_n - x_2}$	Eq. 23.9
$14 \le n \le 25$	τ_{22}	$\frac{x_n - x_{n-2}}{x_n - x_3}$	Eq. 23.10

researcher should select the outlier test he or she is most comfortable with before looking at the data.

As mentioned previously, both Grubbs' and Dixon's tests assume that the population from which the sample was taken is normally distributed. In the case of the Grubbs' test with more than one outlier, the most extreme measurement will tend to be masked by the presence of other possible outliers. **Masking** occurs when two or more outliers have similar values. In a data set, if the two smallest (or largest) values are almost equal, an outlier test for the more extreme of the two values may not be statistically significant. This is especially true for sample sizes less than ten, where the numerator of the ratio for the Dixon Q test is the difference between the two most extreme values. Only a test for both of these two smallest observations will be statistically significant. Plotting the data can sometimes avoid the masking problem. **Swamping** is another problem and is seen when several good data points, that may be close to the suspected outlier, disguise its effect. Using graphing techniques, it is possible to identify a cluster of data points and these might influence tests for outliers.

Hampel's Rule

The underlying assumption with both the Grubbs' and Dixon's tests is that the sample being evaluated comes from population with a normal distribution. Hampel's rule for testing outliers is based on the median and can be used for samples from populations with any type of distribution and is not restricted to only normally distributed populations.

The first step in determining an outlier using Hampel's rule is to calculate an *MAD* value (which is the median for the absolute deviations from the median times a constant). To calculate the *MAD* the median is subtracted from each data point and expressed in absolute terms (called the **absolute deviations**).

$$AD_i = \left| x_i - Md \right| \qquad \text{Eq. 23.11}$$

For example, using our previous data set, the AD_i for 98.0 is:

$$AD_i = \left| 98.0 - 99.2 \right| = \left| -1.2 \right| = 1.2$$

These absolute derivations are presented in the second column of Table 23.5. The next step is to multiply the median for the absolute deviations by a constant 1.483[1] to produce the MAD_i.

$$MAD_i = Median(\, AD_i \,) \cdot 1.483 \qquad \text{Eq. 23.12}$$

The third step is to normalize the MAD_i data. However instead of subtracting each value from the mean and dividing the results by the standard deviation (similar to Grubbs' calculations), each value is subtracted from the median and divided by the MAD_i.

$$NAD_i = \frac{\left| Md - x_i \right|}{MAD_i} \qquad \text{Eq. 23.13}$$

These results are presented in the third column of Table 23.5. In the case of an assumed underlying normal distribution, if the most extreme value is greater than 3.5 it can be rejected as an outlier (more than 3.5 standard deviations based on the normalized median). In this example, 98.0 is once again removed as an outlier. Hampel provides other constants and critical values for nonnormal situations (Hampel, 1985).

[1] The constant 1.483 is the reciprocal of the range of values for a normal standardized distribution between the first and third quartiles. The area between −0.674 and +0.674 is 0.500 (1.483 = 1/0.674).

Table 23.5 Example Using Hampel's Rule

	Data	Absolute Deviations (AD_i)	Absolute Normalized Deviations (NAD_i)
	99.7	0.5	1.686
	99.5	0.3	1.011
	99.4	0.2	0.674
	99.4	0.2	0.674
	99.3	0.1	0.337
	99.3	0.1	0.337
	99.2	0	0.000
	99.2	0	0.000
	99.1	0.1	0.337
	99.0	0.2	0.674
	99.0	0.2	0.674
	98.9	0.3	1.011
	98.8	0.4	1.349
	98.6	0.6	2.023
	98.0	1.2	4.046
Median =	99.2	0.2	
MAD =		0.2966	

Multiple Outliers

Once an initial extreme outlier value has been determined and removed from the data, the researcher can determine if there is a possible second outlier using the same procedures with $n - 1$ data points. Using our data from the previous example (now with only 14 data points) is 98.6 a possible outlier? In this case the mean and standard deviation for the data would be:

	With 98.6	Without 98.6
Mean	99.17	99.22
SD	0.29	0.25
n	14	14

Using the "huge" rule, the value 98.6 is less than four standard deviations below the mean and not an outlier:

$$M = \frac{|x_i - \overline{X}|}{S} = \frac{|99.22 - 98.6|}{0.25} = \frac{1.17}{0.29} = 2.14$$

Using the Grubbs' test we fail to reject 98.6 as an outlier because it does not exceed the critical value of 2.371.

Table 23.6 Example Using Hampel's Rule without 98.0

	Data	Absolute Deviations (AD_i)	Absolute Normalized Deviations (NAD_i)
	99.7	0.5	1.686
	99.5	0.3	1.011
	99.4	0.2	0.674
	99.4	0.2	0.674
	99.3	0.1	0.337
	99.3	0.1	0.337
	99.2	0	0.000
	99.2	0	0.000
	99.1	0.1	0.337
	99.0	0.2	0.674
	99.0	0.2	0.674
	98.9	0.3	1.011
	98.8	0.4	1.349
	98.6	0.6	2.023
Median =	99.2	0.2	
MAD =		0.2966	

$$T = \frac{\overline{X} - x_1}{S} = \frac{99.17 - 98.6}{0.29} = \frac{0.57}{0.29} = 1.97$$

Dixon's test shows similar results, with the calculated ratio not exceeding the critical value of 0.546.

$$\frac{(x_3 - x_1)}{(x_{n-2} - x_1)} = \frac{98.9 - 98.6}{99.4 - 98.6} = \frac{0.3}{0.8} = 0.375$$

Finally, the same results are found with Hampel's rule as seen in Table 23.6 where the absolute normalized deviation for 98.6 is less than 3.5. Note the values in Tables 23.5 and 23.6 are similar because the median value is the same in both cases.

One should use some common sense when dealing with potential multiple outliers. If two or more potential outliers are in opposite directions, maybe this represents only poor precision or large variance in the data. If two or more potential outliers are in the same direction, maybe there is a subpopulation that requires further investigation. Indiscriminant use of outlier tests and rejection of data points may result in the loss of valuable information about the population(s) being studied.

Table 23.7 Data and Residuals Presented in Figure 23.2

x_i concentration	y_i units	y_c	r
2.0	87.1	89.980	–2.840
2.5	95.2	93.165	+2.035
3.0	98.3	96.350	+1.950
3.5	96.7	99.535	–2.835
4.0	100.4	102.720	–2.320
4.5	112.9	105.905	+6.985
5.0	110.7	109.090	+1.610
5.5	108.5	112.275	–3.735
6.0	114.7	115.460	–0.760
		$\sum =$	0.000

Bivariate Outliers in Correlation and Regression Analysis

In the case of correlation or regression, where each data point represents values on different axes, an outlier is a point clearly outside the range of the other data points on the respective axis. Outliers may greatly affect the results of the correlation or regression models. Outliers in regression analysis are data points that fall outside the linear pattern of the regression line. At the same time, many statistical tests for identifying multivariate outliers are prone to problems of masking, swamping, or both; and no single method is adequate for all given situations. For our discussion we will focus only on the simplest situations where we are analyzing just two continuous variables. Obviously problems will compound as additional variables enter into the analysis.

In linear regression-type models, outliers generally do not occur in the independent variable, because the levels for that variable are selected by the researcher and can usually be controlled. Potential problems then exist only with the dependent or response variable. In contrast, with a correlation model both variables can vary greatly and outliers may occur in either variable. As mentioned previously, variables are sometime referred to as the **predictor variable** and the **response variable** depending on the focus of our investigation. For example, as the dose of a medication changes (predictor variable), what type of response do we see in the physiological measure in laboratory animals (response variable)?

Let us first look at the regression model where we can control the independent variable and are interested in possible outliers in the dependent (response) variable. Outlier detecting techniques are based on an evaluation of the residuals. The **residual** is the difference between the observed outcome (y_i) and the predicted outcome (y_c) based on the least square line that best fits the data ($r = y_i - y_c$). In Chapter 14, when evaluating if a linear relationship existed between our independent and dependent variable, we used residuals to explain the error with respect to the deviations about the regression line (Eqs. 14.4 and 14.5):

Table 23.8 Regression Analysis for Figure 23.2

Source	SS	df	MS	F
Linear Regression	608.65	1	608.65	43.79
Residual	97.29	7	13.90	
Total	705.94	8		

$$\Sigma(y_i - \overline{X}_y)^2 = \Sigma(y_c - \overline{X}_y)^2 + \Sigma(y_i - y_c)^2$$

$$SS_{total} = SS_{explained} + SS_{unexplained}$$

An outlier in linear regression is a data point that lies a great distance from the regression line. It can be defined as an observation with an extremely large residual.

To illustrate a potential outlier, consider the following example, where during one step in the synthesis of a biological product there is a brief fermentation period. The concentration (in percent) of one component is evaluated to determine if changes will influence the yield in units produced. The results of the experiment are presented in Table 23.7. If we perform a regression analysis (Table 23.8), as described in Chapter 14, we would reject the null hypothesis and conclude that there is a straight line relationship between our two variables. Therefore, we can draw a straight line through our data and graphically present it (Figure 23.2). Is the data point at the 4.5% concentration an outlier or simply an extreme measurement?

Graphing techniques involving residuals can be useful in identifying potential outliers in one variable. For example if the box-and-whisker plot method were applied

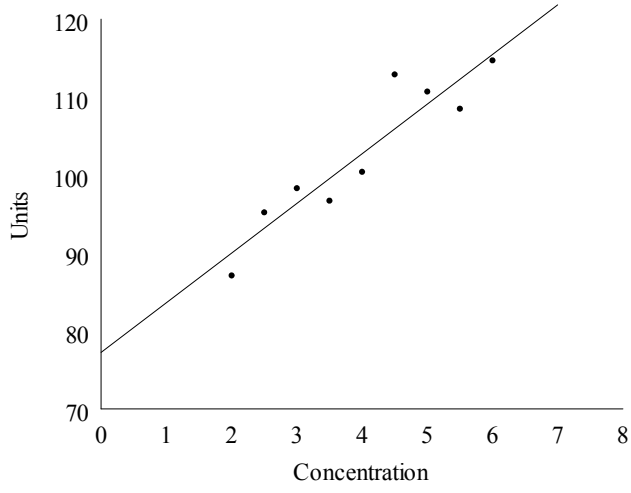


Figure 23.2 Data and best-fit line for yield versus various concentrations.

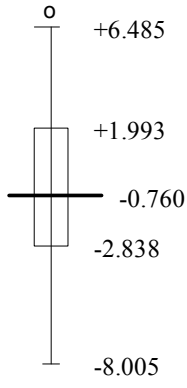


Figure 23.3 Box-and-whisker plot of residuals.

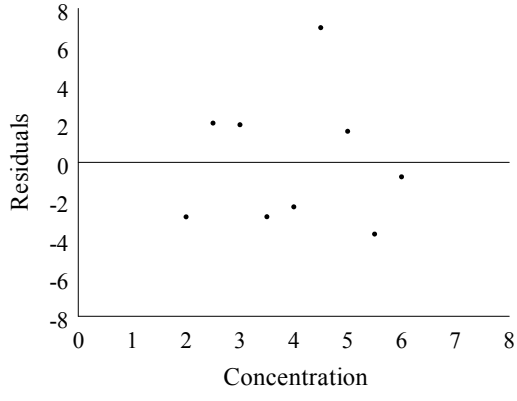


Figure 23.4 Scatter diagram showing residuals.

(Figure 23.3) to the residuals in Table 23.7 we would see that the residual of +6.985 for 4.5% seems to be an outlier. Note that the second largest residual (3.735) does not fall outside the lower whisker and would not be considered an outlier using the visual method.

A second method would be to create a **residuals plot**, which is a scatter plot of the residuals against their corresponding outcomes (dependent variable), where the independent variable is on the *x*-axis and the residuals plotted on the *y*-axis. The residuals seen in Table 23.7 are used and plotted in Figure 23.4. Once again the residual +6.985 visually appears to be an outlier. Similar to univariate outliers, the plotting of residuals can help with subjective decisions about the possibility that a data point is an outlier.

Residual plots, like the one seen in Figure 23.4 should be a random scattering of points and there should be no systematic pattern. There should be approximately as many

positive points as negative ones. Note in Table 23.7 that the sum of the residuals equals zero. Outliers are identified as points far above or below the center line. Instead of plotting the residuals (Figure 23.4), we can plot the **studentized residuals** that are calculated:

$$t = \frac{y_i - y_c}{\sqrt{MS_E}} \qquad \text{Eq. 23.14}$$

where MS_E is the $MS_{residual}$ taken from the ANOVA table used to test for linearity. These studentized values are scaled by the estimate of the standard error so their values follow a Student *t*-distribution (Tables B5 and B6 in Appendix B). Use of the studentized residuals makes systematic trends and potential outliers more obvious. Figure 23.5 shows the studentized residual plot of the same data seen in Figure 23.4. Note that the studentized value at 4.5% concentration does not exceed the critical *t*-value of $t_8(0.975) = 2.306$; therefore, we cannot statistically reject this value as an outlier. One rule of thumb is to consider any point on an outlier if its standardized residual value is greater than 3.3. This would correspond to an $\alpha = 0.001$.

There are more objective statistical procedures available to evaluate such extreme points based on the residuals. One process known as **studentized deleted residuals** is a popular method for identifying outliers when there are multiple continuous variables. It involves deleting the outlying observation and refitting the regression model with the remaining $n - 1$ observations. By refitting the model, it is possible to predict if the observation was deleted as an outlier if the residual was large. It requires calculations involving the standard error estimated for each deleted residual and are best handled through computer manipulation of the data. A detailed explanation of the studentized deleted residual method is found in Mason (1989, pp. 518-521).

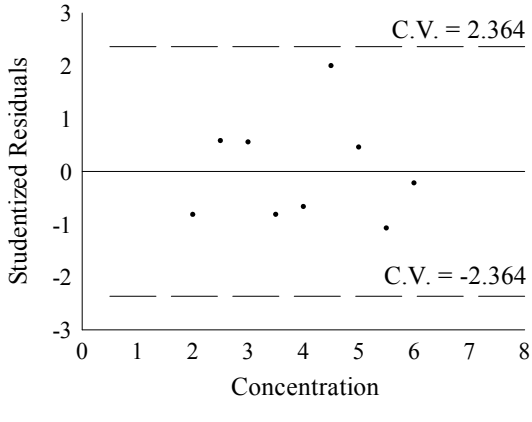


Figure 23.5 Scatter diagram studentized residuals.

For correlation problems, an outlier (represented by a pair of observations that are clearly out of the range of the other pairs) can have a marked effect on the correlation coefficient and often lead to misleading results. Such a paired data point may be extremely large or small compared to the bulk of the other sample data. This does not mean that there should not be a data point that is greatly different from the other data points on one axis as long as there is an equal difference on the second axis, which is consistent with the remainder of the data. For example, look at the two dispersions in Figure 23.6. It appears that the single lone data point (*A*) on the left scatter diagram is consistent with the remainder of the distribution (as x increases, y also appears to increase). In contrast, point (*B*) on the right scatter diagram is going in the opposite direction from the other sample points.

The problem occurs when one data point distorts the correlation coefficient or significantly changes the line of best-fit through the data points. One check for a potential outlier is to remove the single observation and recalculate the correlation coefficient and determine its influence on the outcome of the sample. For example consider the data in Figure 23.7, where the data point at the extreme left side might be an outlier. Without this one point there is virtually no correlation ($r = .07$) and a best-fit line drawn between these points has slight positive slope ($b = +0.426$). However, if this point is added into our calculations, there is a "low" negative correlation ($r = –0.34$) and our best-fit line changes to a negative slope ($b = –0.686$). One method for deciding to classify a data point as an outlier might be to collect more data to determine if the number is a true outlier or just an extreme value of a trend that was not noted in the original data.

Two additional problems may be seen with bivariate outliers. The first is swamping, which was previously described as several good data points that may be close to the suspected outlier and mask its effect. Using graphing techniques, it is possible to identify a cluster of data points and these might influence tests for outliers. The second involves influential observations, which are data points that have a pronounced influence on the position of the regression line. If removed, the remaining data can be refitted and the position of the regression line may shift by a significant amount. An outlier and an influential observation are not necessarily the same. Studentized deleted residuals may be helpful in identifying influential observations.

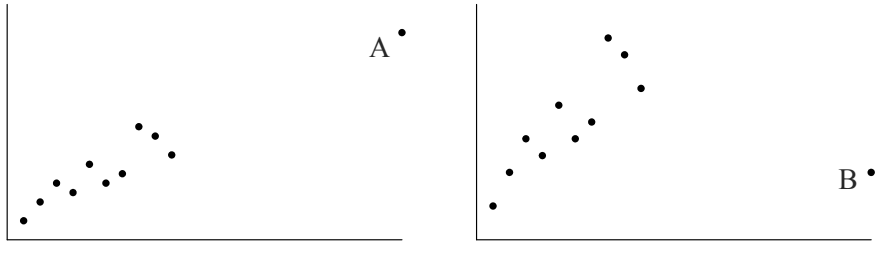


Figure 23.6 Examples of two correlation distributions.

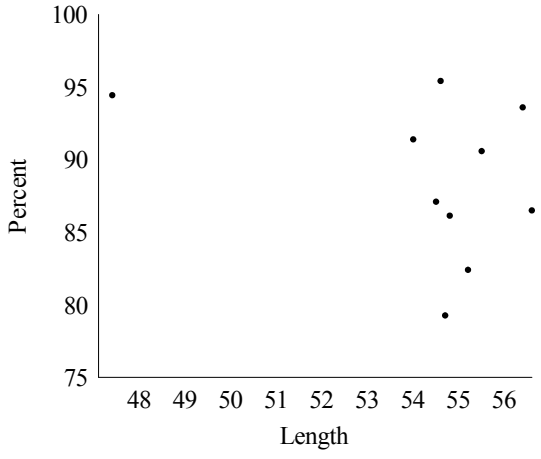


Figure 23.7 Possible outlier with a correlation example.

References

Dixon, W.J. (1953). "Processing data for outliers," *Biometrics* 1:74-89.

Dixon, W.J. and Massey, F.J. (1969). *Introduction to Statistical Analysis*, McGraw-Hill, New York, pp. 330-332.

FDA Draft Guidance (1998). "Investigating Out of Specification (OOS) Test Results for Pharmaceutical Production, Guidance for Industry," FDA, Rockville, MD.

FDA Draft Guidance (2006). "Investigating Out of Specification (OOS) Test Results for Pharmaceutical Production, Guidance for Industry," FDA, Rockville, MD (http://www.fda.gov/downloads/Drugs/Guidances/ucm070287.pdf).

Grubbs, F.E. (1969). "Procedures for detecting outlying observations in samples," *Technometrics* 11:1-21.

Hampel, F.R. (1985). "The breakdown points of the mean combined with some rejection rules," *Technometrics* 27:95–107.

Marascuilo, L.A. (1971). *Statistical Methods for Behavioral Science Research*, McGraw Hill, New York, 1971, p. 199.

Mason, R.L., Gunst, R.F., Hess, J.L. (1989). *Statistical Design and Analysis of Experiments*, John Wiley and Sons, New York, pp. 518, 526.

Rodda, B.E. (1990). "Bioavailability: design and analysis," *Statistical Methodology in the Pharmaceutical Sciences*. Berry, D.A., ed., Marcel Dekker, New York, p. 78.

Taylor, J.K. (1987). *Quality Assurance of Chemical Measures*, Lewis Publishers, Chelsea, MI, pp. 37-38.

USP (2011). <1010> Analytical Data—Interpretation and Treatment, United States Pharmacopeia/National Formulary, Rockville, MD, pp. 419-430.

United States v. Barr Laboratories, Inc., 812 F. Supp. 458, 1993 (U.S. D.C. New Jersey).

Suggested Supplemental Readings

Aggarwal, C.C. (2013). *Outlier Analysis*, Springer, New York.

Barnett V., Lewis, T. (1994). *Outliers in Statistical Data*, Third edition, John Wiley and Sons, New York.

Bolton, S. and Bon, C. (2004). *Pharmaceutical Statistics: Practical and Clinical Applications*, Fourth edition, Marcel Dekker, Inc., New York, pp. 300-309.
Mason, R.L., Gunst, R.F., and Hess, J.L. (1989). *Statistical Design and Analysis of Experiments*, John Wiley and Sons, New York, pp. 510-527.

Example Problems (Answers are provided in Appendix D)

1. Is the data point 12.9 an outlier from the following set of observations?

   12.3, 12.0, 12.9, 12.5, 12.4

2. The analytical laboratory at Acme Chemical assayed a solution that was assumed to be homogeneous, but found the following assay results (in percent). Is 94.673 a possible outlier?

   89.470, 94.673, 89.578, 89.096, 88.975, 89.204
   87.765, 91.993, 89.954, 90.738, 90.122, 89.711

3. An experiment was designed to evaluate different theoretical concentrations of a particular agent. Based on HPLC analysis, the following recoveries were observed. Is the observation at 50% a possible outlier?

Theoretical %	% Recovered	Theoretical %	% Recovered
30	30.4	80	81.6
40	39.7	90	89.3
50	42.0	100	100.1
60	59.1	110	109.7
70	70.8	120	119.4

Table 23.9 Data Produced by Two Different Groups

Group 1	Group 2
0.85	3.15
3.10	3.28
3.25	3.31
3.36	3.41
3.58	3.58
3.41	3.66
3.66	3.73
3.69	3.74
3.74	3.77
3.79	3.80
3.93	4.10
4.20	4.25
4.41	4.36
4.51	4.48
5.00	4.80

4. Data presented in Table 23.9 is collected for comparing two groups of data. The researcher is interested in whether or not there is a difference between the two groups. At the same time she is concerned about the value 0.85 in Group 1. Is this value a statistical outlier? If so, does removing it change the results of the comparison of the two groups?

24

Statistical Errors in the Literature

In the preface to this book, we discussed the need for a better understanding of statistics in order to avoid research mistakes and to be better able to identify possible errors in published documents. It only seems fitting to conclude this book by reviewing the prevalence of these mathematical misadventures and identifying some of the most common types of statistical errors.

The purpose of this chapter is to point out errors that can occur, not to criticize individual authors. It is doubtful that any of the errors described below were the results of intentional manipulation of findings or overt attempts to mislead the reader. More than likely, they are errors committed due to a misunderstanding or misinterpretation of the statistics involved with the evaluating the findings. Therefore, examples will be presented without reference to the specific author(s), articles, or journals of publication. However, the reader should appreciate that these are all genuine errors that have appeared in refereed journals of medicine or pharmacy.

Errors and the Peer Review Process

By the end of the 20th century, the use of statistical analysis in published works had increased greatly, due in no small part to the ease, accessibility, and power of modern desktop and laptop computers. This also led to an increase in the complexity of the procedures performed and reported in the literature. As noted by Altman (1991) there was an increasing trend to use statistics in the medical literature, which were usually not taught to medical students during their education and may not have been taught in postgraduate programs. He found a dramatic decrease between 1978 and 1990 in the percentage of papers that contained no statistics or only descriptive statistics (Table 24.1). The number of simple inferential statistics (e.g., t-test, chi square) remained the same, but more complex statistics increased greatly during that time period. There is no reason not to believe that this trend has continued, especially due to easier access to statistical software. Earlier work by Felson and colleagues (1984), showed an even more dramatic increase in the use of statistics in *Arthritis and Rheumatism*, between the years 1967–1968 and 1982 (Table 24.2).

As pointed out by Glantz (1980), few researchers have had formal training in biostatistics and "assume that when an article appears in a journal, the reviewers and

Table 24.1 Changes in the Use of Statistics in the Literature

	1978	1990
No statistics or descriptive only	27%	11%
t-tests	44%	39%
Chi square	27%	30%
Linear regression	8%	18%
Analysis of variance	8%	14%
Multiple regression	5%	6%
Nonparametric tests	11%	25%

From: Altman, D.G. (1991). "Statistics in medical journals: developments in the 1980s," *Statistics in Medicine* 10:1899.

Table 24.2 Changes in the Use of Common Statistics

	1967-1968	1982
t-tests	17%	50%
Chi square	19%	22%
Linear regression	1%	18%

From: Felson, D.T. et al. (1994). "Misuse of statistical methods in *arthritis and rheumatism*," *Arthritis and Rheumatism* 27:1020.

editors have scrutinized every aspect of the manuscript, including the statistical methods." As he noted this assumption was usually not correct. Have things changed that much in the past 30 years? Are today's researchers any more knowledgeable of statistics, even though they now have the power of very sophisticated software packages in their desktop computers? Most journals do not employ a statistician or involve a statistician in their review process. McGuigan (1995) noted that only a small portion of the articles he reviewed (24% to 30%) employed a statistician as coauthors or acknowledged their help in papers. In fact, in the peer review process, colleagues reviewing articles submitted to journals probably have about the same statistical expertise as the authors submitting the manuscript.

During a 50-year period between 1961 and 2011 there were several articles presented in the medical literature that report the incidence and types of errors seen in publications (Table 24.3). In these papers statisticians review either all the articles published during a given time period (usually one year) in a specific periodical or a random sample of articles from a publication over a longer period. These errors are related to mistakes in the medical literature, because this is an area where most of the research has been conducted. However, it is doubtful that the incidence of these errors is any less frequent in the pharmacy literature.

A problem to consider with the results presented in Table 24.3 was that most of these evaluations used different methods of assessing mistakes and there were no

Table 24.3 Prevalence of Statistical Errors in the Literature (percent of articles with at least one statistical error)

Percent	Journal(s)	Reference
57	*Canadian Medical Association Journal* and *Canadian Journal of Public Health*, 1960	Badgley, 1961
60	*Arthritis and Rheumatism*, 1967-1968	Felson, 1984
42	*British Medical Journal*, 1976	Gore, 1976
44	*Circulation*, 1977	Glantz, 1980
45	*British Journal of Psychiatry*, 1977-1978	White, 1979
66	*Arthritis and Rheumatism*, 1982	Felson, 1984
65	*British Journal of Anaesthesia*, 1990	Goodman and Hughes, 1992
74	*American Journal of Tropical Medicine and Hygiene*, 1988	Cruess, 1989
54	*Clinical Orthopaedics and Related Research*, *Spine*, *Journal of Pediatric Orthopaedics*, *Journal of Orthopaedic Research, Journal of Bone and Joint Surgery* and *Orthopedics*, 1970-1990	Vrbos, 1993
75	*Transfusion*, 1992-1993	Kanter and Taylor, 1994
40	*British Journal of Psychiatry*, 1993	McGuigan, 1995
54	*Infection and Immunology,* 2002	Olsen, 2003
82	*Human Reproduction* and *Fertility and Sterility*, 2001	Vail and Gardener, 2003
79	*Korean Journal of Pain*, 2004-2008	Yim, et al., 2010
52	*The International Journal of Oral and Maxillofacial Implants, The Journal of the American Dental Association,* and 12 other dental journals, 1995-2009	Kim, et al., 2011

standardized criteria for defining statistical errors. Therefore, the same error may be defined differently or the researchers may have been focusing their attentions on different parameters for establishing such errors. As errors are discussed, citations will be made to the articles presented in Table 24.3 and the proportion of such errors identified by the various authors in their research of the medical literature.

Problems with Experimental Design

Many of the problems reported in the literature relate to the design of the studies. Ultimately such experimental design problems will show flawed statistical results. For example, many studies have inadequate or no control groups as part of the design. These types of incidences were reported to be as high as 41% (McGuigan, 1995) and 58% (Glantz, 1980). Outcomes from various medical interventions are extremely difficult to evaluate without a control set of subjects to determine if the outcome

would occur without the intervention.

As discussed in Chapter 3, there are two requirements for any statistical procedure: 1) samples are selected or volunteers assigned by some probabilistic process and 2) each measurement is independent of all others (except in certain repeat measurement designs). Unfortunately McGuigan (1995) and Cruess (1989) found errors related to randomization in 43% and 12%, respectively, of the articles they evaluated. Also there was a disregard for statistical independence in 10% of the articles reviewed by Gore and colleagues (1977) and 5% of those reviewed by Kanter and Taylor (1994).

In one research project it was found that 5% of studies failed to state a null hypotheses (McGuigan, 1995) and in a second study, questionable conclusions were drawn from the results in 47.5% of the articles evaluated (Vrbos, 1993). Excellent books exist on research design studies, especially by Friedman and colleagues (2010), that are more effective in evaluating the desired outcomes.

Another problem commonly seen in the methodology sections of papers, is a failure to state and/or reference statistics used in the article. Failure to cite the specific statistics used were found in 41.5% of the articles reviewed by McGuigan (1995) and 13% of those by Kanter and Taylor (1994). In addition, studies of the medical literature found that many times conclusions were stated without any indication which statistical tests were performed (49% for Kanter and Taylor, 1994; and 35.7% for Vrbos, 1993). Failure to document the statistical method used or using an incorrect method has been noted as one of the more common errors in the literature (Murphy, 2004).

Another common problem is a failure of authors to cite references for lesser known statistical procedures employed in their data analysis. Commonly used procedures (t-tests, ANOVA, correlation, linear regression, and even some of the popular nonparametric tests) need not be referenced. But lesser used procedures should be referenced so readers can understand the inferential statistic(s) involved. Nothing is more frustrating than to have a colleague or student ask about A-B-C statistical procedure, then: 1) to search Medline or PubMed for references to that test and find 10 to 15 articles mentioning the A-B-C test in the online abstract; 2) to retrieve all the articles from the library; and 3) to find that not one of the authors cited a source for the A-B-C test in the methodology sections. More than likely the A-B-C test was part of a printout involved with a sophisticated software package and referenced somewhere in that software's reference manual. Even referencing the software would help readers seeking more information about a specific test.

Standard Deviations versus Standard Error of the Mean

When reporting continuous data, it is important to describe the centers of the distribution and provide information about the dispersion of observations around the center(s). Unfortunately, studies by Gore and colleagues (1977) and White (1979) reported inadequate description of basic data, including centers and dispersions in 16.1% and 12.9% of the articles they reviewed, respectively.

As discussed in Chapter 5, the standard deviation (*S*) measures dispersion of the sample and provides an estimate of the dispersion of the population from which the sample was taken. In contrast the standard error of the mean (*SEM*), or standard error

Table 24.4 Examples of Failure to Identify *S* or *SEM* (n = 45)

Parameter	Mean Baseline Value	Mean Value at 4-8 years (mean = 5.3 years)
Total cholesterol (nmol/L)	7.17 ± 0.83	7.01 ± 0.92
HDL cholesterol (nmol/L)	1.17 ± 0.41	1.39 ± 0.36*
Triglycerides (nmol/L)	1.38 ± 0.63	1.35 ± 0.61

*Statistically significant increase ($p < 0.05$).

HDL = high-density lipoprotein.

(*SE*), is a measure of how all possible sample means might vary around the population mean. As seen in the following equation (Eq. 7.3), the *SEM* will always be smaller than *S*.

$$SEM = \frac{S}{\sqrt{n}}$$

Because *SEM* is smaller, investigators will often report that value because it gives the perception of greater precision.

Often authors fail to state the measurement to the right of the ± symbol (7.1% from White's research, 1979; 13% for Felson et al., 1984; and 24% for Kanter and Taylor, 1994). Is it the *S* or the *SE*, or even relative standard deviation (*RSD*)? If a parameter is not stated, the reader cannot adequately interpret the results. Even if the authors state in the methodology what is represented by the value to the right of the ± symbol, tables should still be self-explanatory, so readers can evaluate the results. For example, in an article evaluating serum lipid levels after long-term therapy with a calcium channel blocking agent, the author made the following statement: "After a mean treatment period of 5.3 years, total cholesterol and triglyceride levels were not significantly different from baseline, whereas the mean high-density lipoprotein cholesterol value increased significantly from 1.17 ± 0.41 nmol/L at the initiation of treatment to 1.39 ± 0.36 nmol/l at 5.3 years ($p < 0.05$)." The findings were presented in a table and an abbreviated version of this table is presented in Table 24.4. Unfortunately, nowhere in the article did the author state whether the values to the right of the ± symbol in the table or the text represent the standard deviation or the standard error of the mean. Only after recalculating the statistics is it possible to determine that the values reflect the standard deviation. Looking solely at the HDL cholesterol data in Table 24.4, if the measure of dispersion was the standard deviation, a two-sample t-test produces a *t*-value of 2.705, $p < 0.003$. In contrast, if the figure to the right of the ± symbol was the SEM, the two-sample t-test result would be $t = 0.40$, $p > 0.35$. Thus, data in the original table represents the mean ± standard deviation. However, the only way to determine this is to actually recalculate the statistical outcome. Murphy lists "not identifying or properly labeling the type of

Table 24.5 Examples of Skewed Data Evaluated Using ANOVA

Nasal EDN (ng/ml)	Drug A (n = 16)	Drug B (n = 14)	Placebo (n = 15)
Original information cited in article (mean ± SE):			
Treatment day 1	245 ± 66	147 ± 49	275 ± 133
Treatment day 15	78 ± 34*	557 ± 200	400 ± 159
Data modified to reflect dispersion of the sample (mean ± SD)			
Treatment day 1	245 ± 264	147 ± 183	275 ± 515
Treatment day 1	78 ± 136*	557 ± 748	400 ± 615

* $p < 0.05$ versus Drug B or placebo based on change from day 1 to day 15.

variance estimate" as one of the more common errors in the medical literature (2004).

Another potential problem is using the standard deviation for nonnormal data. As discussed in Chapter 6, the standard deviation reflects certain mathematical characteristics associated with normally distributed data. The median and quartiles are more appropriate measures for skewed distributions. However, McGuigan (1995) reported that 39 of the 164 papers he reviewed (24%) used the mean and standard deviation for describing skewed or ordinal data. This occurred with less frequency (19%) in the work by Kanter and Taylor (1994). An example of skewed data can be seen in a recent article comparing two drugs and their effects on the amount of eosinophil-derived neurotoxin (EDN). Part of the results is presented in the upper half of Table 24.5 and the authors report that they "compared between treatment groups using t-tests." Also, "values of $p < 0.05$ were considered statistically significant." Note that the outcomes are reported as mean ± standard error. Converting the dispersion to standard deviations ($S = SEM \cdot \sqrt{n}$) we find the results presented in the lower portion of Table 24.5. Note in all cases that the standard deviation is larger than the mean, indicating data that is positively skewed. A nonparametric procedure or log transformation of the original data would have been the preferred method for analyzing the data.

Another problem with data dispersion is the evaluation of ordinal data by calculating a mean and standard deviation (Kim, 2011; Shott, 2011). This was identified in 25% of articles reviewed by Avram and colleagues (1985). An example of the use of parametric procedures to evaluate ordinal data is presented in a publication from the 1980s, where women who received a lumpectomy or mastectomy for breast cancer were asked to rate their feelings of femininity. The authors used a simple three-level ordinal scale (0 = no change, 1 = a little less feminine, and 2 = moderately less feminine). Unfortunately, the authors took the responses, calculated means and standard deviations for women with lumpectomies versus those with mastectomies, and evaluated the data using a two-sample t-test ("$t = 4.35$, $p < 0.01$" after 14 months). The more appropriate assessment would have been a chi square test of independence with frequencies of responses in each of the following cells:

	No Change	A Little Less Feminine	Moderately Less Feminine
Lumpectomy			
Mastectomy			

Problems with Hypothesis Testing

We know from our previous discussions in Chapter 8 that the Type I error rate can be expressed as either α or p (*a priori* or *post hoc*, respectively) and provides the researcher with a certain degree of confidence ($1 - \alpha$) in statistics. Unfortunately in Vrbos' (1993) review of the literature there was confusion over the level of significance or meaning of p in 46% of the articles surveyed.

A second problem, which appears less frequently, is assuming the null hypothesis is true simply because the researcher fails to reject the null hypothesis. As discussed in Chapter 8, the null hypothesis is never proven; we only fail to reject it.

A third problem is the correct identification or proper use of one-tailed or two-tailed statistical procedures (Rigby, 1998). In some cases the reader is not informed which type of test is performed or the incorrect test may be used based on the hypotheses tested.

A fourth problem related to hypothesis testing is the failure to perform a prestudy power calculation or the failure to have an adequate sample size. This was observed in 50% of the articles reviewed by McGuigan (1995). For example, in a study comparing two routes of administration of a hematopoietic growth factor the authors reported the data in Table 24.6. Note the small sample size, n = 4. If there was a significant difference (e.g., 20%) at the <100 U/Kg/wk dosage, how many subjects would be required to detect such a difference? The authors used an ANOVA to evaluate the results. Since there are only two levels of the independent variable, we can use the formula presented in Chapter 8 (Eq. 8.2) as a quick estimate of the number of subjects required to detect a 20% difference with 80% power. Performing the calculations found that the required number of subjects would be 188 per delivery system. This large number is due primarily to the large variance in the sample data. In the study of published articles Vail and Gardener found that 54% of studies they reviewed "gave no statistical consideration of sample size" (2003).

The following is an example of a 1998 clinical trial protocol where the researchers clearly attempted to control the Type II error rate. "A sample size of 28 healthy males will be enrolled in this study to ensure study completion by at least 24

Table 24.6 Comparison of Mean Posologies at the End (Day 120) of Study

Dosage	Time	IV Group (n = 4)	SC Group (n = 4)	Statistical Difference
>150 U/Kg/wk	Day 120	255 ± 131	138 ± 105	$P < 0.01$
<100 U/Kg/wk	Day 120	69 ± 45	58 ± 43	ns

Table 24.7 Volunteer Demographics

	Group A	Group B
Age (yr)	67.4 ± 5.8	61.4 ± 8.6 *

* $p = 0.0539$

patients. Based on (*a previous study)* a sample size of 24 patients can provide at least 80% probability to show that the 90% confidence interval of the mean AUC value for the clinical lot of *Drug B* is within ±20% of the reference (commercial lot) mean AUC value."

Readers should be cautious of papers that report unnecessarily small and overly exact probabilities. For example, in a 1988 publication the authors were reporting the difference in parasitic infection rates in children in a developing country and the change in the frequencies of infections before and after their particular intervention. The change reported "for prevalence in 1984 versus 1985, $\chi^2 = 624$, $df = 1$, $p < 10^{-11}$)." In other words, the Type I error rate was less than 0.00000000001! This paper clearly overstates the obvious. A second example, illustrating probabilities that are too exact, comes from a 1993 article presenting volunteer demographics (Table 24.7). Good luck finding a statistical table that provides a column for $p = 0.0539$! Also, note that the authors failed to indicate what the values were to the right of the ± symbol. In both cases, it appears that the authors were simply reporting results directly from the computer printout, without any attempt to apply a reasonable explanation to their results. This type of presentation of statistical results should warn the reader to read the article with extreme caution to ensure that the appropriate analysis was performed and correct interpretation stated. According to Rigby, in reporting extreme p-values "this degree of exactness is very difficult to conceptualize and it could be argued that this kind of precision has limited scientific value" (1988, p. 124).

There are situations where computer programs may truncate the *p*-value. For example, many times Minitab will report the results as $p = 0.000$. In these cases the reportable *p*-value is $p < 0.001$ because the researcher has no knowledge of values past the third zero.

Problems with Parametric Statistics

As discussed in Chapter 9, the two additional underlying requirements for performing a parametric statistic (t-tests, F-tests, correlation, and regression) are that the data: 1) come from populations that are normally distributed and 2) that sample variances (which are reflective of the population variances) be approximately equal (homogeneity of variance). The use of statistical tests that require an underlying normal distribution where data are not normally distributed is a commonly identified error in the literature (Olsen, 2003; Murphy, 2004).

One common error is to perform a parametric test on data that is obviously skewed. The incidence of such mistakes ranges from 8% (Kanter and Taylor, 1994) and 17.7% (Gore, 1977), to as large as 54% (McGuigan, 1995). Note in the data cited

Table 24.8 Comparison of Eight Subjects Following a Single Oral Dose of a Drug at 10 and 22 Hours

	C_{max} ng/ml^{-1}	
Subject	10.00 h	22.00 h
1	59.5	18.6
2	75.2	7.5
3	33.6	18.9
4	37.6	33.9
5	27.8	20.8
6	28.4	14.9
7	76.8	29.7
8	37.5	15.0
Mean (SD)	47.1 (20.4)	19.9 (8.4)*

* $p < 0.05$ compared to 10.00 h (analysis of variance).

in Table 24.5 that the standard deviations are greater than the means that would indicate that the data is positively skewed.

One method for correcting this problem is to transform the data so the resultant distribution is approximately normal (Chapter 6), for example, the log transformation of data from a positively skewed distribution. This is illustrated in the statistical analysis section of a paper by Cohn and colleagues (1993), where they evaluate cardiac function: "Because values were extremely skewed to the right, the Holter monitor results were transformed using the logarithmic transformation…." An alternative approach would be to perform one of the nonparametric procedures.

A second type of error related to parametric and nonparametric procedures is confusing paired vs. unpaired data and performing an inappropriate statistical test (e.g., an ANOVA instead of a randomized block design or a paired t-test for unpaired data). Paired data obviously has advantages in clinical trials where each person serves as his or her own control and it provides a more rigorous test, because we are evaluating changes within individual subjects. Kanter and Taylor (1994) noted that in 15% of the articles they studied, the wrong t-test (paired/unpaired) was used and McGuigan (1995) found that in 26% of the papers he studied the type of t-test (paired/unpaired) was not mentioned. For example, a comparison of the pharmacokinetic results between two time periods is presented in Table 24.8. As indicated in the table and the methodology section of the original paper, "the statistical analysis employed analysis of variance." As seen in Table 24.8 this clearly represents paired data (each subject serves as his own control, measured at two separate time periods). The authors obviously established a decision rule and rejected the results for any $p < 0.05$. Recalculating the statistics we find the results to be even more significant than reported in the article: $F = 12.09$, $df = 1,14$, $p < 0.0037$. Obviously a two-sample t-test would produce the identical results: $t = 3.48$, $df = 14$, $p < 0.0037$. However, a more rigorous paired t-test shows that there is more Type I error when such a design is employed: paired-$t = 3.41$, $df = 7$, $p < 0.0113$. Unfortunately, in this particular

Table 24.9 Effects of Three Different Mouth Guards on Air Flow (n = 17)

	FEV_1 (liters)	PEF (l/min)
No mouth guard	3.46 (0.70)	508.65 (70.25)
Mouth guard 1	3.17 (0.16)†	472.88 (68.44) †
Mouth guard 2	2.97 (0.19) †	432.31 (78.99) †
Mouth guard 3	3.04 (0.86) †	428.38 (65.02) †

* Values represent means (s.d.)
† Values are significantly different ($p < 0.05$; ANOVA) from the values recorded with no mouth guard.

example the author failed to observe the requirement of homogeneity of variance in order to perform an ANOVA. Note that $S^2_{10h} = 416.16$ and $S^2_{22h} = 70.60$ are not close to establishing homogeneity. Thus, the most appropriate statistic would have been a paired t-test looking at the difference for each subject or a nonparametric Wilcoxon matched-pairs test; the results of such a procedure would be $Z = 2.52$, $p < 0.02$.

Another common error, discussed in Chapter 11, is the use of multiple t-tests to address a significant ANOVA where H_0: $\mu_1 = \mu_2 = \mu_3 \ldots = \mu_k$ is false. The compounding of the error using multiple t-tests was defined as experimentwise error rate (Eq. 11.2):

$$a_{ew} = 1 - (1 - \alpha)^C$$

To correct this problem, multiple comparison procedures were presented in Chapter 11. The incidence of this type of error has been fairly consistent around one of every four articles reviewed (27% for Glantz, 1980; 24% for Altman, 1991; and 22% for Kanter and Taylor, 1994). More recently it has been noted as a continuing problem by Murphy (2004) and Shott (2011). An example of the misinterpretation of data due to experimentwise error is illustrated in an article evaluating different athletic mouth guards and their effects on air flow in young adults (ages 20 – 36). The authors' findings are presented in Table 24.9. They concluded, based on this table, "that each of the three athletic mouth guards used in this study significantly reduced air flow ($p < 0.05$) .... Similarly, peak expiratory flow rates were significantly reduced by the different mouth guards ($p < 0.05$)." The authors clearly state in their table that the measure of dispersion is the standard deviation. Therefore, it is a relatively easy process to re-evaluate their data using the ANOVA formulas presented in Chapter 10 and the multiple comparison procedures in Chapter 11. This re-evaluation finds that there was in fact a significant difference with respect to the mouth guards tested and the outcome measures for only the *PEF*. The calculated *F*-value was 4.85 where the critical F-value for 95% confidence is 2.53. In fact the outcome was significant at $p < 0.005$. Assume the original hypothesis of equality was tested ($\alpha = 0.05$) the Scheffé *post hoc* pairwise comparisons with the same error rate find that there were only two significant differences: no mouth guard > mouth guard 2 and no mouth guard > mouth guard 3. Unlike the authors' findings, there was no significant difference

Table 24.10 Original Table Reporting Susceptibility Scores and Annual Frequency of BSE

	Perceived Susceptibility Scores			
	High (15 to 19)	Moderate (9 to 14)	Low (9)	Total
More than monthly	9	1	0	10
Monthly	31	5	0	36
6-11 times	11	3	0	14
1-15 times	19	3	0	22
Less than yearly	5	1	0	6
Never	13	10	1	24
Total	88	23	1	112

between the PEF for mouth guard 1 and no mouth guard. How could the authors have found a significant difference for all three mouth guards? If one calculates three separate two-sample t-tests comparing each mouth guard to no mouth guard, there is still no significant difference (t = 1.05). It appears that after finding a significant ANOVA, the authors simply assumed that all the mouth guards provided significantly less air flow. Without a statement in the methodology section on how significant ANOVAs were evaluated, the question must remain unanswered.

Errors with the Chi Square Test of Independence

As discussed in Chapter 16 the chi square test of independence is used to evaluate the independence or relationship (lack of independence) between two discrete variables. Overall problems with chi square analysis were identified in 15% of the articles reviewed by McGuigan (1995).

Two criteria are required in order to perform this test: 1) there cannot be any empty cells (a cell within the matrix where the observed frequency equals zero); and 2) the expected value for each cell must be equal to or greater than five. A common mistake in the literature is to proceed with the statistical analysis even though one or both of these criteria are violated. An excellent example of this type of error appears in an article evaluating the practice of breast self-examination (BSE) in relationship to "susceptibility" scores (risk factors) for developing breast cancer. The authors concluded the following: "Forty-one (36%) participants with high susceptibility scores practiced BSE monthly or more frequently (Table 24.10). However, chi square analysis showed no statistically significant difference in the level of perceived susceptibility of students and the frequency of BSE, $\chi^2(10) = 13.1925$, $p = 0.2131$, $\alpha = 0.05$". Note that 24% (5/21) of the cells are empty. If we calculated the expected values for each cell under complete independence we would determine that 67% of the cells fail to meet the criteria of expected values greater or equal to five. Clearly the use of the chi square test of independence was inappropriate for this contingency table. If we modify the data by collapsing the adjacent rows or columns in a logical

Table 24.11 Data Modified from Table 24.10 to Meet Criteria for the Chi Square Test of Independence

	High (15 to 19)	Low and Moderate (less than 15)	Total
12 or more times per year	40	6	46
1 to 11 times per year	30	6	36
Less than yearly or never	18	11	30
Total	88	23	112

order, we can create a matrix which fulfills the criteria required (Table 24.11). However, in doing this, we arrive at a decision exactly opposite that of the authors ($\chi^2(2) = 7.24$, $p < 0.05$). With $\alpha = 0.05$ there is a significant relationship between risk factors and the volunteers practice of BSE. Also, note in the original table that the frequency of the BSE variable did not represent mutually exclusive and exhaustive categories. It is assumed that this was a typographical error and the mid-range values should have been 1 to 5 times and 6 to 11 times, but it was presented in the article that the two categories overlapped.

If the sample size is too small or data fails to meet the required criteria, a Fisher's exact test should be utilized. The percent of articles with this type of error is approximately 5% (5% by Kanter and Taylor, 1994; and 6% by Felson, 1984). For example, Cruess (1989) discussed an article reporting a significant relationship between reactivity with parasite isolates based on primary or multiple attacks of malaria in subjects studied and presented the following results:

	Reactivity		
	Positive	Negative	
Primary Attack	1	2	3
Multiple Attacks	5	0	5
	6	2	8

The authors used a chi square test and reported a significant relationship ($p = 0.03$). However, if the more appropriate Fisher's exact test is performed (since there is one empty cell and all expected values are less than five), the result is no significant relationship exists ($p = 0.107$). An example of the appropriate use of Fisher's exact test is described in the methodology section of an article in *Gastroenterology*: "The responses to interferon were compared between the cirrhotic and noncirrhotic patients at various times of treatment and follow up, using χ^2 method or Fisher's exact test when appropriate" (Jouet, 1994).

Another type of problem with the chi square test of independence is the correction for continuity when there is only one degree of freedom. This type of error was identified with a frequency of occurring between 2.8% (McGuigan, 1995) and 4.8% (Gore, 1977). The following is a simple clarification in the methodology section by Parsch et al. (1997), which assists the reader in understanding the statistics

involved in the manuscript: "Categorical demographic data and differences in clinical outcome were analyzed by χ^2 with Yates correction factor … Statistical significance was established at a *p*-value of less than 0.05."

Summary

The purpose of this chapter has been to identify the most frequent statistical errors seen in the literature to better identify these mistakes in your own readings and assist you in avoiding them as you prepare written reports or publishable manuscripts.

One should always use caution when reading published articles in the literature. Make sure that the drug design and statistical tests are clearly described in the methodology section of the article. Altman (1991), George (1985), and McGuigan (1995) have indicated methods for improving the peer review process. These include requiring authors to indicate who performed the statistical analysis on submissions. Journals should clearly state minimum requirements for submission, even provide a standardized format regarding the nature of the research, the research design and the statistical analyses used in preparing the manuscript. Lastly, papers should be more extensively reviewed by statisticians and possibly include a statistician among the reviewers for papers submitted for publication. An incorrect or inappropriate statistical analysis can lead to the wrong conclusions and all false credibility (White, 1979).

Additional information on the types of statistical errors can be found in the classic publication by Huff (1954) or a more recent publication by Spirer et al. (1998), which are listed in the suggested supplemental readings. For specific information on designing and evaluation of clinical trails, the reader is referred to the book by Friedman and colleagues (1998), also listed in the suggested readings.

References

Altman, D.G. (1991). "Statistics in medical journals: developments in the 1980s," *Statistics in Medicine* 10:1897-1913.

Avram, M.J., Shanks, C.A., Dykes, M.H., Ronai, A.K., and Stiers, W.M. (1985). "Statistical methods in anesthesia articles: an evaluation of two American journals during two six-month periods," *Anesthesiology and Anal*gesia, 64:607-611.

Badgley, R.F. (1961). "An assessment of research methods reported in 103 scientific articles in two Canadian medical journals," *Canadian Medical Association Journal*, 85:246-250.

Cohn, J.B., Wilcox, C.S., and Goodman, L.I. (1993). "Antidepressant efficacy and cardiac safety of trimipramine in patients with mild heart disease," *Clinical Therapeutics* 15:114-122.

Cruess, D.F. (1989). "Review of use of statistics in the *American Journal of Tropical Medicine and Hygiene* for January-December 1988," *American Journal of Tropical Medicine and Hygiene* 41:619-626.

Felson, D.T., Cupples, L.A., and Meenan R.F. (1984). "Misuse of statistical methods in *Arthritis and Rheumatism,* 1882 versus 1967-68," *Arthritis and Rheumatism* 27:1018-1022.

Glantz, S.A. (1980). "Biostatistics: how to detect, correct and prevent errors in the medical literature," *Circulation* 61:1-7.

Goodman, N.W. and Hughes, A.O. (1992). "Statistical awareness of research workers in British anaesthesia," *British Journal of Anaesthesia* 68:321-324.

Gore, S.M., Jones, I.G., and Rytter, E.C. (1977). "Misuse of statistical methods: critical assessment of articles in BMJ from January to March 1976," *British Medical Journal* 1:85-87.

Jouet, P. et al. (1994). "Comparative efficacy of interferon alfa in cirrhotic and noncirrhotic patients with non-A, non-B, C hepatitis," *Gastroenterology* 106:686-690.

Kanter, M.H. and Taylor, J.R. (1994). "Accuracy of statistical methods in *Transfusion*: a review of articles from July/August 1992 through June 1993," *Transfusion* 34:687-701.

Kim, J.S., Kim D-K. and Hong S.J. (2011). "Assessment of errors and misused statistics in dental research," *International Dental Journal* 61:163-167.

McGuigan, S.M. (1995). "The use of statistics in the *British Journal of Psychiatry*," *British Journal of Psychiatry* 167:683-688.

Murphy, J.R. (2004). "Statistical errors in immunologic research," *Journal of Allergy and Clinical Immunology* 114:1259-1263.

Olsen, C.H. (2003). "Review of the use of statistics in *Infection and Immunity,*" *Infection and Immunity* 71:6689-6692.

Parsch, D.J. and Paladino, J.A. (1997) "Economics of sequential ofloxacin versus switch therapy," *Annals of Pharmacotherapy* 31:1137-1145.

Rigby, A.S. (1998). "Statistical methods in epidemiology: I. Statistical errors in hypothesis testing," *Disability and Rehabilitation* 20:(4)121-126.

Shott, S. (2011). "Detecting statistical errors in veterinary research," *Journal of the American Veterinary Medicine Association* 238:305-308.

Vail, A. and Gardener, E. (2003). "Common statistical errors in the design and analysis of subfertility trials," *Human Reproduction* 18:1000-1004.

Vrbos, L.A., Lorenz, M.A., Peabody, E.H., et al. (1993). "Clinical methodologies and incidence of appropriate statistic testing in orthopaedic spine literature: are statistics misleading?" *Spine* 18:1021-1029.

White, S.J. (1979). "Statistical errors in papers in the *British Journal of Psychiatry*," *British Journal of Psychiatry* 135:336-342.

Yim, K.H., Hahm, F.S., Han, K.A. and Park. S.Y. (2010). "Analysis of statistical methods and errors in the articles published in the *Korean Journal of Pain,*" *Korean Journal of Pain* 23:(1)35-41.

Supplemental Suggested Readings

Friedman, L.M., Furberg, C.D. and DeMets, D.L. (2010). *Fundamentals of Clinical Trials*, Fourth edition, Springer-Verlag, New York.

Huff, D. (1954). *How to Lie with Statistics*, W.W. Norton and Company, New York.

Murphy, J.R. (2004). "Statistical errors in immunologic research," *Journal of Allergy and Clinical Immunology* 114:1259-1263.

Shott, S. (2011). "Detecting statistical errors in veterinary research," *Journal of the American Veterinary Medicine Association* 238:305-308.

Spirers, H.F., Spirers, L. and Jaffee, A.J. (1998). *Misused Statistics: Straight Talk for Twisted Numbers*, Second edition, Marcel Dekker, Inc., New York.

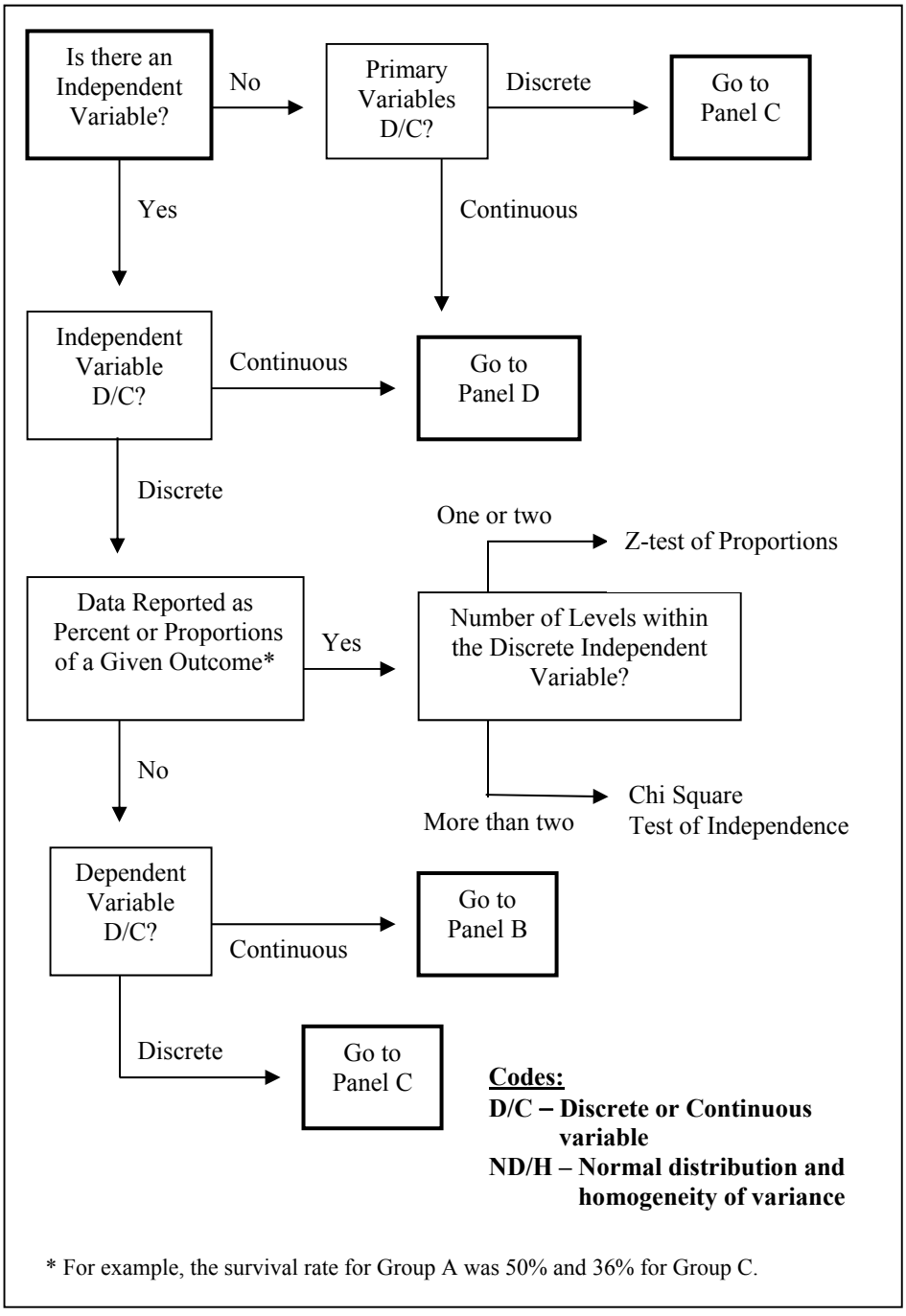
Appendix A

Flow Charts for Selection of Appropriate Inferential Tests

On the following pages are a series of panels that give direction on selecting the most appropriate inferential statistical test to use, based on the types of variables involved in the outcomes measurement.

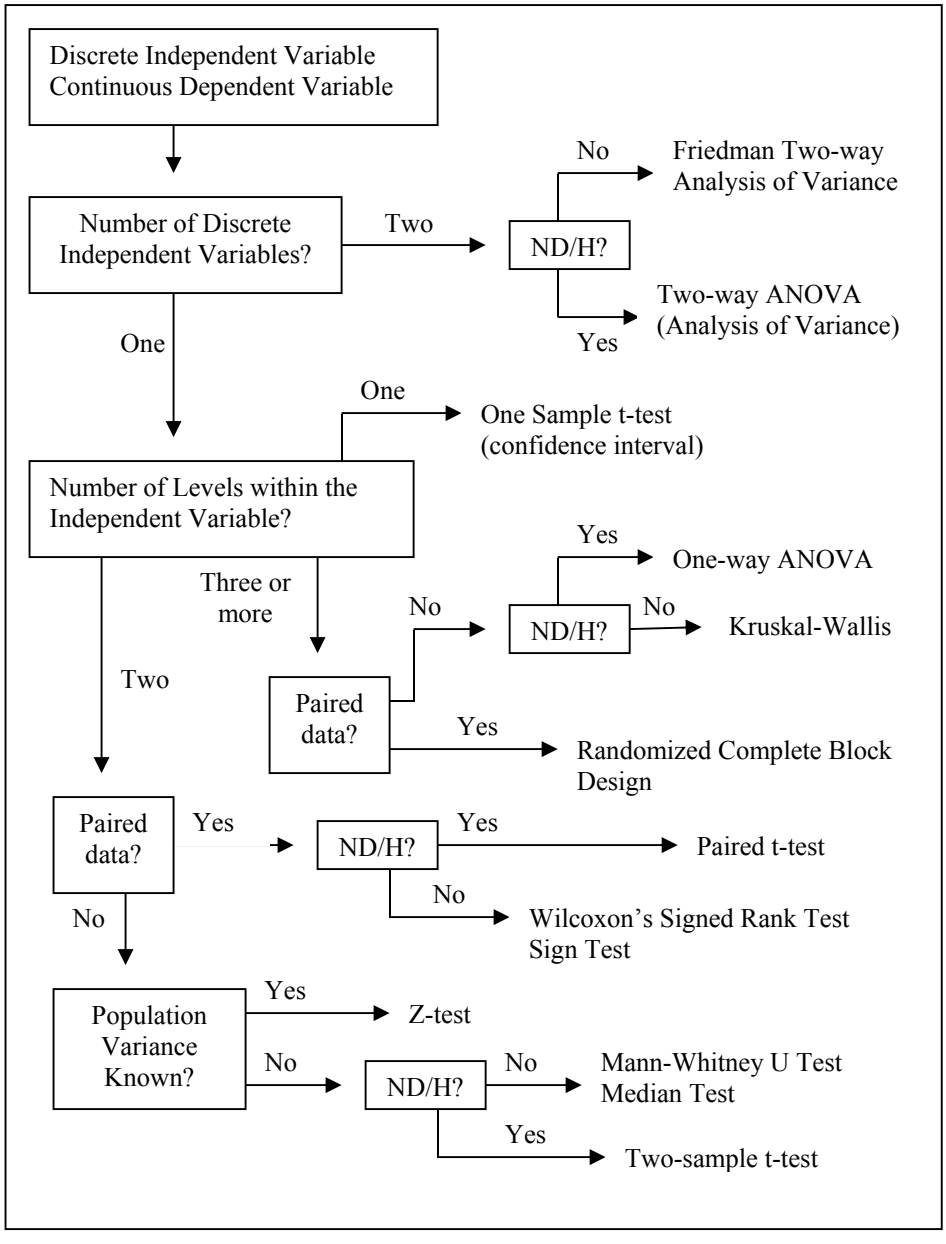
For any given hypothesis being tested, the researcher must first identify the independent variable(s) and/or dependent variable(s). This begins the process seen in Panel A. Next the researcher must consider if the data presented by the respective variables involves discrete or continuous data (D/C?). Lastly, at various points in the decision making process the researcher must determine if the sample data comes from populations that are normally distributed and, if more than one level of a discrete independent variable, does there appear to be homogeneity of variance (ND/H?).

Panel A

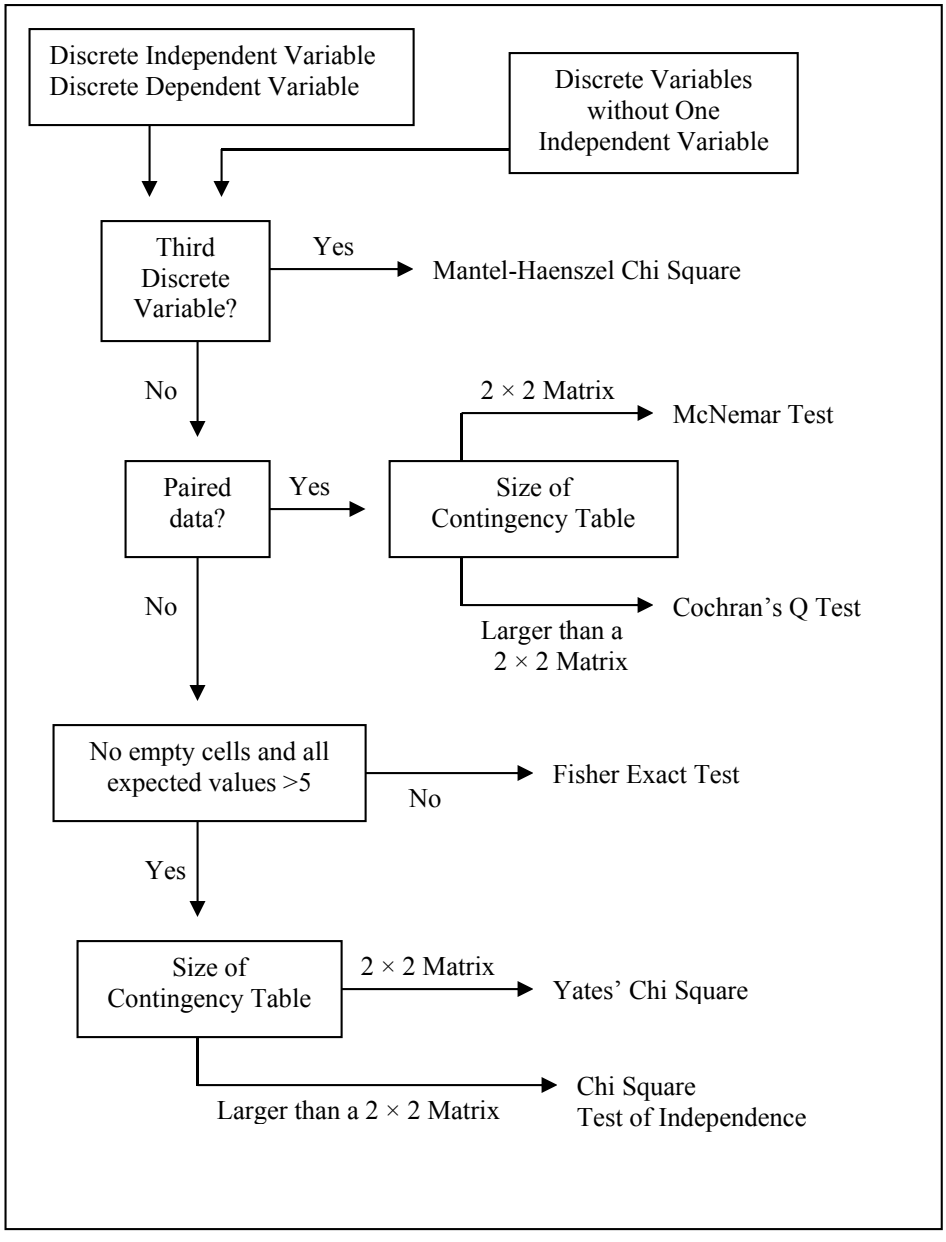


* For example, the survival rate for Group A was 50% and 36% for Group C.

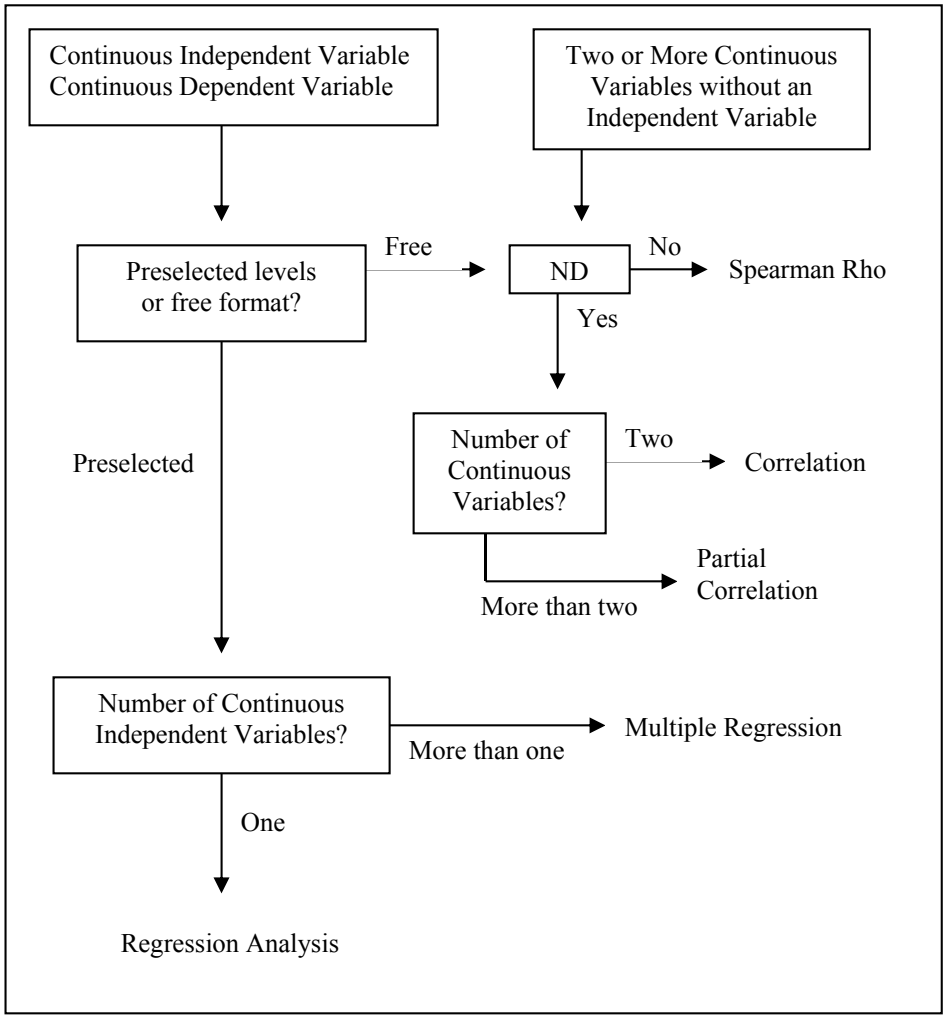
Panel B


Discrete Independent Variable
Continuous Dependent Variable
Number of Discrete Independent Variables?
Two
ND/H?
No
Friedman Two-way Analysis of Variance
Yes
Two-way ANOVA (Analysis of Variance)
One
Number of Levels within the Independent Variable?
One
One Sample t-test (confidence interval)
Three or more
Yes
One-way ANOVA
No
ND/H?
No
Kruskal-Wallis
Two
Paired data?
Yes
Randomized Complete Block Design
No
Paired data?
Yes
ND/H?
Yes
Paired t-test
No
Wilcoxon's Signed Rank Test
Sign Test
No
Population Variance Known?
Yes
Z-test
No
ND/H?
No
Mann-Whitney U Test
Median Test
Yes
Two-sample t-test

Panel C


Discrete Independent Variable
Discrete Dependent Variable
Discrete Variables without One Independent Variable
Third Discrete Variable?
Yes
Mantel-Haenszel Chi Square
No
2 × 2 Matrix
McNemar Test
Paired data?
Yes
Size of Contingency Table
No
Cochran's Q Test
Larger than a 2 × 2 Matrix
No empty cells and all expected values >5
Fisher Exact Test
No
Yes
Size of Contingency Table
2 × 2 Matrix
Yates' Chi Square
Larger than a 2 × 2 Matrix
Chi Square Test of Independence

Panel D


Continuous Independent Variable
Continuous Dependent Variable
Two or More Continuous Variables without an Independent Variable
Preselected levels or free format?
Free
ND
No
Spearman Rho
Yes
Preselected
Number of Continuous Variables?
Two
Correlation
Partial Correlation
More than two
Number of Continuous Independent Variables?
More than one
Multiple Regression
One
Regression Analysis

Appendix B
Statistical Tables

Table B1 Random Numbers Table

42505	29928	18850	17263	70236	35432	61247	38337	87214	68897
32654	33712	97303	74982	30341	17824	38448	96101	58318	84892
09241	92732	66397	91735	20477	88736	14252	65579	71724	41661
60481	36875	52880	38061	76675	97108	70738	13808	86470	81613
00548	99401	29620	77382	62582	90279	51053	55882	23689	42138
14935	30864	23867	91238	43732	41176	27818	99720	82276	58577
01517	25915	86821	20550	13767	19657	39114	88111	62768	42600
85448	28625	27677	13522	00733	23616	45170	78646	77552	01582
11004	06949	40228	95804	06583	10471	83884	27164	50516	89635
38507	11952	75182	03552	58010	94680	28292	65340	34292	05896
99452	62431	36306	44997	71725	01887	74115	88038	98193	80710
87961	20548	03520	81159	62323	95340	10516	91057	64979	15326
91695	49105	11072	41328	45844	15199	52172	24889	99580	65735
90335	66089	33914	13927	17168	96354	35817	55119	77894	86274
74775	37096	60407	78405	04361	55394	09344	45095	88789	73620
65141	71286	54481	68757	28095	62329	66628	01479	47433	76801
30755	11466	35367	84313	19280	37714	06161	48322	23077	63845
40192	33948	28043	88427	73014	40780	16652	20279	09418	60695
94528	98786	62495	60668	41998	39213	17701	91582	91659	03018
21917	16043	24943	93160	97513	76195	08674	74415	81408	66525
36632	18689	89137	46685	11119	75330	03907	73296	43519	66437
90668	57765	80858	07179	35167	49098	57371	51101	08015	41710
71063	60441	53750	08240	85269	01440	04898	57359	55221	64656
21036	16589	79605	10277	52852	40111	77130	38429	31212	41578
88085	84496	81220	51929	00903	39425	61281	02201	03726	95044
27162	31340	60963	14372	21057	19015	14858	26932	85648	43430
12046	49063	03168	64138	55123	29232	59462	29850	79201	18349
33052	11252	53477	65078	09199	58814	07790	36148	18962	85602
84187	61668	03267	75095	13486	05438	01962	13994	16834	60262
67887	50033	32275	68259	05930	74797	66309	66181	37093	31528
70457	55716	87554	47943	42819	98810	02729	94043	54642	37974
86336	64926	01880	41598	64455	88602	81755	74262	74591	58802
94323	92053	79740	92794	69032	62871	07447	14192	16290	11747
13869	60770	04022	91154	72841	17275	52936	76317	89963	73241
94585	85528	41527	05795	59929	25458	38851	87484	18897	61470

Table B2 Normal Standardized Distribution

(Area under the curve between 0 and z)

z	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
0.0	.0000	.0040	.0080	.0120	.0160	.0199	.0239	.0279	.0319	.0359
0.1	.0398	.0438	.0478	.0517	.0557	.0596	.0636	.0675	.0714	.0753
0.2	.0793	.0832	.0871	.0910	.0948	.0987	.1026	.1064	.1103	.1141
0.3	.1179	.1217	.1255	.1293	.1331	.1368	.1406	.1443	.1480	.1517
0.4	.1554	.1591	.1628	.1664	.1700	.1736	.1772	.1808	.1844	.1879
0.5	.1915	.1950	.1985	.2019	.2054	.2088	.2123	.2157	.2190	.2224
0.6	.2257	.2291	.2324	.2357	.2389	.2422	.2454	.2486	.2517	.2549
0.7	.2580	.2611	.2642	.2673	.2704	.2734	.2764	.2794	.2823	.2852
0.8	.2881	.2910	.2939	.2967	.2995	.3023	.3051	.3078	.3106	.3133
0.9	.3159	.3186	.3212	.3238	.3264	.3289	.3315	.3340	.3365	.3389
1.0	.3413	.3438	.3461	.3485	.3508	.3531	.3554	.3577	.3599	.3621
1.1	.3643	.3665	.3686	.3708	.3729	.3749	.3770	.3790	.3810	.3830
1.2	.3849	.3869	.3888	.3907	.3925	.3944	.3962	.3980	.3997	.4015
1.3	.4032	.4049	.4066	.4082	.4099	.4115	.4131	.4147	.4162	.4177
1.4	.4192	.4207	.4222	.4236	.4251	.4265	.4279	.4292	.4306	.4319
1.5	.4332	.4345	.4357	.4370	.4382	.4394	.4406	.4418	.4429	.4441
1.6	.4452	.4463	.4474	.4484	.4495	.4505	.4515	.4525	.4535	.4545
1.7	.4554	.4564	.4573	.4582	.4591	.4599	.4608	.4616	.4625	.4633
1.8	.4641	.4649	.4656	.4664	.4671	.4678	.4686	.4693	.4699	.4706
1.9	.4713	.4719	.4726	.4732	.4738	.4744	.4750	.4756	.4761	.4767
2.0	.4772	.4778	.4783	.4788	.4793	.4798	.4803	.4808	.4812	.4817
2.1	.4821	.4826	.4830	.4834	.4838	.4842	.4846	.4850	.4854	.4857
2.2	.4861	.4864	.4868	.4871	.4875	.4878	.4881	.4884	.4887	.4890
2.3	.4893	.4896	.4898	.4901	.4904	.4906	.4909	.4911	.4913	.4916
2.4	.4918	.4920	.4922	.4925	.4927	.4929	.4931	.4932	.4934	.4936
2.5	.4938	.4940	.4941	.4943	.4945	.4946	.4948	.4949	.4951	.4952
2.6	.4953	.4955	.4956	.4957	.4959	.4960	.4961	.4962	.4963	.4964
2.7	.4965	.4966	.4967	.4968	.4969	.4970	.4971	.4972	.4973	.4974
2.8	.4974	.4975	.4976	.4977	.4977	.4978	.4979	.4979	.4980	.4981
2.9	.4981	.4982	.4982	.4983	.4984	.4984	.4985	.4985	.4986	.4986
3.0	.4987	.4987	.4987	.4988	.4988	.4989	.4989	.4989	.4990	.4990
3.1	.4990	.4991	.4991	.4991	.4992	.4992	.4992	.4992	.4993	.4993
3.2	.4993	.4993	.4994	.4994	.4994	.4994	.4994	.4995	.4995	.4995
3.3	.4995	.4995	.4995	.4996	.4996	.4996	.4996	.4996	.4996	.4997
3.4	.4997	.4997	.4997	.4997	.4997	.4997	.4997	.4997	.4997	.4998
3.5	.4998	.4998	.4998	.4998	.4998	.4998	.4998	.4998	.4998	.4998
3.6	.4998	.4998	.4999	.4999	.4999	.4999	.4999	.4999	.4999	.4999

This table was created with Microsoft® Excel 2010 using function command NORM.S.DIST(*value*)-0.5.

Table B3 K-Values for Calculating Tolerance Limits (Two-Tailed)

	90% Confidence			95% Confidence			99% Confidence		
n	95%	99%	99.9%	95%	99%	99.9%	95%	99%	99.9%
2	18.22	23.42	29.36	36.52	46.94	58.84	182.7	234.9	294.4
3	6.823	8.819	11.10	9.789	12.64	15.92	22.13	28.58	35.98
4	4.913	6.372	8.046	6.341	8.221	10.38	11.12	14.41	18.18
5	4.142	5.387	6.816	5.077	6.598	8.345	7.870	10.22	12.92
6	3.723	4.850	6.146	4.422	5.758	7.294	6.373	8.292	10.50
7	3.456	4.508	5.720	4.020	5.241	6.647	5.520	7.191	9.114
8	3.270	4.271	5.423	3.746	4.889	6.206	4.968	6.479	8.220
9	3.132	4.094	5.203	3.546	4.633	5.885	4.581	5.980	7.593
10	3.026	3.958	5.033	3.393	4.437	5.640	4.292	5.610	7.127
12	2.871	3.759	4.785	3.175	4.156	5.287	3.896	5.096	6.481
15	2.720	3.565	4.541	2.965	3.885	4.949	3.529	4.621	5.883
18	2.620	3.436	4.380	2.828	3.709	4.727	3.297	4.321	5.505
20	2.570	3.372	4.299	2.760	3.621	4.616	3.184	4.175	5.321
25	2.479	3.254	4.151	2.638	3.462	4.416	2.984	3.915	4.993
30	2.417	3.173	4.050	2.555	3.355	4.281	2.851	3.742	4.775
35	2.371	3.114	3.975	2.495	3.276	4.182	2.756	3.618	4.618
40	2.336	3.069	3.918	2.448	3.216	4.105	2.684	3.524	4.499
50	2.285	3.003	3.834	2.382	3.129	3.995	2.580	3.390	4.328
60	2.250	2.956	3.775	2.335	3.068	3.918	2.509	3.297	4.210
80	2.203	2.895	3.697	2.274	2.988	3.816	2.416	3.175	4.055
100	2.172	2.855	3.646	2.234	2.936	3.750	2.357	3.098	3.956
120	2.151	2.826	3.610	2.206	2.899	3.703	2.315	3.043	3.887
150	2.128	2.796	3.572	2.176	2.859	3.652	2.271	2.985	3.812
200	2.102	2.763	3.529	2.143	2.816	3.598	2.223	2.921	3.732
300	2.073	2.725	3.481	2.106	2.767	3.535	2.169	2.850	3.641
400	2.057	2.703	3.453	2.084	2.739	3.499	2.138	2.810	3.589
500	2.046	2.689	3.435	2.070	2.721	3.476	2.117	2.783	3.555
1000	2.019	2.654	3.390	2.036	2.676	3.418	2.068	2.718	3.473
∞	1.960	2.576	3.291	1.960	2.576	3.291	1.960	2.576	3.291

Modified from: Odeh, R.E. and Owen, D.B. (1980). *Tables for Normal Tolerance Limits, Sampling Plans, and Screening*, Marcel Dekker, Inc., New York, pp. 90-93 and 98-105. Reproduced with permission of the publisher.

Table B4 K-Values for Calculating Tolerance Limits (One-Tailed)

	90% Confidence			95% Confidence			99% Confidence		
n	95%	99%	99.9%	95%	99%	99.9%	95%	99%	99.9%
2	13.09	18.50	24.58	26.26	37.09	49.28	131.4	185.6	246.6
3	5.311	7.340	9.651	7.656	10.55	13.86	17.37	23.90	31.35
4	3.957	5.438	7.129	5.144	7.042	9.214	9.083	12.39	16.18
5	3.400	4.666	6.111	4.203	5.741	7.502	6.578	8.939	11.65
6	3.092	4.243	5.556	3.708	5.062	6.612	5.406	7.335	9.550
7	2.894	3.972	5.202	3.399	4.642	6.063	4.728	6.412	8.346
8	2.754	3.783	4.955	3.187	4.354	5.688	4.285	5.812	7.564
9	2.650	3.641	4.771	3.031	4.143	5.413	3.972	5.389	7.014
10	2.568	3.532	4.629	2.911	3.981	5.203	3.738	5.074	6.605
12	2.448	3.371	4.420	2.736	3.747	4.900	3.410	4.633	6.035
15	2.329	3.212	4.215	2.566	3.520	4.607	3.102	4.222	5.504
18	2.249	3.105	4.078	2.453	3.370	4.415	2.905	3.960	5.167
20	2.208	3.052	4.009	2.396	3.295	4.318	2.808	3.832	5.001
25	2.132	2.952	3.882	2.292	3.158	4.142	2.633	3.601	4.706
30	2.080	2.884	3.794	2.220	3.064	4.022	2.515	3.447	4.508
35	2.041	2.833	3.729	2.167	2.995	3.934	2.430	3.334	4.364
40	2.010	2.793	3.679	2.125	2.941	3.865	2.364	3.249	4.255
50	1.965	2.735	3.605	2.065	2.862	3.766	2.269	3.125	4.097
60	1.933	2.694	3.552	2.022	2.807	3.695	2.202	3.038	3.987
80	1.890	2.638	3.482	1.964	2.733	3.601	2.114	2.924	3.842
100	1.861	2.601	3.435	1.927	2.684	3.539	2.056	2.850	3.748
120	1.841	2.574	3.402	1.899	2.649	3.495	2.015	2.797	3.682
150	1.818	2.546	3.366	1.870	2.611	3.448	1.971	2.740	3.610
200	1.793	2.514	3.326	1.837	2.570	3.395	1.923	2.679	3.532
300	1.765	2.477	3.280	1.800	2.522	3.335	1.868	2.608	3.443
400	1.748	2.456	3.253	1.778	2.494	3.300	1.836	2.567	3.392
500	1.736	2.442	3.235	1.763	2.475	3.277	1.814	2.540	3.358
1000	1.697	2.392	3.172	1.727	2.430	3.220	1.740	2.446	3.240
∞	1.645	2.326	3.090	1.645	2.326	3.090	1.645	2.326	3.090

Modified from: Odeh, R.E. and Owen, D.B. (1980). *Tables for Normal Tolerance Limits, Sampling Plans, and Screening*, Marcel Dekker, Inc., New York, pp. 22-25 and 98-107. Reproduced with permission of the publisher.

Table B5 Student t-Distribution $(1 - \alpha/2)$

d.f.	$t_{.80}$	$t_{.90}$	$t_{.95}$	$t_{.975}$	$t_{.99}$	$t_{.995}$	$t_{.9975}$	$t_{.9995}$
1	0.7265	3.0777	6.3137	12.706	31.821	63.656	127.32	636.58
2	0.6172	1.8856	2.9200	4.3027	6.9645	9.9250	14.089	31.600
3	0.5844	1.6377	2.3534	3.1824	4.5407	5.8408	7.4532	12.924
4	0.5686	1.5332	2.1318	2.7765	3.7469	4.6041	5.5975	8.6101
5	0.5594	1.4759	2.0150	2.5706	3.3649	4.0321	4.7733	6.8685
6	0.5534	1.4398	1.9432	2.4469	3.1427	3.7074	4.3168	5.9587
7	0.5491	1.4149	1.8946	2.3646	2.9979	3.4995	4.0294	5.4081
8	0.5459	1.3968	1.8595	2.3060	2.8965	3.3554	3.8325	5.0414
9	0.5435	1.3830	1.8331	2.2622	2.8214	3.2498	3.6896	4.7809
10	0.5415	1.3722	1.8125	2.2281	2.7638	3.1693	3.5814	4.5868
11	0.5399	1.3634	1.7959	2.2010	2.7181	3.1058	3.4966	4.4369
12	0.5386	1.3562	1.7823	2.1788	2.6810	3.0545	3.4284	4.3178
13	0.5375	1.3502	1.7709	2.1604	2.6503	3.0123	3.3725	4.2209
14	0.5366	1.3450	1.7613	2.1448	2.6245	2.9768	3.3257	4.1403
15	0.5357	1.3406	1.7531	2.1315	2.6025	2.9467	3.2860	4.0728
16	0.5350	1.3368	1.7459	2.1199	2.5835	2.9208	3.2520	4.0149
17	0.5344	1.3334	1.7396	2.1098	2.5669	2.8982	3.2224	3.9651
18	0.5338	1.3304	1.7341	2.1009	2.5524	2.8784	3.1966	3.9217
19	0.5333	1.3277	1.7291	2.0930	2.5395	2.8609	3.1737	3.8833
20	0.5329	1.3253	1.7247	2.0860	2.5280	2.8453	3.1534	3.8496
21	0.5325	1.3232	1.7207	2.0796	2.5176	2.8314	3.1352	3.8193
22	0.5321	1.3212	1.7171	2.0739	2.5083	2.8188	3.1188	3.7922
23	0.5317	1.3195	1.7139	2.0687	2.4999	2.8073	3.1040	3.7676
24	0.5314	1.3178	1.7109	2.0639	2.4922	2.7970	3.0905	3.7454
25	0.5312	1.3163	1.7081	2.0595	2.4851	2.7874	3.0782	3.7251
30	0.5300	1.3104	1.6973	2.0423	2.4573	2.7500	3.0298	3.6460
40	0.5286	1.3031	1.6839	2.0211	2.4233	2.7045	2.9712	3.5510
50	0.5278	1.2987	1.6759	2.0086	2.4033	2.6778	2.9370	3.4960
60	0.5272	1.2958	1.6706	2.0003	2.3901	2.6603	2.9146	3.4602
80	0.5265	1.2922	1.6641	1.9901	2.3739	2.6387	2.8870	3.4164
100	0.5261	1.2901	1.6602	1.9840	2.3642	2.6259	2.8707	3.3905
120	0.5258	1.2886	1.6576	1.9799	2.3578	2.6174	2.8599	3.3734
160	0.5254	1.2869	1.6544	1.9749	2.3499	2.6069	2.8465	3.3523
200	0.5252	1.2858	1.6525	1.9719	2.3451	2.6006	2.8385	3.3398
∞	0.5244	1.2816	1.6450	1.9602	2.3267	2.5763	2.8076	3.2915

This table was created with Microsoft® Excel 2010, function command T.INV.2T (alpha,df).

Table B6 Comparison of One-Tailed versus Two-Tailed t-Distributions

df	95% Confidence		99% Confidence	
	Two-Tailed ($\alpha/2$)	One-Tailed (α)	Two-Tailed ($\alpha/2$)	One-Tailed (α)
1	12.706	6.314	63.657	31.821
2	4.302	2.920	9.924	6.985
3	3.182	2.353	5.840	4.541
4	2.776	2.131	4.604	3.747
5	2.570	2.015	4.032	3.365
6	2.446	1.943	3.707	3.143
7	2.364	1.894	3.499	2.998
8	2.306	1.859	3.355	2.896
9	2.262	1.833	3.249	2.821
10	2.228	1.812	3.169	2.764
11	2.201	1.795	3.105	2.718
12	2.178	1.782	3.054	2.681
13	2.160	1.770	3.012	2.650
14	2.144	1.761	2.976	2.624
15	2.131	1.753	2.946	2.602
20	2.086	1.724	2.845	2.528
25	2.059	1.708	2.787	2.485
30	2.042	1.697	2.750	2.457
40	2.021	1.683	2.704	2.423
50	2.008	1.675	2.677	2.403
60	2.000	1.670	2.660	2.390
80	1.990	1.664	2.638	2.374
100	1.984	1.660	2.626	2.364
120	1.979	1.657	2.617	2.358
160	1.974	1.654	2.607	2.350
200	1.971	1.652	2.600	2.345
∞	1.960	1.645	2.576	2.326

This table was created with Microsoft® Excel 2010, function command T.INV(alpha,df) for one-tailed values and T.INV.2T (alpha,df) for two-tailed values.

Table B7 Analysis of Variance F-Distribution

ν_1	ν_2	$F_{.80}$	$F_{.90}$	$F_{.95}$	$F_{.975}$	$F_{.99}$	$F_{.999}$	$F_{.9999}$
	1	9.4722	39.864	161.45	647.79	4052.2	4×10^5	4×10^7
	2	3.5556	8.5263	18.513	38.506	98.502	998.38	1×10^4
	3	2.6822	5.5383	10.128	17.443	34.116	167.06	784.17
	4	2.3507	4.5448	7.7086	12.218	21.198	74.127	241.68
	5	2.1782	4.0604	6.6079	10.007	16.258	47.177	124.80
	6	2.0729	3.7760	5.9874	8.8131	13.745	35.507	82.422
	7	2.0020	3.5894	5.5915	8.0727	12.246	29.246	62.166
	8	1.9511	3.4579	5.3176	7.5709	11.259	25.415	50.699
	9	1.9128	3.3603	5.1174	7.2093	10.562	22.857	43.481
	10	1.8829	3.2850	4.9646	6.9367	10.044	21.038	38.592
	11	1.8589	3.2252	4.8443	6.7241	9.6461	19.687	35.041
	12	1.8393	3.1766	4.7472	6.5538	9.3303	18.645	32.422
	13	1.8230	3.1362	4.6672	6.4143	9.0738	17.815	30.384
1	14	1.8091	3.1022	4.6001	6.2979	8.8617	17.142	28.755
	15	1.7972	3.0732	4.5431	6.1995	8.6832	16.587	27.445
	16	1.7869	3.0481	4.4940	6.1151	8.5309	16.120	26.368
	17	1.7779	3.0262	4.4513	6.0420	8.3998	15.722	25.437
	18	1.7699	3.0070	4.4139	5.9781	8.2855	15.380	24.651
	19	1.7629	2.9899	4.3808	5.9216	8.1850	15.081	23.982
	20	1.7565	2.9747	4.3513	5.8715	8.0960	14.819	23.399
	22	1.7457	2.9486	4.3009	5.7863	7.9453	14.381	22.439
	24	1.7367	2.9271	4.2597	5.7166	7.8229	14.028	21.653
	26	1.7292	2.9091	4.2252	5.6586	7.7213	13.739	21.042
	30	1.7172	2.8807	4.1709	5.5675	7.5624	13.293	20.096
	35	1.7062	2.8547	4.1213	5.4848	7.4191	12.897	19.267
	40	1.6980	2.8353	4.0847	5.4239	7.3142	12.609	18.670
	45	1.6917	2.8205	4.0566	5.3773	7.2339	12.393	18.219
	50	1.6867	2.8087	4.0343	5.3403	7.1706	12.222	17.884
	60	1.6792	2.7911	4.0012	5.2856	7.0771	11.973	17.375
	90	1.6668	2.7621	3.9469	5.1962	6.9251	11.573	16.589
	120	1.6606	2.7478	3.9201	5.1523	6.8509	11.380	16.204
	240	1.6515	2.7266	3.8805	5.0875	6.7416	11.099	15.658
	∞	1.6423	2.7053	3.8415	5.0239	6.6349	10.828	15.134

continued

Table B7 Analysis of Variance F-Distribution (continued)

ν_1	ν_2	$F_{.80}$	$F_{.90}$	$F_{.95}$	$F_{.975}$	$F_{.99}$	$F_{.999}$	$F_{.9999}$
	2	4.000	9.000	19.00	39.00	99.00	998.8	1×10^4
	3	2.886	5.462	9.552	16.04	30.82	148.5	694.8
	4	2.472	4.325	6.944	10.65	18.00	61.25	197.9
	5	2.259	3.780	5.786	8.434	13.27	37.12	97.09
	6	2.130	3.463	5.143	7.260	10.92	27.00	61.58
	8	1.981	3.113	4.459	6.059	8.649	18.49	35.97
2	10	1.899	2.924	4.103	5.456	7.559	14.90	26.54
	12	1.846	2.807	3.885	5.096	6.927	12.97	21.86
	15	1.795	2.695	3.682	4.765	6.359	11.34	18.10
	20	1.746	2.589	3.493	4.461	5.849	9.953	15.12
	24	1.722	2.538	3.403	4.319	5.614	9.340	13.85
	30	1.699	2.489	3.316	4.182	5.390	8.773	12.72
	40	1.676	2.440	3.232	4.051	5.178	8.251	11.70
	60	1.653	2.393	3.150	3.925	4.977	7.768	10.78
	120	1.631	2.347	3.072	3.805	4.787	7.321	9.954
	∞	1.609	2.303	2.996	3.689	4.605	6.908	9.211
	2	4.1563	9.1618	19.164	39.166	99.164	999.31	1×10^4
	3	2.9359	5.3908	9.2766	15.439	29.457	141.10	659.38
	4	2.4847	4.1909	6.5914	9.9792	16.694	56.170	181.14
	5	2.2530	3.6195	5.4094	7.7636	12.060	33.200	86.380
	6	2.1126	3.2888	4.7571	6.5988	9.7796	23.705	53.667
	8	1.9513	2.9238	4.0662	5.4160	7.5910	15.829	30.443
3	10	1.8614	2.7277	3.7083	4.8256	6.5523	12.553	22.032
	12	1.8042	2.6055	3.4903	4.4742	5.9525	10.805	17.899
	15	1.7490	2.4898	3.2874	4.1528	5.4170	9.3351	14.639
	20	1.6958	2.3801	3.0984	3.8587	4.9382	8.0981	12.049
	24	1.6699	2.3274	3.0088	3.7211	4.7181	7.5543	10.965
	30	1.6445	2.2761	2.9223	3.5893	4.5097	7.0545	9.9972
	40	1.6195	2.2261	2.8387	3.4633	4.3126	6.5947	9.1277
	60	1.5950	2.1774	2.7581	3.3425	4.1259	6.1714	8.3528
	120	1.5709	2.1300	2.6802	3.2269	3.9491	5.7812	7.6579
	∞	1.5472	2.0838	2.6049	3.1162	3.7816	5.4220	7.0359

Continued

Table B7 Analysis of Variance F-Distribution (continued)

ν_1	ν_2	$F_{.80}$	$F_{.90}$	$F_{.95}$	$F_{.975}$	$F_{.99}$	$F_{.999}$	$F_{.9999}$
	2	4.2361	9.2434	19.247	39.248	99.251	999.31	1×10^4
	3	2.9555	5.3427	9.1172	15.101	28.710	137.08	640.75
	4	2.4826	4.1072	6.3882	9.6045	15.977	53.435	171.83
	5	2.2397	3.5202	5.1922	7.3879	11.392	31.083	80.559
	6	2.0924	3.1808	4.5337	6.2271	9.1484	21.922	49.418
	8	1.9230	2.8064	3.8379	5.0526	7.0061	14.392	27.474
4	10	1.8286	2.6053	3.4780	4.4683	5.9944	11.283	19.631
	12	1.7684	2.4801	3.2592	4.1212	5.4119	9.6334	15.789
	15	1.7103	2.3614	3.0556	3.8043	4.8932	8.2528	12.777
	20	1.6543	2.2489	2.8661	3.5147	4.4307	7.0959	10.419
	24	1.6269	2.1949	2.7763	3.3794	4.2185	6.5893	9.4224
	30	1.6001	2.1422	2.6896	3.2499	4.0179	6.1245	8.5420
	40	1.5737	2.0909	2.6060	3.1261	3.8283	5.6980	7.7598
	60	1.5478	2.0410	2.5252	3.0077	3.6491	5.3069	7.0577
	120	1.5222	1.9923	2.4472	2.8943	3.4795	4.9472	6.4356
	∞	1.4972	1.9449	2.3719	2.7858	3.3192	4.6166	5.8790
	2	4.2844	9.2926	19.296	39.298	99.302	999.31	1×10^4
	3	2.9652	5.3091	9.0134	14.885	28.237	134.58	627.71
	4	2.4780	4.0506	6.2561	9.3645	15.522	51.718	166.24
	5	2.2275	3.4530	5.0503	7.1464	10.967	29.751	76.834
	6	2.0755	3.1075	4.3874	5.9875	8.7459	20.802	46.741
	8	1.9005	2.7264	3.6875	4.8173	6.6318	13.484	25.640
5	10	1.8027	2.5216	3.3258	4.2361	5.6364	10.481	18.132
	12	1.7403	2.3940	3.1059	3.8911	5.0644	8.8921	14.465
	15	1.6801	2.2730	2.9013	3.5764	4.5556	7.5670	11.627
	20	1.6218	2.1582	2.7109	3.2891	4.1027	6.4606	9.3860
	24	1.5933	2.1030	2.6207	3.1548	3.8951	5.9767	8.4547
	30	1.5654	2.0492	2.5336	3.0265	3.6990	5.5338	7.6325
	40	1.5379	1.9968	2.4495	2.9037	3.5138	5.1282	6.8976
	60	1.5108	1.9457	2.3683	2.7863	3.3389	4.7567	6.2464
	120	1.4841	1.8959	2.2899	2.6740	3.1735	4.4156	5.6662
	∞	1.4579	1.8473	2.2141	2.5665	3.0172	4.1030	5.1477

continued

Table B7 Analysis of Variance F-Distribution (continued)

ν_1	ν_2	$F_{.80}$	$F_{.90}$	$F_{.95}$	$F_{.975}$	$F_{.99}$	$F_{.999}$	$F_{.9999}$
	2	4.3168	9.3255	19.329	39.331	99.331	999.31	1×10^4
	3	2.9707	5.2847	8.9407	14.735	27.911	132.83	620.26
	4	2.4733	4.0097	6.1631	9.1973	15.207	50.524	162.05
	5	2.2174	3.4045	4.9503	6.9777	10.672	28.835	74.506
	6	2.0619	3.0546	4.2839	5.8197	8.4660	20.031	44.936
	8	1.8826	2.6683	3.5806	4.6517	6.3707	12.858	24.360
6	10	1.7823	2.4606	3.2172	4.0721	5.3858	9.9262	17.084
	12	1.7182	2.3310	2.9961	3.7283	4.8205	8.3783	13.562
	15	1.6561	2.2081	2.7905	3.4147	4.3183	7.0913	10.819
	20	1.5960	2.0913	2.5990	3.1283	3.8714	6.0186	8.6802
	24	1.5667	2.0351	2.5082	2.9946	3.6667	5.5506	7.7926
	30	1.5378	1.9803	2.4205	2.8667	3.4735	5.1223	6.9995
	40	1.5093	1.9269	2.3359	2.7444	3.2910	4.7307	6.3010
	60	1.4813	1.8747	2.2541	2.6274	3.1187	4.3719	5.6825
	120	1.4536	1.8238	2.1750	2.5154	2.9559	4.0436	5.1332
	∞	1.4263	1.7741	2.0986	2.4082	2.8020	3.7430	4.6421
	2	4.3401	9.3491	19.353	39.356	99.357	999.31	1×10^4
	3	2.9741	5.2662	8.8867	14.624	27.671	131.61	614.67
	4	2.4691	3.9790	6.0942	9.0741	14.976	49.651	159.26
	5	2.2090	3.3679	4.8759	6.8530	10.456	28.165	72.643
	6	2.0508	3.0145	4.2067	5.6955	8.2600	19.463	43.539
	8	1.8682	2.6241	3.5005	4.5285	6.1776	12.398	23.429
7	10	1.7658	2.4140	3.1355	3.9498	5.2001	9.5170	16.327
	12	1.7003	2.2828	2.9134	3.6065	4.6395	8.0008	12.893
	15	1.6368	2.1582	2.7066	3.2934	4.1416	6.7412	10.230
	20	1.5752	2.0397	2.5140	3.0074	3.6987	5.6921	8.1563
	24	1.5451	1.9826	2.4226	2.8738	3.4959	5.2351	7.2978
	30	1.5154	1.9269	2.3343	2.7460	3.3045	4.8171	6.5374
	40	1.4861	1.8725	2.2490	2.6238	3.1238	4.4356	5.8644
	60	1.4572	1.8194	2.1665	2.5068	2.9530	4.0864	5.2678
	120	1.4287	1.7675	2.0868	2.3948	2.7918	3.7669	4.7385
	∞	1.4005	1.7167	2.0096	2.2875	2.6393	3.4745	4.2673

continued

Table B7 Analysis of Variance F-Distribution (continued)

ν_1	ν_2	$F_{.80}$	$F_{.90}$	$F_{.95}$	$F_{.975}$	$F_{.99}$	$F_{.999}$	$F_{.9999}$
	5	2.2021	3.3393	4.8183	6.7572	10.289	27.649	71.246
	10	1.7523	2.3771	3.0717	3.8549	5.0567	9.2041	15.745
	15	1.6209	2.1185	2.6408	3.1987	4.0044	6.4706	9.7789
	20	1.5580	1.9985	2.4471	2.9128	3.5644	5.4401	7.7562
8	30	1.4968	1.8841	2.2662	2.6513	3.1726	4.5816	6.1809
	40	1.4668	1.8289	2.1802	2.5289	2.9930	4.2071	5.5261
	60	1.4371	1.7748	2.0970	2.4117	2.8233	3.8649	4.9477
	120	1.4078	1.7220	2.0164	2.2994	2.6629	3.5518	4.4329
	∞	1.3788	1.6702	1.9384	2.1918	2.5113	3.2655	3.9781
	5	2.1963	3.3163	4.7725	6.6810	10.158	27.241	70.082
	10	1.7411	2.3473	3.0204	3.7790	4.9424	8.9558	15.280
	15	1.6076	2.0862	2.5876	3.1227	3.8948	6.2560	9.4224
	20	1.5436	1.9649	2.3928	2.8365	3.4567	5.2391	7.4397
9	30	1.4812	1.8490	2.2107	2.5746	3.0665	4.3929	5.8972
	40	1.4505	1.7929	2.1240	2.4519	2.8876	4.0243	5.2569
	60	1.4201	1.7380	2.0401	2.3344	2.7185	3.6873	4.6912
	120	1.3901	1.6842	1.9588	2.2217	2.5586	3.3792	4.1910
	∞	1.3602	1.6315	1.8799	2.1136	2.4073	3.0975	3.7471
	5	2.1914	3.2974	4.7351	6.6192	10.051	26.914	69.267
	10	1.7316	2.3226	2.9782	3.7168	4.8491	8.7539	14.901
	15	1.5964	2.0593	2.5437	3.0602	3.8049	6.0809	9.1313
	20	1.5313	1.9367	2.3479	2.7737	3.3682	5.0754	7.1814
10	30	1.4678	1.8195	2.1646	2.5112	2.9791	4.2387	5.6643
	40	1.4365	1.7627	2.0773	2.3882	2.8005	3.8744	5.0350
	60	1.4055	1.7070	1.9926	2.2702	2.6318	3.5416	4.4820
	120	1.3748	1.6524	1.9105	2.1570	2.4721	3.2371	3.9909
	∞	1.3442	1.5987	1.8307	2.0483	2.3209	2.9588	3.5561

continued

Table B7 Analysis of Variance F-Distribution (continued)

ν_1	ν_2	$F_{.80}$	$F_{.90}$	$F_{.95}$	$F_{.975}$	$F_{.99}$	$F_{.999}$	$F_{.9999}$
	5	2.1835	3.2682	4.6777	6.5245	9.8883	26.419	67.987
	10	1.7164	2.2841	2.9130	3.6210	4.7058	8.4456	14.334
	15	1.5782	2.0171	2.4753	2.9633	3.6662	5.8121	8.6875
	20	1.5115	1.8924	2.2776	2.6758	3.2311	4.8231	6.7812
12	30	1.4461	1.7727	2.0921	2.4120	2.8431	4.0006	5.3078
	40	1.4137	1.7146	2.0035	2.2882	2.6648	3.6425	4.6966
	60	1.3816	1.6574	1.9174	2.1692	2.4961	3.3153	4.1582
	120	1.3496	1.6012	1.8337	2.0548	2.3363	3.0161	3.6816
	∞	1.3177	1.5458	1.7522	1.9447	2.1847	2.7425	3.2614
	5	2.1751	3.2380	4.6188	6.4277	9.7223	25.910	66.590
	10	1.7000	2.2435	2.8450	3.5217	4.5582	8.1291	13.752
	15	1.5584	1.9722	2.4034	2.8621	3.5222	5.5352	8.2291
	20	1.4897	1.8449	2.2033	2.5731	3.0880	4.5616	6.3737
15	30	1.4220	1.7223	2.0148	2.3072	2.7002	3.7528	4.9386
	40	1.3883	1.6624	1.9245	2.1819	2.5216	3.4004	4.3456
	60	1.3547	1.6034	1.8364	2.0613	2.3523	3.0782	3.8217
	120	1.3211	1.5450	1.7505	1.9450	2.1915	2.7833	3.3597
	∞	1.2874	1.4871	1.6664	1.8326	2.0385	2.5132	2.9504
	5	2.1660	3.2067	4.5581	6.3285	9.5527	25.393	65.193
	10	1.6823	2.2007	2.7740	3.4185	4.4054	7.8035	13.155
	15	1.5367	1.9243	2.3275	2.7559	3.3719	5.2487	7.7562
	20	1.4656	1.7938	2.1242	2.4645	2.9377	4.2901	5.9517
20	30	1.3949	1.6673	1.9317	2.1952	2.5487	3.4927	4.5547
	40	1.3596	1.6052	1.8389	2.0677	2.3689	3.1450	3.9763
	60	1.3241	1.5435	1.7480	1.9445	2.1978	2.8265	3.4688
	120	1.2882	1.4821	1.6587	1.8249	2.0346	2.5344	3.0177
	∞	1.2519	1.4206	1.5705	1.7085	1.8783	2.2658	2.6193

This table was created using Microsoft® Excel 2010, function command F.INV.RT (alpha,df1,df2).

Table B8 Upper Percentage Points of the F_{max} Statistic

		K = number of variances										
n – 1	α	2	3	4	5	6	7	8	9	10	11	12
4	.05	9.60	15.5	20.6	25.2	29.5	33.6	37.5	41.4	44.6	48.0	51.4
	.01	23.2	37	49	59	69	79	89	97	106	113	120
5	.05	7.15	10.8	13.7	16.3	18.7	20.8	22.9	24.7	26.5	28.2	29.9
	.01	14.9	22	28	33	38	42	46	50	54	57	60
6	.05	5.82	8.38	10.4	12.1	13.7	15.0	16.3	17.5	18.6	19.7	20.7
	.01	11.1	15.5	19.1	22	25	27	30	32	34	36	37
7	.05	4.99	6.94	8.44	9.70	10.8	11.8	12.7	13.5	14.3	15.1	15.8
	.01	8.89	12.1	14.5	16.5	18.4	20	22	23	24	26	27
8	.05	4.43	6.00	7.18	8.12	9.03	9.78	10.5	11.1	11.7	12.2	12.7
	.01	7.50	9.9	11.7	13.2	14.5	15.8	16.9	17.9	18.9	19.8	21
9	.05	4.03	5.34	6.31	7.11	7.80	8.41	8.95	9.45	9.91	10.3	10.7
	.01	6.54	8.5	9.9	11.1	12.1	13.1	13.9	14.7	15.3	16.0	16.6
10	.05	3.72	4.85	5.67	6.34	6.92	7.42	7.87	8.28	8.66	9.01	9.34
	.01	5.85	7.4	8.6	9.6	10.4	11.1	11.8	12.4	12.9	13.4	13.9
12	.05	3.28	4.16	4.79	5.30	5.72	6.09	6.42	6.72	7.00	7.25	7.48
	.01	4.91	6.1	6.9	7.6	8.2	8.7	9.1	9.5	9.9	10.2	10.6
15	.05	2.86	3.54	4.01	4.37	4.68	4.95	5.19	5.40	5.59	5.77	5.93
	.05	4.07	4.9	5.5	6.0	6.4	6.7	7.1	7.3	7.5	7.8	8.0
20	.05	2.46	2.95	3.29	3.54	3.76	3.94	4.10	4.24	4.37	4.49	4.59
	.01	3.32	3.8	4.3	4.6	4.9	5.1	5.3	5.5	5.6	5.8	5.9
30	.05	2.07	2.40	2.61	2.78	2.91	3.02	3.12	3.21	3.29	3.36	3.39
	.01	2.63	3.0	3.3	3.4	3.6	3.7	3.8	3.9	4.0	4.1	4.2
60	.05	1.67	1.85	1.96	2.04	2.11	2.17	2.22	2.26	2.30	2.33	2.36
	.01	1.96	2.2	2.3	2.4	2.4	2.5	2.5	2.6	2.6	2.7	2.7
∞	.05	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	.01	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Modified from: Pearson, E.S. and Hartley, H.O. (1970). *Biometrika Tables for Statisticians*, Vol. 1 (Table 31), Biometrika Trustees at the University Press, Cambridge, London. Reproduced with permission of the Biometrika Trustees.

Table B9 Upper Percentage Points of the Cochran C Test for Homogeneity of Variance

n-1	α	k = levels of independent variable								
		2	3	4	5	6	7	8	9	10
1	.05	.999	.967	.907	.841	.781	.727	.680	.639	.602
	.01	.999	.993	.968	.928	.883	.838	.795	.754	.718
2	.05	.975	.871	.768	.684	.616	.561	.516	.478	.445
	.01	.995	.942	.864	.789	.722	.664	.615	.573	.536
3	.05	.939	.798	.684	.598	.532	.480	.438	.403	.373
	.01	.979	.883	.781	.696	.626	.569	.521	.481	.447
4	.05	.906	.746	.629	.544	.480	.431	.391	.358	.331
	.01	.959	.834	.721	.633	.564	.508	.463	.425	.393
5	.05	.877	.707	.590	.507	.445	.397	.360	.329	.303
	.01	.937	.793	.676	.588	.520	.466	.423	.387	.357
6	.05	.853	.677	.560	.478	.418	.373	.336	.307	.282
	.01	.917	.761	.641	.553	.487	.435	.393	.359	.331
7	.05	.833	.653	.537	.456	.398	.354	.319	.290	.267
	.01	.899	.734	.613	.526	.461	.411	.370	.338	.311
8	.05	.816	.633	.518	.439	.382	.338	.304	.277	.254
	.01	.882	.711	.590	.504	.440	.391	.352	.321	.295
9	.05	.801	.617	.502	.424	.368	.326	.293	.266	.244
	.05	.867	.691	.570	.485	.423	.375	.337	.307	.281
16	.05	.734	.547	.437	.364	.314	.276	.246	.223	.203
	.01	.795	.606	.488	.409	.353	.311	.278	.251	.230
36	.05	.660	.475	.372	.307	.261	.228	.202	.182	.166
	.01	.707	.515	.406	.335	.286	.249	.221	.199	.181
144	.05	.581	.403	.309	.251	.212	.183	.162	.145	.131
	.01	.606	.423	.325	.264	.223	.193	.170	.152	.138

Modified from: Eisenhart, C., Hastay, M.W., and Wallis W.A., eds. (1947). *Techniques of Statistical Analysis* (Tables 15.1 and 15.2), McGraw-Hill Book Company, New York. Reproduced with permission of the publisher.

Table B10 Percentage Point of the Studentized Range (q)

df	α	k (or p)								
		2	3	4	5	6	7	8	9	10
10	.05	3.15	3.88	4.33	4.65	4.91	5.12	5.30	5.46	5.60
	.01	4.48	5.27	5.77	6.14	6.43	6.67	6.87	7.05	7.21
12	.05	3.08	3.77	4.20	4.51	4.75	4.95	5.12	5.27	5.39
	.01	4.32	5.05	5.50	5.84	6.10	6.32	6.51	6.67	6.81
14	.05	3.03	3.70	4.11	4.41	4.64	4.83	4.99	5.13	5.25
	.01	4.21	4.89	5.32	5.63	5.88	6.08	6.26	6.41	6.54
16	.05	3.00	3.65	4.05	4.33	4.56	4.74	4.90	5.03	5.15
	.01	4.13	4.79	5.19	5.49	5.72	5.92	6.08	6.22	6.35
18	.05	2.97	3.61	4.00	4.28	4.49	4.67	4.82	4.96	5.07
	.01	4.07	4.70	5.09	5.38	5.60	5.79	5.94	6.08	6.20
20	.05	2.95	3.58	3.96	4.23	4.45	4.62	4.77	4.90	5.01
	.01	4.02	4.64	5.02	5.29	5.51	5.69	5.84	5.97	6.09
24	.05	2.92	3.53	3.90	4.17	4.37	4.54	4.68	4.81	4.92
	.01	3.96	4.55	4.91	5.17	5.37	5.54	5.69	5.81	5.92
30	.05	2.89	3.49	3.85	4.10	4.30	4.46	4.60	4.72	4.82
	.01	3.89	4.45	4.80	5.05	5.24	5.40	5.54	5.65	5.76
40	.05	2.86	3.44	3.79	4.04	4.23	4.39	4.52	4.63	4.73
	.01	3.82	4.37	4.70	4.93	5.11	5.26	5.39	5.50	5.60
60	.05	2.83	3.40	3.74	3.98	4.16	4.31	4.44	4.55	4.65
	.01	3.76	4.28	4.59	4.82	4.99	5.13	5.25	5.36	5.45
120	.05	2.80	3.36	3.68	3.92	4.10	4.24	4.36	4.47	4.56
	.01	3.70	4.20	4.50	4.71	4.87	5.01	5.12	5.21	5.30
∞	.05	2.77	3.31	3.63	3.86	4.03	4.17	4.29	4.39	4.47
	.01	3.64	4.12	4.40	4.60	4.76	4.88	4.99	5.08	5.16

Modified from: Pearson, E.S. and Hartley, H.O. (1970). *Biometrika Tables for Statisticians*, Vol. 1 (Table 29), Biometrika Trustees at the University Press, Cambridge, London. Reproduced with permission of the Biometrika Trustees.

Table B11 Percentage Points of the Dunn Multiple Comparisons

Number of Comparisons (C)	α	(N – K) degrees of freedom								
		10	15	20	24	30	40	60	120	∞
2	.05	2.64	2.49	2.42	2.39	2.36	3.33	2.30	2.27	2.24
	.01	3.58	3.29	3.16	3.09	3.03	2.97	2.92	2.86	2.81
3	.05	2.87	2.69	2.61	2.58	2.54	2.50	2.47	2.43	2.39
	.01	3.83	3.48	3.33	3.26	3.19	3.12	3.06	2.99	2.94
4	.05	3.04	2.84	2.75	2.70	2.66	2.62	2.58	2.54	2.50
	.01	4.01	3.62	3.46	3.38	3.30	3.23	3.16	3.09	3.02
5	.05	3.17	2.95	2.85	2.80	2.75	2.71	2.66	2.62	2.58
	.01	4.15	3.74	3.55	3.47	3.39	3.31	3.24	3.16	3.09
6	.05	3.28	3.04	2.93	2.88	2.83	2.78	2.73	2.68	2.64
	.01	4.27	3.82	3.63	3.54	3.46	3.38	3.30	3.22	3.15
7	.05	3.37	3.11	3.00	2.94	2.89	2.84	2.79	2.74	2.69
	.01	4.37	3.90	3.70	3.61	3.52	3.43	3.34	3.27	3.19
8	.05	3.45	3.18	3.06	3.00	2.94	2.89	2.84	2.79	2.74
	.01	4.45	3.97	3.76	3.66	3.57	3.48	3.39	3.31	3.23
9	.05	3.52	3.24	3.11	3.05	2.99	2.93	2.88	2.83	2.77
	.01	4.53	4.02	3.80	3.70	3.61	3.51	3.42	3.34	3.26
10	.05	3.58	3.29	3.16	3.09	3.03	2.97	2.92	2.86	2.81
	.01	4.59	4.07	3.85	3.74	3.65	3.55	3.46	3.37	3.29
15	.05	3.83	3.48	3.33	3.26	3.19	3.12	3.06	2.99	2.94
	.01	4.86	4.29	4.03	3.91	3.80	3.70	3.59	3.50	3.40
20	.05	4.01	3.62	3.46	3.38	3.30	3.23	3.16	3.09	3.02
	.01	5.06	4.42	4.15	4.04	3.90	3.79	3.69	3.58	3.48
30	.05	4.27	3.82	3.63	3.54	3.46	3.38	3.30	3.22	3.15
	.01	5.33	4.61	4.33	4.2	4.13	3.93	3.81	3.69	3.59

Modified from: Dunn, O.J. (1961). "Multiple Comparisons among Means," *Journal of the American Statistical Association*, 56:62-64. Reproduced with permission of the American Statistical Association.

Table B12 Critical Values of q for the Two-Tailed Dunnett's Test

N – k df	α	Number of means with the range from the control inclusive (*p*)								
		2	3	4	5	6	7	8	9	10
10	.05	2.23	2.57	2.81	2.97	3.11	3.21	3.31	3.39	3.46
	.01	3.17	3.53	3.78	3.95	4.01	4.21	4.31	4.40	4.47
12	.05	2.18	2.50	2.72	2.88	3.00	3.10	3.18	3.25	3.32
	.01	3.05	3.39	3.61	3.76	3.89	3.99	4.08	4.15	4.22
14	.05	2.14	2.46	2.67	2.81	2.93	3.02	3.10	3.17	3.23
	.01	2.98	3.29	3.49	3.64	3.75	3.84	3.92	3.99	4.05
16	.05	2.12	2.42	2.63	2.77	2.88	2.96	3.04	3.10	3.16
	.01	2.92	3.22	3.41	3.55	3.65	3.74	3.82	3.88	3.93
18	.05	2.10	2.40	2.59	2.73	2.84	2.92	2.99	3.05	3.11
	.01	2.88	3.17	3.35	3.48	3.58	3.67	3.74	3.80	3.85
20	.05	2.09	2.38	2.57	2.70	2.81	2.89	2.96	3.02	3.07
	.01	2.85	3.13	3.31	3.43	3.53	3.61	3.67	3.73	3.78
24	.05	2.06	2.35	2.53	2.66	2.76	2.84	2.91	2.96	3.01
	.01	2.80	3.07	3.24	3.36	3.45	3.52	3.58	3.64	3.69
30	.05	2.04	2.32	2.50	2.62	2.72	2.79	2.86	2.91	2.96
	.01	2.75	3.01	3.17	3.28	3.37	3.44	3.50	3.55	3.59
40	.05	2.02	2.29	2.47	2.58	2.67	2.75	2.81	2.86	2.90
	.01	2.70	2.95	3.10	3.21	3.29	3.36	3.41	3.46	3.50
60	.05	2.00	2.27	2.43	2.55	2.63	2.70	2.76	2.81	2.85
	.01	2.66	2.90	3.04	3.14	3.22	3.28	3.33	3.38	3.42
120	.05	1.98	2.24	2.40	2.51	2.59	2.66	2.71	2.76	2.80
	.01	2.62	2.84	2.98	3.08	3.15	3.21	3.25	3.30	3.33
∞	.05	1.96	2.21	2.37	2.47	2.55	2.62	2.67	2.71	2.75
	.01	2.58	2.79	2.92	3.01	3.08	3.14	3.18	3.22	3.25

Modified from: Dunnett, C.W. (1955) "A multiple comparison procedure for comparing several treatments with a control," *Journal of the American Statistical Association*, 50:1119-1120. Reprinted with permission from *The Journal of the American Statistical Association*.

Table B13 Critical Values of q for the One-Tailed Dunnett's Test

N – k df	α	Number of means with the range from the control inclusive (p)								
		2	3	4	5	6	7	8	9	10
10	.05	1.81	2.15	2.34	2.47	2.56	2.64	2.70	2.76	2.81
	.01	2.76	3.11	3.31	3.45	3.56	3.64	3.71	3.78	3.83
12	.05	1.78	2.11	2.29	2.41	2.50	2.58	2.64	2.69	2.74
	.01	2.68	3.01	3.19	3.32	3.42	3.50	3.56	3.62	3.67
14	.05	1.76	2.08	2.25	2.37	2.46	2.53	2.59	2.64	2.69
	.01	2.62	2.94	3.11	3.23	3.32	3.40	3.46	3.51	3.56
16	.05	1.75	2.06	2.23	2.34	2.43	2.50	2.56	2.61	2.65
	.01	2.58	2.88	3.05	3.17	3.26	3.33	3.39	3.44	3.48
18	.05	1.73	2.04	2.21	2.32	2.41	2.48	2.53	2.58	2.62
	.01	2.55	2.84	3.01	3.12	3.21	3.27	3.33	3.38	3.42
20	.05	1.72	2.03	2.19	2.30	2.39	2.46	2.51	2.56	2.60
	.01	2.53	2.81	2.97	3.08	3.17	3.23	3.29	3.34	3.38
24	.05	1.71	2.01	2.17	2.28	2.36	2.43	2.48	2.53	2.57
	.01	2.49	2.77	2.92	3.03	3.11	3.17	3.22	3.27	3.31
30	.05	1.70	1.99	2.15	2.25	2.33	2.40	2.45	2.50	2.54
	.01	2.46	2.72	2.87	2.97	3.05	3.11	3.16	3.21	3.24
40	.05	1.68	1.97	2.13	2.23	2.31	2.37	2.42	2.47	2.51
	.01	2.42	2.68	2.82	2.92	2.99	3.05	3.10	3.14	3.18
60	.05	1.67	1.95	2.10	2.21	2.28	2.35	2.39	2.44	2.48
	.01	2.39	2.64	2.78	2.87	2.94	3.00	3.04	3.08	3.12
120	.05	1.66	1.93	2.08	2.18	2.26	2.32	2.37	2.41	2.45
	.01	2.36	2.60	2.73	2.82	2.89	2.94	2.99	3.03	3.06
∞	.05	1.64	1.92	2.06	2.16	2.23	2.29	2.34	2.38	2.42
	.01	2.33	2.56	2.68	2.77	2.84	2.89	2.93	2.97	3.00

Modified from: Dunnett, C.W. (1955) "A multiple comparison procedure for comparing several treatments with a control," *Journal of the American Statistical Association*, 50:1117-1118. Reprinted with permission from *The Journal of the American Statistical Association*.

Table B14 Values of r at Different Levels of Significance

d.f.	.01	.05	.01	.001
1	.988	.997	.999	1.00
2	.900	.950	.990	.999
3	.805	.878	.959	.991
4	.730	.811	.917	.974
5	.669	.755	.875	.951
6	.622	.707	.834	.925
7	.582	.666	.798	.898
8	.549	.632	.765	.872
9	.521	.602	.735	.847
10	.497	.576	.708	.823
11	.476	.553	.684	.801
12	.458	.532	.661	.780
13	.441	.514	.641	.760
14	.426	.497	.623	.742
15	.412	.482	.606	.725
16	.400	.468	.590	.708
17	.389	.456	.575	.693
18	.378	.444	.561	.679
19	.369	.433	.549	.665
20	.360	.423	.537	.652
25	.323	.381	.487	.597
30	.296	.349	.449	.554
35	.275	.325	.418	.519
40	.257	.304	.393	.490
50	.231	.273	.354	.443
60	.211	.250	.325	.408
80	.183	.217	.283	.357
100	.164	.195	.254	.321
150	.134	.159	.208	.264
200	.116	.138	.181	.230

Modified from: Pearson, E.S. and Hartley, H.O. (1970). *Biometrika Tables for Statisticians*, Vol. 1 (Table 13), Biometrika Trustees at the University Press, Cambridge, London. Reproduced with permission of the Biometrika Trustees.

Table B15 Chi Square Distribution

d.f.	$\alpha = 0.10$	0.05	0.025	0.01	0.005	0.001	0.0001
1	2.7055	3.8415	5.0239	6.6349	7.8794	10.827	15.134
2	4.6052	5.9915	7.3778	9.2104	10.597	13.815	18.425
3	6.2514	7.8147	9.3484	11.345	12.838	16.266	21.104
4	7.7794	9.4877	11.143	13.277	14.860	18.466	23.506
5	9.2363	11.070	12.832	15.086	16.750	20.515	25.751
6	10.645	12.592	14.449	16.812	18.548	22.457	27.853
7	12.017	14.067	16.013	18.475	20.278	24.321	29.881
8	13.362	15.507	17.535	20.090	21.955	26.124	31.827
9	14.684	16.919	19.023	21.666	23.589	27.877	33.725
10	15.987	18.307	20.483	23.209	25.188	29.588	35.557
11	17.275	19.675	21.920	24.725	26.757	31.264	37.365
12	18.549	21.026	23.337	26.217	28.300	32.909	39.131
13	19.812	22.362	24.736	27.688	29.819	34.527	40.873
14	21.064	23.685	26.119	29.141	31.319	36.124	42.575
15	22.307	24.996	27.488	30.578	32.801	37.698	44.260
16	23.542	26.296	28.845	32.000	34.267	39.252	45.926
17	24.769	27.587	30.191	33.409	35.718	40.791	47.559
18	25.989	28.869	31.526	34.805	37.156	42.312	49.185
19	27.204	30.144	32.852	36.191	38.582	43.819	50.787
20	28.412	31.410	34.170	37.566	39.997	45.314	52.383
21	29.615	32.671	35.479	38.932	41.401	46.796	53.960
22	30.813	33.924	36.781	40.289	42.796	48.268	55.524
23	32.007	35.172	38.076	41.638	44.181	49.728	57.067
24	33.196	36.415	39.364	42.980	45.558	51.179	58.607
25	34.382	37.652	40.646	44.314	46.928	52.619	60.136

This table was created with Microsoft® Excel 2010, function command CHI.INV.RT (alpha,df).

Table B16 Binomial Distributions where $p = 0.50$

$$p(x) = \binom{n}{x} p^x q^{n-x}$$

	x						
n	0	1	2	3	4	5	6
1	0.5000	0.5000	…	…	…	…	…
2	0.2500	0.5000	0.2500	…	…	…	…
3	0.1250	0.3750	0.3750	0.1250	…	…	…
4	0.0625	0.2500	0.3750	0.2500	0.0625	…	…
5	0.0313	0.1563	0.3125	0.3125	0.1563	0.0313	…
6	0.0156	0.0938	0.2344	0.3125	0.2344	0.0938	0.0156
7	0.0078	0.0547	0.1641	0.2734	0.2734	0.1641	0.0547
8	0.0039	0.0313	0.1094	0.2188	0.2734	0.2188	0.1094
9	0.0020	0.0176	0.0703	0.1641	0.2461	0.2461	0.1641
10	0.0010	0.0098	0.0439	0.1172	0.2051	0.2461	0.2051
11	0.0005	0.0054	0.0269	0.0806	0.1611	0.2256	0.2256
12	0.0002	0.0029	0.0161	0.0537	0.1208	0.1934	0.2256
13	0.0001	0.0016	0.0095	0.0349	0.0873	0.1571	0.2095
14	0.0001	0.0009	0.0056	0.0222	0.0611	0.1222	0.1833
15	<0.0001	0.0005	0.0032	0.0139	0.0417	0.0916	0.1527
16	<0.0001	0.0002	0.0018	0.0085	0.0278	0.0667	0.1222
17	<0.0001	0.0001	0.0010	0.0052	0.0182	0.0472	0.0944
18	<0.0001	0.0001	0.0006	0.0031	0.0117	0.0327	0.0708
19	<0.0001	<0.0001	0.0003	0.0018	0.0074	0.0222	0.0518
20	<0.0001	<0.0001	0.0002	0.0011	0.0046	0.0148	0.0370

n	6	7	8	9	10	11	12
6	0.0156	…	…	…	…	…	…
7	0.0547	0.0078	…	…	…	…	…
8	0.1094	0.0313	0.0039	…	…	…	…
9	0.1641	0.0703	0.0176	0.0020	…	…	…
10	0.2051	0.1172	0.0439	0.0098	0.0010	…	…
11	0.2256	0.1611	0.0806	0.0269	0.0054	0.0005	…
12	0.2256	0.1934	0.1208	0.0537	0.0161	0.0029	0.0002
13	0.2095	0.2095	0.1571	0.0873	0.0349	0.0095	0.0016
14	0.1833	0.2095	0.1833	0.1222	0.0611	0.0222	0.0056
15	0.1527	0.1964	0.1964	0.1527	0.0916	0.0417	0.0139
16	0.1222	0.1746	0.1964	0.1746	0.1222	0.0667	0.0278
17	0.0944	0.1484	0.1855	0.1855	0.1484	0.0944	0.0472
18	0.0708	0.1214	0.1669	0.1855	0.1669	0.1214	0.0708
19	0.0518	0.0961	0.1442	0.1762	0.1762	0.1442	0.0961
20	0.0370	0.0739	0.1201	0.1602	0.1762	0.1602	0.1201

This table was created with Microsoft® Excel 2010 using the formula:
(FACT(n)/(FACT(x)*FACT(n-x))*(0.5^x)*(0.5^n-x)

Table B17 Critical Values of the Wilcoxon T Distribution

				x		
	α =	0.10	0.05	0.02	0.01	0.05
n	α/2 =	0.05	0.025	0.01	0.005	0.0025
5		0				
6		2	0			
7		3	2	0		
8		5	3	1	0	
9		8	5	3	1	0
10		10	8	5	3	1
11		13	10	7	5	3
12		17	13	9	7	5
13		21	17	12	9	7
14		25	21	15	12	9
15		30	25	19	15	12
16		35	29	23	19	15
17		41	34	27	23	19
18		47	40	32	27	23
19		53	46	37	32	27
20		60	52	43	37	32
21		67	58	49	42	37
22		75	65	55	48	42
23		83	73	62	54	48
24		91	81	69	61	54
25		100	89	76	68	60
26		110	98	84	75	67
27		119	107	92	83	74
28		130	116	101	91	82
29		140	126	110	100	90
30		151	137	120	109	98

Modified from: McCornack, R.J. (1965) "Extended tables of the Wilcoxon matched pair signed rank statistic," *Journal of the American Statistical Association*, 60:864-871. Reprinted with permission from *The Journal of the American Statistical Association*.

Table B18 Critical Values for Kolmogorov Goodness-of-Fit Test ($\alpha = 0.05$)

n	One-Tailed Test	Two-Tailed Test
1	0.950	0.975
2	0.776	0.842
3	0.636	0.708
4	0.565	0.624
5	0.509	0.563
6	0.468	0.519
7	0.436	0.483
8	0.410	0.454
9	0.387	0.430
10	0.369	0.409
11	0.352	0.391
12	0.338	0.375
13	0.325	0.361
14	0.314	0.349
15	0.304	0.338
16	0.295	0.327
17	0.286	0.318
18	0.279	0.309
19	0.271	0.301
20	0.265	0.294
25	0.238	0.264
30	0.218	0.242
35	0.202	0.224
40	0.189	0.210
Approximation for >40	$1.22/\sqrt{n}$	$1.36/\sqrt{n}$

Modified from: Miller L.H. (1956). "Table of percentage points of Kolmogorov statistics," *Journal of the American Statistical Association* 51:111-121. Reprinted with permission from *The Journal of the American Statistical Association*. Copyright 1956 by the American Statistical Association. All rights reserved.

Table B19 Critical Values for Smirnov Test Statistic ($\alpha = 0.05$)

n	One-Tailed Test	Two-Tailed Test
3	2/3	…
4	3/4	3/4
5	3/5	4/5
6	4/6	4/6
7	4/7	5/7
8	4/8	5/8
9	5/9	5/9
10	5/10	6/10
11	5/11	6/11
12	5/12	6/12
13	6/13	6/13
14	6/14	7/14
15	6/15	7/15
16	6/16	7/16
17	7/17	7/17
18	7/18	8/18
19	7/19	8/19
20	7/20	8/20
25	8/25	9/25
30	9/30	10/30
35	10/35	11/35
40	10/40	12/40
Approximation for >40	$1.73/\sqrt{n}$	$1.92/\sqrt{n}$

Modified from: Birnbaum, Z.W. and Hall, R.A. (1960). "Small-sample distribution for multiple sample statistics of the Smirnov type," *Annals of Mathematical Statistics* 31:710-720. Permission to reprint was granted by the Institute of Mathematical Statistics.

Table B20 Critical Values for the Runs Test ($\alpha = 0.05$)

Reject H_0 if r is < Lower or > Upper Limits

n_1	n_2	Lower	Upper	n_1	n_2	Lower	Upper
6	6	4	10	11	16	9	19
	7-8	4	11		17-18	10	19
	9-12	5	12		19-20	10	20
	13-18	6	13	12	12	8	18
	19-20	7	13		13	9	18
7	7	4	12		14	9	19
	8	5	12		15	9	20
	9	5	13		16-18	10	20
	10-12	6	13		19-20	11	21
	13-14	6	14	13	13	9	19
	15	7	14		14	10	19
	16-20	7	15		15-16	10	20
8	8	5	13		17-18	11	21
	9	6	13		19-20	11	22
	10-11	6	14	14	14	10	20
	12-15	7	15		15-16	10	21
	16	7	16		17-18	11	22
	17-20	8	16		19	12	22
9	9	6	14		20	12	23
	10	6	15	15	15	11	21
	11-12	7	15		16	11	22
	13	7	16		17	12	22
	14	8	16		18-19	12	23
	15	8	17		20	13	24
	18-20	9	17	16	16	12	22
10	10	7	15		17	12	23
	11	7	16		18	12	24
	12	8	16		19-20	13	24
	13-15	8	17	17	17	12	24
	16-18	9	18		18	13	24
	19	9	19		19-20	13	25
	20	10	19	18	18	13	25
11	11	8	16		19	14	25
	12	8	17		20	14	26
	13	8	18	19	19-20	14	26
	14-15	9	18	20	20	15	27

Where n_1 is the small number of observations and n_2 is the larger

Modified from: Swed, F.S. and Eisenbar C. (1943). "Tables for testing randomness of grouping in a sequence of alternatives," *Annals of Mathematical Statistics* 14:84-86. Permission to reprint was granted by the Institute of Mathematical Statistics.

Table B21 Critical Values for T_1 Range Test ($\alpha = 0.05$)

	One-Tailed Test		Two-Tailed Test	
n	$\alpha = 0.05$	$\alpha = 0.01$	$\alpha = 0.05$	$\alpha = 0.01$
2	3.157	15.910	6.353	31.828
3	0.885	2.111	1.304	3.008
4	0.529	1.023	0.717	1.316
5	0.388	0.685	0.507	0.843
6	0.312	0.523	0.399	0.628
7	0.263	0.429	0.333	0.507
8	0.230	0.366	0.288	0.429
9	0.205	0.322	0.255	0.374
10	0.186	0.288	0.230	0.333
11	0.170	0.262	0.210	0.302
12	0.158	0.241	0.194	0.277
13	0.147	0.224	0.181	0.256
14	0.138	0.209	0.170	0.239
15	0.131	0.197	0.160	0.224
16	0.124	0.186	0.151	0.212
17	0.118	0.177	0.144	0.201
18	0.113	0.168	0.137	0.191
19	0.108	0.161	0.131	0.182
20	0.104	0.154	0.126	0.175

Modified from: Lord, E. (1947). "The use of range in place of standard deviation in the t-test," *Biometrika* 34:66. Reproduced with permission of the Biometrika Trustees.

Table B22 Critical Values for the F_R Test for Dispersion

w_1 = w_2	2	3	4	5	6	7	8	9	10
2	12.66	19.23	25.64	27.78	29.41	31.25	32.26	33.33	35.71
3	3.23	4.35	5.00	5.56	6.25	6.67	7.14	7.14	7.69
4	2.00	2.70	3.13	3.45	3.70	3.85	4.00	4.17	4.35
5	1.61	2.04	2.38	2.50	2.78	2.86	3.03	3.13	3.23
6	1.35	1.75	2.00	2.17	2.33	2.44	2.50	2.63	2.70
7	1.25	1.56	1.75	1.92	2.04	2.13	2.22	2.27	2.33
8	1.16	1.43	1.61	1.75	1.85	1.96	2.00	2.08	2.13
9	1.10	1.33	1.49	1.64	1.72	1.82	1.89	1.92	1.96
10	1.05	1.25	1.43	1.43	1.64	1.70	1.75	1.82	1.85

Modified from: Link, R.F. (1950). "The sampling distribution of the ratio of two ranges from independent samples," *Annals of Mathematical Statistics* 21:112-116. (These represent 1/*R*-values in original table to account for ratios >1.) Permission to reprint was granted by the Institute of Mathematical Statistics.

Table B23 Critical Values for Grubbs' Test (One-Sided Test for T)

	α			
n	0.1%	0.5%	1%	5%
3	1.155	1.155	1.155	1.153
4	1.499	1.496	1.492	1.463
5	1.780	1.764	1.749	1.672
6	2.011	1.973	1.944	1.822
7	2.201	2.139	2.097	1.938
8	2.358	2.274	2.221	2.032
9	2.492	2.387	2.323	2.110
10	2.606	2.482	2.410	2.176
11	2.705	2.564	2.485	2.234
12	2.791	2.636	2.550	2.285
13	2.867	2.699	2.607	2.331
14	2.935	2.755	2.659	2.371
15	2.997	2.806	2.705	2.409
16	3.052	2.852	2.747	2.443
17	3.103	2.894	2.785	2.475
18	3.149	2.932	2.821	2.504
19	3.191	2.968	2.854	2.532
20	3.230	3.001	2.884	2.557
21	3.266	3.031	2.912	2.580
22	3.300	3.060	2.939	2.603
23	3.332	3.087	2.963	2.624
24	3.362	3.112	2.987	2.644
25	3.389	3.135	3.009	2.663
30	3.507	3.236	3.103	2.745
35	3.599	3.316	3.178	2.811
40	3.673	3.381	3.240	2.866
45	3.736	3.435	3.292	2.914
50	3.789	3.483	3.336	2.956

Modified from: Grubbs, F.E. and Beck, G. (1972). "Extension of sample size and percentage points for significance tests of outlying observations," *Technometrics*, 14:847-54. Reproduced with permission of the American Statistical Association.

Table B24 Values for Use in Dixon Test for Outlier (α)

Statistic	n	0.5%	1%	5%
	3	.994	.988	.941
	4	.926	.889	.765
τ_{10}	5	.821	.780	.642
	6	.740	.698	.560
	7	.680	.637	.507
	8	.725	.683	.554
τ_{11}	9	.677	.635	.512
	10	.639	.597	.477
	11	.713	.679	.576
τ_{21}	12	.675	.642	.546
	13	.649	.615	.521
	14	.674	.641	.546
	15	.647	.616	.525
	16	.624	.595	.507
τ_{22}	17	.605	.577	.490
	18	.589	.561	.475
	19	.575	.547	.462
	20	.562	.535	.450
	21	.551	.524	.440
	22	.541	.514	.430
	23	.532	.505	.421
	24	.524	.497	.413
	25	.516	.489	.406

From: Dixon, W.J. and Massey, F.J. (1983). *Introduction to Statistical Analysis* (Table A-8e), McGraw-Hill Book Company, New York. Reproduced with permission of the publisher.

Appendix C

Summary of Commands for Excel® and Minitab®

ANOVA, complete randomized block design (Chapter 10)
Excel: Data ➤ Data Analysis ➤ Anova: Two-Factor Without Replicates

ANOVA, one-way design (Chapter 10)
Excel: Data ➤ Data Analysis ➤ Anova: Single Factor
Minitab: Stat ➤ ANOVA ➤ One-way
Stat ➤ ANOVA ➤ One-way (Unstacked)

ANOVA, two-way design (Chapter 12)
Excel: Data ➤ Data Analysis ➤ Anova: Two-Factor With Replication
Minitab: Stat ➤ ANOVA ➤ Two-way

Bar chart (Chapter 4)
Excel: Insert ➤ Chart ➤ Column (vertical)
Insert ➤ Chart ➤ Bar (horizontal)
Minitab: Graph ➤ Bar Chart

Binomial distribution - probability (Chapter 2)
Excel Function: BINOM.DIST or BINOMDIST

Box-and-whisker plot (Chapter 4)
Minitab: Graph ➤ Boxplot

Chi square distribution, critical values (Chapter 16)
Excel fx: **CHISQ.INV.RT** (**CHIINV** in versions before 2010)
CHISQ.INV (left–tail of the distribution)

Chi square distribution, p-values (Chapter 16)
Excel fx: **CHISQ.DIST.RT** (**CHIDIST** in versions before 2010)
CHISQ.DIST (left–tail of the distribution)

Chi square tests (Chapter 16)
Excel *f*x: **CHISQ.TEST** (**CHITEST** in versions before 2010)
Minitab: Stat ➤ Tables ➤ Chi-Square Test (Table in Worksheet)
Stat ➤ Tables ➤ Cross Tabulation and Chi-Square
Stat ➤ Tables ➤ Chi-Square Goodness-of-Fit Test (one variable)

Coefficient of variation – See Descriptive statistics

Combinations (Chapter 2)
Excel *f*x: COMBIN

Confidence intervals
Z-distribution – See Z-test, one sample
t-distribution – See t-test, one sample
Test of proportions – See Z-test of proportions, one sample

Correlation (Chapter 13)
Excel *f*x: **CORREL**
Excel: Data ➤ Data Analysis ➤ Correlation
Minitab: Stat ➤ Basic Statistics ➤ Correlation

Covariance (Chapter 13)
Excel *f*x: **COVARIANCE.S**
Excel: Data ➤ Data Analysis ➤ Covariance
Minitab: Stat ➤ Basic Statistics ➤ Covariance

Critical values
Chi square distribution – See chi square, critical values
F-distribution – See t-distribution, critical values
t-distribution – See t-distribution, critical values
Z-distribution – See Z-distribution, critical values

Descriptive statistics (Chapter 5)
Excel *f*x: See Table C.1
Excel: Data ➤ Data Analysis ➤ Descriptive Statistics ➤ Summary Statistics
Minitab: Stat ➤ Basic Statistics ➤ Display Descriptive Statistics

Dot chart (Chapter 4)
Minitab: Graph ➤ Dotplot

Factorials (Chapter 2)
Excel *f*x: FACT

F-distribution, critical values (Chapter 10)
Excel *f*x: **F.INV.RT** (**FINV** in versions before 2010)
F.INV (left–tail of the distribution)

Table C.1 Excel Function Commands for Descriptive Statistics

Statistics	Excel Function
Mode	MODE
Median	MEDIAN
Mean	AVERAGE
Smallest Data Point	MIN
Largest Data Point	MAX
Range	=MAX-MIN
Variance (sample)	VAR
Variance (population)	VAR.P
Standard Deviation (sample)	STDEV
Standard Deviation (population)	STDEV.P
Coefficient of Variation	=STDEV/AVERAGE

F-distribution, p-values (Chapter 10)
Excel fx: **F.DIST.RT** (**FDIST** in versions before 2010)
F.DIST (left–tail of the distribution)

Friedman test (Chapter 21)
Minitab: Stat ➤ Nonparametrics ➤ Friedman

Histogram (Chapter 4)
Minitab: Graph ➤ Histogram

Homogeneity of variance (Chapter 10) - Bartlett's and Levene's tests
Minitab: Stat ➤ ANOVA ➤ Test for equal variances

Kruskal-Wallis test (Chapter 21)
Minitab: Stat ➤ Nonparametrics ➤ Kruskal-Wallis

Kurtosis (Chapter 6)
Excel fx: KURT
Minitab: Stat ➤ Basic Statistics ➤ Display Description Statistics

Mann-Whitney test (Chapter 21)
Minitab: Stat ➤ Nonparametrics ➤ Mann-Whitney

Mean – See Descriptive statistics

Median – See Descriptive statistics

Mode – See Descriptive statistics

Mood's median test (Chapter 21)
Minitab: Stat ➤ Nonparametrics ➤ Mood's Median Test

Normality tests (Chapter 6)
Anderson-Darling, Kolmogorov-Smirnov and Ryan-Joiner
Minitab: Stat ➤ Basic Statistics ➤ Normality Test

Permutations (Chapter 2)
Excel *f*x: PERMUT

Pie chart (Chapter 4)
Excel: Insert ➤ Chart ➤ Pie
Minitab: Graph ➤ Pie Chart

Poisson distribution - probability (Chapter 2)
Excel *f*x: POISSON or POISSON.DIST

***Post hoc* procedures** (Chapter 11)
Tukey's HSD, Fisher's LSD, Dunnette's and Hsu's MCB tests
Minitab: Stat ➤ ANOVA ➤ One-way ➤ *Comparisons…*
Stat ➤ ANOVA ➤ One-way (Unstacked) ➤ *Comparisons…*

Probability – binomial distribution (Chapter 2)
Excel *f*x: BINOM.DIST or BINOMDIST

Probability – Poisson distribution (Chapter 2)
Excel *f*x: POISSON or POISSON.DIST

p-Values
Chi square distribution – See chi square, p-values
F-distribution – See t-distribution, p-values
t-distribution – See t-distribution, p-values
Z-distribution – See Z-distribution, p-values

Quality control (Chapter 7)
Minitab: Stat ➤ Control Charts ➤ Variables Charts for Subgroups
Stat ➤ Control Charts ➤ Variables Charts for Individuals
Stat ➤ Quality Tools ➤ Capacity Sixpack ➤ Normal

Random sampling (Chapter 3)
Excel *f*x: RAND and RANDBETWEEN
Minitab: Calc ➤ Random Data ➤ Sample from Columns

Range – See Descriptive statistics

Regression (Chapter 14)
Excel: Data ➤ Data Analysis ➤ Regression
Minitab: Stat ➤ Regression ➤ Regression
Stat ➤ Regression ➤ Stepwise
Stat ➤ Regression ➤ Fitted line plot

Repeatability and Reproducibility (Chapter 12)
Minitab: Stat ➤ Quality Tools ➤ Gage Study ➤ Gage R&R study (crossed)

Runs test (Chapter 21)
Minitab: Stat ➤ Nonparametrics ➤ Runs Test

Scatter plot (Chapter 4)
Excel Insert ➤ Chart ➤> Scatter
Minitab: Graph ➤Scatterplot

Sign test, one-sample (Chapter 21)
Minitab: Stat ➤ Nonparametrics ➤ 1-Sample Sign

Skew (Chapter 6)
Excel *f*x: SKEW
Minitab: Stat ➤ Basic Statistics ➤ Display Description Statistics

Spearman rho test (Chapter 21)
Minitab: Stat ➤ Tables ➤ Cross tabulation and Chi Square ➤ *Other Stats…*

Standard deviation – See descriptive statistics

Stem-and-leaf plot (Chapter 4)
Minitab: Graph ➤ Stem-and-Leaf

Survival statistics (Chapter 20)
Minitab: Stat ➤ Reliability/Survival ➤
Distribution Analysis (Right Censoring) ➤
Nonparametric Distribution Analysis

t-distribution, critical values (Chapter 9)
Excel *f*x: **T.INV.2T** (two-tailed distribution); **TINV** (in versions before 2010)
T.INV (one-tailed distribution)

t-distribution, p-values (Chapter 9)
Excel *f*x: **T.DIST.2T** (two-tailed distribution);
TDIST (in versions before 2010)
T.DIST.RT (one-tailed distribution)

Time series plot (Chapter 4)
Minitab: Graph ➤ Time Series Plot

Tolerance Limits (Chapter 7)
Minitab: Stat ➤ Quality Tools ➤ Tolerance Interval

t-test, one-sample (Chapter 9)
Excel *f*x: **CONFIDENCE.T –** creates portion of the equation that includes the reliability coefficient and error only
Minitab: Stat ➤ Basic Statistics ➤ 1-sample t… one sample CI

t-test, paired data (Chapter 9)
Excel: Data ➤ Data Analysis ➤ t-test: Paired Two Sample for Means
Minitab: Stat ➤ Stat ➤ Basic Statistics ➤ Paired t… one sample CI

t-test, two-sample (Chapter 9)
Excel: Data ➤ Data Analysis ➤ t-test: Two-Sample Assuming Unequal Variance
Data ➤ Data Analysis ➤ t-test: Two-Sample Assuming Equal Variances
Minitab: Stat ➤ Basic Statistics ➤ 2-sample t… two-sample t-test

Variance – See Descriptive statistics

Wilcoxon test, one-sample (Chapter 21)
Minitab: Stat ➤ Nonparametrics ➤ 1-Sample Wilcoxon

Z-distribution, critical values (Chapter 7)
Excel *f*x: **NORM.S.INV** (**NORMSINV** in versions before 2010)
NORM.INV (**NORMINV** in versions before 2010)

Z-distribution, p-values (Chapter 7)
Excel *f*x: **NORM.S.DIST** (**NORMSDIST** in versions before 2010)
NORM.DIST (**NORMDIST** is versions before 2010)

Z-test of proportions, one-sample (Chapter 15)
Minitab: Stat ➤ Basic Statistics ➤ 1-proportion

Z-test of proportions, two-sample (Chapter 15)
Minitab: Stat ➤ Basic Statistics ➤ 2-proportions

Z-test, one sample (Chapter 7)
Excel *f*x: **CONFIDENCE.NORM** (**CONFIDENCE** in versions before 2010) creates portion of the equation that includes the reliability coefficient and error only
Minitab: Stat ➤ Basic Statistics ➤ 1-sample Z

Appendix D

Answers to Example Problems

Chapter 1 – Introduction

1. **Discrete variables:** experimental versus controls (placebo); dosage form – table/capsule/other; test drug versus reference standard; fed versus fasted state (before/after meals); manufacturer (generic versus brand); male versus female subjects; "normal" versus geriatric population
   Continuous variables: bioavailability measurements (C_{max}, T_{max}, AUC); prolactin levels (ng/l); age (in years); smoking history (cigarettes per day)

2. **Discrete variables:** dissolution – pass or fail criteria; friability – pass or fail criteria; impurities – present or absent; change in manufacturing process – old or new process; immediate release or sustained release; formulation A, B, or C
   Continuous variables: amount of active ingredient (content uniformity); disintegration rate; hardness; size – thickness/diameter; tablet weight

3. a. Independent variable: Two manufacturers (Innovator, Acme); Discrete
      Dependent variable: Pharmacokinetic measure (C_{max}); Continuous
   b. Independent variable: Nutritional status (poor versus good); Discrete
      Dependent variable: Survival (lived versus died); Discrete
   c. Independent variable: Laboratory (manufacturer, contract lab); Discrete
      Dependent variable: Assay results (% labeled amount); Continuous
   d. Independent variable: Temperature (39°C versus 35°C); Discrete
      Dependent variable: Disintegration results (pass versus fail); Discrete
   e. Independent variable: Physician (A versus B versus C); Discrete
      Dependent variable: Length of stay in hospital (days); Continuous
   f. Independent variable: Batch of raw material (batch A, B or C); Discrete
      Dependent variable: Viscosity; Continuous
   g. Independent variable: Method A (gold standard); Continuous
      Dependent variable: Method B; Continuous

Chapter 2 – Probability

1. 150 healthy female volunteers in a multicenter study for a new pregnancy test. The probability of randomly selecting one volunteer:

a. Who is pregnant

$$p(PG) = \frac{m(PG)}{N} = \frac{75}{150} = 0.500$$

b. Who has acidic urine

$$p(pH \downarrow) = \frac{m(pH \downarrow)}{N} = \frac{62}{150} = 0.413$$

c. Who has nonacidic urine

$$p(pH \uparrow) = 1 - p(pH \downarrow) = 1 - 0.413 = 0.587$$

d. Who is both pregnant and has acidic urine

$$p(PG \cap pH \downarrow) = \frac{m(PG \cap pH \downarrow)}{N} = \frac{36}{150} = 0.240$$

e. Who is either pregnant or has acidic urine

$$p(PG \cup pH \downarrow) = p(PG) + p(pH \downarrow) - p(PG \cap pH \downarrow)$$

$$p(PG \cup pH \downarrow) = 0.500 + 0.413 - 0.240 = 0.673$$

f. Who is pregnant among women with acidic urine

$$p(PG \mid pH \downarrow) = \frac{p(PG \cap pH \downarrow)}{p(pH \downarrow)} = \frac{0.24}{0.413} = 0.581$$

g. Who has nonacidic urine among women who are pregnant

$$p(pH \uparrow \mid PG) = \frac{p(PG \cap pH \uparrow)}{p(PG)} = \frac{0.260}{0.500} = 0.520$$

2. The ways of assigning three laboratory technicians to five pieces of equipment:

$$\binom{n}{x} = \frac{n!}{x!(n-x)!} = \binom{5}{3} = \frac{5!}{3!2!} = \frac{5 \cdot 4 \cdot 3 \cdot 2 \cdot 1}{(3 \cdot 2 \cdot 1)(2 \cdot 1)} = 10$$

3. The possible ways to sample five out of ten tablets:

$$\binom{n}{x} = \frac{n!}{x!(n-x)!} = \binom{10}{5} = \frac{10!}{5!5!} = \frac{10 \cdot 9 \cdot 8 \cdot 7 \cdot 6 \cdot 5!}{(5 \cdot 4 \cdot 3 \cdot 2 \cdot 1)(5!)} = 252$$

4. The outcomes for eight patients where the survival rate is 0.60:
   a. That all eight patients will survive:

$$p(8) = \binom{8}{0}(0.60)^8 (0.40)^0 = (1)(0.0168)(1) = 0.017$$

   b. That half will die:

$$p(4) = \binom{8}{4}(0.60)^4 (0.40)^4 = (70)(0.1296)(0.0256) = 0.232$$

5. Two hundred containers of an old and new design were subjected to identical rigorous abuse. The probability of randomly selecting a container:
   a. Of the new design:

$$p(New) = \frac{m(New)}{N} = \frac{100}{200} = 0.500$$

b. That is a "failure":

$$p(F) = \frac{m(F)}{N} = \frac{15}{200} = 0.075$$

c. That is a "success":

$$p(S) = 1 - p(F) = 1 - 0.075 = 0.925$$

d. That is both an old container design and a "failure":

$$p(Old \cap F) = \frac{m(Old \cap F)}{N} = \frac{12}{200} = 0.060$$

e. That is either an old design or a "failure":

$$p(Old \cup F) = p(Old) + p(F) - p(Old \cap F)$$

$$p(Old \cup F) = 0.500 + 0.075 - 0.060 = 0.515$$

f. That the container is a "failure" if selected from only the new containers:

$$p(F \mid New) = \frac{p(F \cap New)}{p(New)} = \frac{0.015}{0.500} = 0.030$$

g. That the container is a "success" if selected from only the old containers:

$$p(S \mid Old) = \frac{p(S \cap Old)}{p(Old)} = \frac{0.440}{0.500} = 0.880$$

6. An in-service director for Galaxy Drugs prepared a program for new employees. She had eight topics to cover, and they could be covered in any order.

a. How many different programs is it possible for her to prepare?

$$8! = 40{,}320$$

b. At the last minute she finds that she has time for only six topics. How many different programs is it possible for her to present if all are equally important?

If order is important, a permutation:

$$\frac{8!}{(8-6)!} = \frac{8!}{2!} = 20{,}160$$

If order is not important, combination:

$$\binom{8}{6} = \frac{8!}{6!\,2!} = 28$$

7. Calculate the following:

a. $\binom{6}{2} = \frac{6!}{2!\,4!} = \frac{6 \times 5 \times 4!}{2 \times 1 \times 4!} = \frac{30}{2} = 15$

b. $\binom{9}{5} = \frac{9!}{5!\,4!} = \frac{9 \times 8 \times 7 \times 6 \times 5!}{4 \times 3 \times 2 \times 1 \times 5!} = 126$

c. $\binom{30}{3} = \frac{30!}{3!\,27!} = \frac{30\times 29\times 28\times 27!}{3\times 2\times 1\times 27!} = 4060$

Chapter 3 – Sampling

1. Sample results will vary based on the five random samples.

2. The averages should be different. The reason will be covered in Chapter 7.

3. $\binom{50}{5} = \frac{50!}{5!\,45!} = \frac{50\times 49\times 48\times 47\times 46\times 45!}{5\times 4\times 3\times 2\times 1\times 45!} = 2{,}118{,}760$

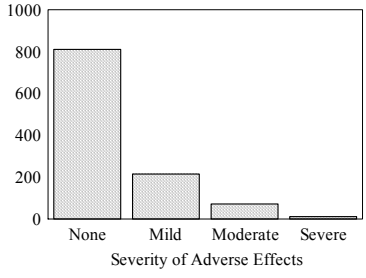
Chapter 4 – Presentation Modes

1. Incidence of reported adverse drug effects:
   a. Tabular results

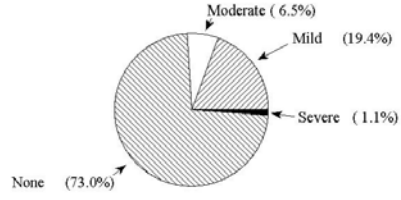
Severity of Adverse Effects

Severity	n	%	Cum. %
None	810	73.0	73.0
Mild	215	19.4	92.4
Moderate	72	6.5	98.9
Severe	12	1.1	100.0
	1109	100.0	

   b. Bar graph

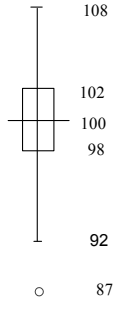


   c. Pie Chart



2. Distribution of assay results:

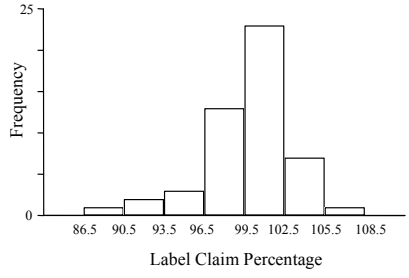
   a. Box-and-whisker plot



b. Stemplot

Frequency	Stem		Leaves
0	8		
1			7
2	9		23
16		Q	5667778888999999
28	10	MQ	00000000001111111112222233344
3			557
50			

c. Histogram



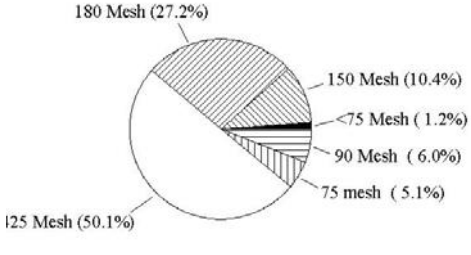
3. Particle size determination:

a. Tabular results

Percent of Particles Retained on Various Sieve Screens

Mesh Size (μM)	% Retained	Cum. % Retained
425	50.1	50.1
180	27.2	77.3
150	10.4	87.7
90	6.0	93.7
75	5.1	98.8
pan (<75)	1.2	100.0
	100.0	

b. Pie chart



c. Bar chart

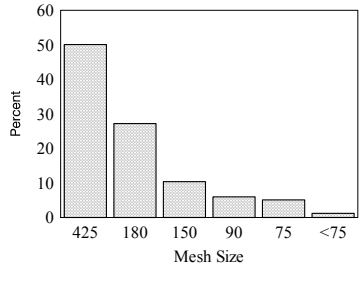
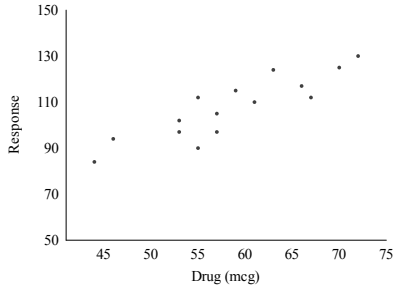


Table D.1 Examination Results Ranked in Descending Order

98	87	84	77
96	86	83	77
95	86	82	76
92	85	80	75
90	85	80	72
90	85	79	70
88	84	78	69
87	84	78	60

4. Scatter plot displaying relationship between two variables



Chapter Five – Central Tendency

1. Final examination results for a pharmacokinetics course (Table D.1):
   a. Range: Highest to lowest grade = 98 – 60 = 38%
   b. Median: Value between 16th and 17th observation = 84%
   c. Sample mean

$$\overline{X} = \frac{\sum x}{n} = \frac{85 + 79 + 98 + \ldots 85 + 80}{32} = 82.4\,\%$$

   d. Sample variance

$$S^2 = \frac{(85 - 82.4)^2 + (79 - 82.4)^2 + \ldots (80 - 82.4)^2}{31} = 67.16$$

   e. Sample standard deviation

$$S = \sqrt{S^2} = \sqrt{67.16} = 8.19\ \%$$

2. Noradrenaline levels obtained during a clinical trial.

$\sum x =$ 37.4 93.60 $= \sum x^2$

Mean: $\overline{X} = \frac{\sum x}{n} = \frac{37.4}{15} = 2.49\ nmol/L$

Variance: $S^2 = \frac{n(\sum X^2) - (\sum X)^2}{n(n-1)} = \frac{15(93.6) - (37.4)^2}{(15)(14)} = 0.025$

Standard deviation: $S = \sqrt{S^2} = \sqrt{0.025} = 0.158 \text{ nmol / L}$

3. Prolactin levels obtained during a clinical trial.

$\sum x =$ 81.3 679.83 $= \sum x^2$

Mean: $\overline{X} = \frac{\sum x}{n} = \frac{81.3}{10} = 8.13 \text{ ng/L}$

Variance: $S^2 = \frac{\sum (x_i - \overline{X})^2}{n-1}$

$S^2 = \frac{(9.4 - 8.13)^2 + (8.6 - 8.13)^2 \ldots + (9.4 - 8.13)^2}{9} = 2.096$

Standard deviation: $S = \sqrt{S^2} = \sqrt{2.096} = 1.45 \text{ ng / L}$

4. Randall-Selitto paw pressure test and the following results (in grams) were observed.

$\sum x = 3{,}810$ $\sum x^2 = 972{,}800$ n = 15

Sample mean: $\overline{X} = \frac{\sum x}{n} = \frac{3810}{15} = 254 \text{ grams}$

Sample variance:

$S^2 = \frac{n(\sum X^2) - (\sum X)^2}{n(n-1)} = \frac{15(972{,}800) - (3{,}810)^2}{15(14)} = 361.4$

Sample standard deviation: $S = \sqrt{S^2} = \sqrt{361.4} = 19.0 \text{ grams}$

Median = eighth response in rank order = 250 grams

5. Measures of central tendency for Table 4.1
Excel:

Column1	
Mean	250
Standard Error	0.404002959
Median	250
Mode	250
Standard Deviation	2.212815339
Sample Variance	4.896551724
Kurtosis	-0.11377813
Skewness	-0.204588377
Range	9
Minimum	245
Maximum	254
Sum	7500
Count	30

Minitab:

Descriptive Statistics: Assay

Variable	N	Mean	StDev	Variance	CoefVar	Mode	N for Mode
Assay	30	250.00	2.21	4.90	0.89	250	7

Variable	Q1	Median	Q3	IQR
Assay	248.75	250.00	251.25	2.50

Variable	TrMean	Minimum	Maximum	Range
Assay	250.04	245.00	254.00	9.00

6. Measures of central tendency for the C_{max} results in the six healthy male volunteers are as follows:
Excel:

Column1	
Mean	77.5
Standard Error	9.6910612
Median	76
Mode	#N/A
Standard Deviation	23.738155
Sample Variance	563.5
Kurtosis	-0.8338413
Skewness	0.1587865
Range	65
Minimum	46
Maximum	111
Sum	465
Count	6

Minitab:

Descriptive Statistics: Cmax

Variable	N	Mean	StDev	Variance	CoefVar	Mode	N for Mode
Cmax	6	77.50	23.74	563.50	30.63	*	0

Variable	Q1	Median	Q3	IQR
Cmax	56.50	76.00	99.75	43.25

Variable	TrMean	Minimum	Maximum	Range
Cmax	*	46.00	111.00	65.00

7. IgA analysis at a Midwestern CRO (sample data).
Mode = 141 (n = 2)
Median = 141+144/2 = 142.5 mcg/ml
Sample mean: $\overline{X} = \frac{\sum x}{n} = \frac{150+135...+141}{8} = 142.1\ mcg/ml$
Range = 162-117 = 45 mcg/ml
Variance: $S^2 = \frac{\sum (x_i - \overline{X})^2}{n-1} = \frac{(150-142.1)^2 + ...(141-142.1)^2}{7} = 166.98$

Standard deviation: $S = \sqrt{S^2} = \sqrt{166.98} = 12.9\ mcg/ml$

Coefficient of variation: $C..V. = \frac{S}{\overline{X}} = \frac{12.9}{142.1} = 0.0909$

Relative standard deviation: $RSD = C.V.x100 = 0.0909x100 = 9.09\%$

8. First-time-in-humans clinical trial of a new agent ($\Sigma x = 17.66$; $\Sigma x^2 = 26.389$)

Median: $Median = \frac{1.40 + 1.44}{2} = 1.42$

Mean: $\overline{X} = \frac{\sum x}{n} = \frac{17.66}{12} = 1.47$

Variance: $S^2 = \frac{n(\sum X^2) - (\sum X)^2}{n(n-1)} = \frac{12(26.389) - (17.66)^2}{12(11)} = 0.036$

Standard deviation: $S = \sqrt{S^2} = \sqrt{0.036} = 0.190$

Example of an Excel output

Mean	1.471667
Standard Error	0.055005
Median	1.42
Mode	#N/A
Standard Deviation	0.190541
Sample Variance	0.036306
Kurtosis	0.564721
Skewness	1.071118
Range	0.63
Minimum	1.26
Maximum	1.89
Sum	17.66
Count	12

Chapter 6 – The Normal Distribution and Data Transformation

1. Clinical trials data with possible skewed data (Table 6.4):

Median = 1.51 hours

Arithmetic mean: $\overline{X} = \frac{1.41 + 1.81 + ...0.91}{15} = 1.66\ hours$

Geometric mean: $\overline{X}_G = \sqrt[15]{1.41\,x\,1.81\,x\,...0.91} = 1.54$

It can be assumed that the distribution is positively skewed, because the arithmetic mean is larger than the median (pulled to the right) and the geometric mean is much closer to the median.

2. Clinical trials data with possible skewed data, values for alternative transformations appear in Table 6.4:

a. Mean based on the square-root transformation

$$\overline{X}_{transformed} = \frac{\sum x_i'}{n} = \frac{18.96}{15} = 2.37$$

$$\overline{X} = (\overline{X}_{transformed})^2 = (2.37)^2 = 5.62 \text{ hours}$$

Note, since there were no zeros in the responses Eq. 6.10 was used in the transformation. If Eq. 6.11 were used the result would be 6.92. The lognormal transformation would be preferable because the transformed mean is closest to the median.

b. Mean based on the reciprocal transformation

$$\overline{X}_{transformed} = \frac{\sum x_i'}{n} = \frac{10.44}{15} = 1.31$$

$$\overline{X} = \frac{1}{\overline{X}_{transformed}} = \frac{1}{1.31} = 0.766 \text{ hours}$$

Since there were no zeros in the responses Eq. 6.12 was used in the transformation. If Eq. 6.13 were used the result would be 0.34. The lognormal transformation would be preferable because the transformed mean is closest to the median. Notice that the reciprocal transformation creates the greatest change in the mean (intended for the extremely positive skewed data) and the square root the last change in the mean (intended to lesser positively skewed distributions).

3. The geometric mean is closer to the median than the arithmetic mean.

GEOMEAN =	1.461054

Chapter 7 – Confidence Intervals and Tolerance Limits

1. Results of different samples will vary based on the random numbers selected from the table. Results can vary from the smallest possible mean outcome of 72.3 to the largest possible mean of 79.0. Assume that our results for Sample C were tablets 05, 16, and 27. The mean assay result would be:

$$\overline{X} = \frac{78+74+73}{3} = 75 \text{ mg}$$

a. The 95% confidence interval would be:

$$\mu = \bar{x} \pm Z_{(1-\alpha/2)} x \frac{\sigma}{\sqrt{n}}$$

$$\mu = 75 \pm 1.96 x \frac{2.01}{\sqrt{3}} = 75 \pm 2.27$$

$$72.73 < \mu < 77.27 \text{ mg}$$

b. Using the above sample the 90% and 99% confidence intervals for the population mean would be:

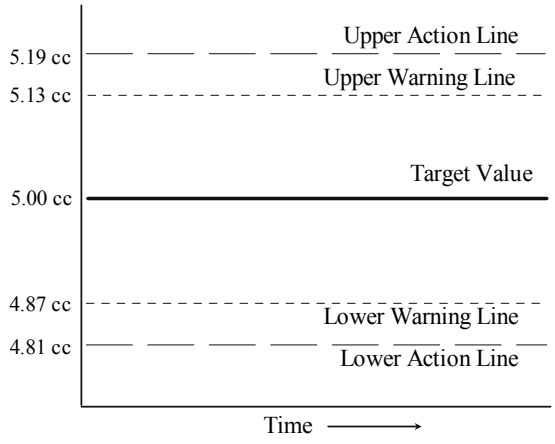


Figure D.1 Warning and action line for question 3.

$$90\%\ CI: \qquad \mu = 75 \pm 1.64 \times \frac{2.01}{\sqrt{3}}$$

$$73.10 < \mu < 76.90\ mg$$

$$99\%\ CI: \qquad \mu = 75 \pm 2.57 \times \frac{2.01}{\sqrt{3}}$$

$$72.02 < \mu < 77.98\ mg$$

As expected the interval becomes much wider (includes more possible results) when we wish to be 99% certain and becomes smaller as we accept a greater amount of error.

c. Since the true population mean (μ) is 75.47 mg, this represents the most frequent outcome, but very few samples will produce 75.47. Instead we would see a clustering of means around that center point for the population.

2. With μ = 75.47, σ = 2.01, and N = 30:
   a. There are a possible 4060 different samples of n = 3:
   $$\binom{n}{x} = \frac{n!}{x!(n-x)!} = \frac{30!}{3!\,27!} = 4060$$
   b. The grand mean for all 4060 possible sample means is 75.47 mg:
   $$\mu_{\overline{X}} = \mu = 75.47\ mg$$
   c. The standard deviation for all 4060 possible sample means is 1.16 mg:
   $$\sigma_{\overline{X}} = \frac{\sigma}{\sqrt{n}} = \frac{2.01}{\sqrt{3}} = 1.16\ mg$$
3. Creation of a quality control chart with the target μ = 5 cc, σ = 0.2 cc, and n = 10:
   Warning lines (Figure D.1)
   $$\mu_w = \mu_0 \pm \frac{2\sigma}{\sqrt{n}} = 5 \pm \frac{2(0.2)}{\sqrt{10}} = 5 \pm 0.13 \quad ; \quad \mu_w = 5.13 \text{ and } 4.87$$

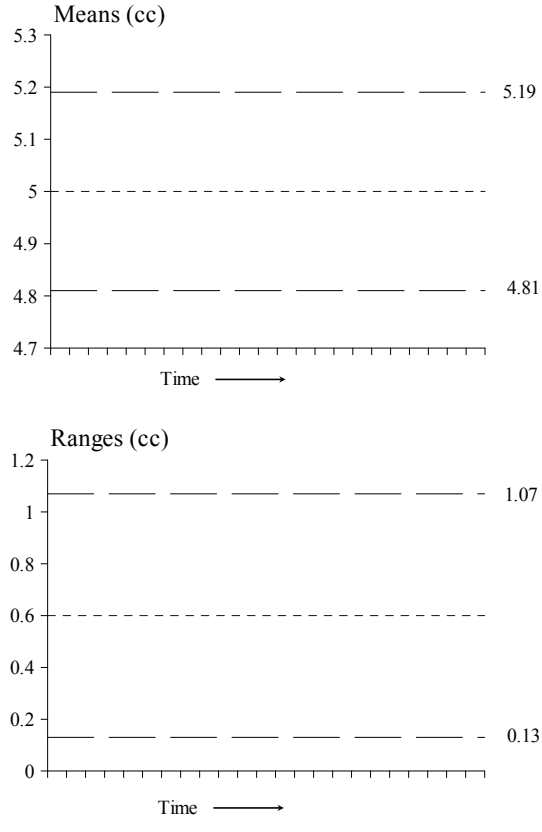


Figure D.2 Action and range action lines using range formulas.

$$\mu_a = \mu_0 \pm \frac{3\sigma}{\sqrt{n}} = 5 \pm \frac{3(0.2)}{\sqrt{10}} = 5 \pm 0.19$$

$$\mu_a = 5.19 \text{ and } 4.81$$

Action lines using range formulas (Figure D.2):

$$AL = \overline{\overline{X}} \pm A\overline{R} = 5 \pm 0.31(0.6) = 5 \pm 0.19$$

$$A_U L = 5.19 \qquad A_L L = 4.81$$

Range action lines:

$$\text{Upper action line} = D_U \overline{R} = 1.78(0.6) = 1.07$$

$$\text{Lower action line} = D_L \overline{R} = 0.22(0.6) = 0.13$$

4. Measures of central tendency associated with volumes of the ampules are:

   Mean ($\overline{X}$) = 2.000 ml
   Standard deviation (S) = 0.014 ml

   The K-value from Table B3 for 99% confidence (γ) for 99% of the batch (p) is 4.161. The tolerance limits based on n = 20 are:

$$LTL = \overline{X} - KS = 2.000 - (4.161)(0.014) = 1.942\ ml$$
$$UTL = \overline{X} + KS = 2.000 + (4.161)(0.014) = 2.058\ ml$$

Thus, with 99% confidence we would expect 99% of all ampules to have between 1.942 and 2.058 ml of volume.

5. Calculation of capacity indices:

$$\hat{C}_p = \frac{USL - LSL}{6S} = \frac{1.20 - 0.80}{6(0.06)} = 1.11$$
$$m = \frac{USL + LSL}{2} = \frac{1.20 + 0.80}{2} = 1.00$$
$$k = \frac{\left|m - \overline{X}\right|}{\frac{USL - LSL}{2}} = \frac{|1.00 - 0.95|}{\frac{1.20 - 0..80}{2}} = \frac{0.05}{0.20} = 0.25$$
$$\hat{C}_{pk} = \hat{C}_p(1 - \hat{k}) = 1.11(1 - 0.25) = 1.11(0.75) = 0.83$$
$$\hat{C}_{pm} = \frac{USL - LSL}{6\sqrt{S^2 + (\overline{X} - T)^2}} = \frac{1.20 - 0.80}{6\sqrt{(0.06)^2 + (0.95 - 1.00)^2}} = 0.85$$

The process is capable by the C_p index, but not by the C_{pk} and C_{pm} indices because sample mean is off center from the target.

$$\delta = \frac{C_{pk}}{C_p} = \frac{0.83}{1.11} x100\% = 74.7\%$$

If the sample mean can be shifted back to the center there will be an almost 75% increase in the process capability.

The 95% confidence interval for the C_{pk} is:

$$C_{pk} = \hat{C}_{pk} \pm z_{1-\alpha/2}\sqrt{\frac{1}{9n} + \frac{\hat{C}_{pk}^2}{2(n-1)}}$$
$$C_{pk} = 0.83 \pm 1.96\sqrt{\frac{1}{9(100)} + \frac{(0.83)^2}{2(99)}} = 0.83 \pm 0.13$$
$$0.70 < C_{pk} < 0.96$$

6. Creation of a 95% confidence interval where $\overline{X}$ = 48.3, σ = 3.5 and n = 20:

$$\mu = \bar{x} \pm Z_{(1-\alpha/2)}\ x\ \frac{\sigma}{\sqrt{n}}$$
$$\mu = 48.3 \pm 1.96 \cdot \frac{3.5}{\sqrt{20}} = 48.3 \pm 1.53$$
$$44.77 < \mu < 49.83$$

Based on a sample of only 20 tablets, there is 95% certainty that the true population mean (strength) is between 44.77 and 49.83 mg. The goal of 50 mg

does not fall within this confidence interval; therefore, it is assumed that Batch #1234 is subpotent.

Chapter 8 – Hypothesis Testing

1. The null hypothesis and alternative hypothesis must create mutually exclusive and exhaustive statements.
   a. H_0: $\mu_A = \mu_B$
      H_1: $\mu_A \neq \mu_B$
   b. H_0: $\mu_H \geq \mu_L$
      H_1: $\mu_H < \mu_L$
   c. H_0: $\mu_1 = \mu_2 = \mu_3 = \mu_4 = \mu_5 = \mu_6$
      H_1: H_0 is false
      (As will be discussed in Chapter 10, traditional tests do not allow us to immediately identify which population means are different, only that at least two of the six means are significantly different. Further testing is required to determine the exact source of the difference(s).)
   d. H_0: $\mu_A \leq \mu_B$
      H_1: $\mu_A > \mu_B$
   e. H_0: $\mu = 125$
      H_1: $\mu \neq 125$
   f. H_0: Populations C, D, E, F, and G are the same
      H_1: Populations C, D, E, F, and G are not the same
      (Similar to c above, we do not identify the specific difference(s).)
   g. H_0: Both samples come from the same population
      H_1: Both samples do not come from the same population

2. In the first part of the question: α (Type I error) = 0.05; confidence level ($1 - \alpha$) = 0.95; power ($1 - \beta$) = 85%; therefore, β (Type II error) = 0.15. If the power happens to be only 72% or 0.72, then the other outcomes in our test of null hypotheses are: β (Type II error) = 1 − 0.72 = 0.28; α (Type I error) = 0.05; confidence level ($1 - \alpha$) = 0.95. Note that α and $1 - \alpha$ did not change because the researcher would have set these parameters prior to the statistical test.

3. This is an example of a propagation of error involving division. The appropriate approach involves combining the relative standard deviations. Where the average density is:

$$\overline{X}_D = \frac{10.6}{4.9} = 2.16\ g/ml$$

Calculation of RSDs:

$$Weight\ RSD = \frac{0.6}{10.6} \cdot 100\% = 5.66\%$$

$$Volume\ RSD = \frac{0.3}{4.9} \cdot 100\% = 6.12\%$$

Calculation of measure of dispersion:

$$RSD_{Total} = \sqrt{(5.66)^2 + (6.12)^2} = 8.34\%$$

$$S_D = \frac{(8.34)(2.16)}{100} = 0.18$$

Result for the density: 2.16 ± 0.18 g/ml

4. We will assume the variability in example is additive (sample + reference variability). The six unknowns have a standard deviation of 2.42%. The reference standard has a standard deviation of 2%. Therefore propagating the error for the total amount of uncertainty would be:

$$S_{Total} = \sqrt{S_1^2 + S_2^2 + S_3^2 + ...S_K^2}$$

$$S_{Total} = \sqrt{(2.42)^2 + (2.00)^2} = \sqrt{9.86} = 3.14\%$$

Chapter 9 – t-Tests

1. Comparison of two groups of physical therapy patients.
   Independent variable: group 1 versus group 2 (discrete)
   Dependent variable: percent range of motion (continuous)
   Statistical test: two-sample t-test

	Group 1	Group 2
Mean =	81.44	83.91
S.D. =	8.08	6.80
n =	9	11

   Hypotheses: H_0: $\mu_1 = \mu_2$
   H_1: $\mu_1 \neq \mu_2$
   Decision rule: With $\alpha = .05$, reject H_0 if $t > t_{18}(0.025)$ or $t < -t_{18}(0.025)$.
   With $\alpha = .05$, reject H_0 if $t > 2.12$ or $t < -2.12$.

$$S_p^2 = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2} = \frac{8(8.08)^2 + 10(6.80)^2}{9 + 11 - 2} = 54.70$$

$$t = \frac{\overline{X_1} - \overline{X_2}}{\sqrt{\frac{S_p^2}{n_1} + \frac{S_p^2}{n_2}}} = \frac{81.44 - 83.91}{\sqrt{\frac{54.7}{9} + \frac{54.7}{11}}} = \frac{-2.47}{3.32} = -0.74$$

   Decision: With $t > -2.12$ we cannot reject H_0 and conclude that there is no significant difference between the two types of treatment regimens.

2. Clinical trial to evaluate the effectiveness of a new bronchodilator.
   Independent variable: two time periods (patient serves as own control)
   Dependent variable: forced expiratory volume (continuous)
   Test statistic: paired t-test – Table D.2
   Mean difference and standard deviation difference:

Table D.2 Effectiveness of a New Bronchodilator

Subject number	FEV_1 before administration	Three hours after administration	d	d^2
1	3.0	3.1	+0.1	0.01
2	3.6	3.9	+0.3	0.09
3	3.5	3.7	+0.2	0.04
4	3.8	3.8	0	0
5	3.3	3.2	–0.1	0.01
6	3.9	3.8	–0.1	0.01
7	3.1	3.4	+0.3	0.09
8	3.2	3.3	+0.1	0.01
9	3.5	3.6	+0.1	0.01
10	3.4	3.4	0	0
11	3.5	3.7	+0.2	0.04
12	3.6	3.5	–0.1	0.01
		$\sum =$	+1.0	0.32

$$\overline{X_d} = \frac{\sum d}{n} = \frac{+1.0}{12} = 0.083$$

$$S_d^2 = \frac{n(\sum d^2) - (\sum d)^2}{n(n-1)} = \frac{12(0.32) - (1.0)^2}{12(11)} = 0.022$$

$$S_d = \sqrt{S_d^2} = \sqrt{0.022} = 0.148$$

a. What is $t_{(1-\alpha/2)}$ for $\alpha = 0.05$? $t_{11}(0.975) = 2.201$

b. Construct a 95% confidence interval for the difference between population means.

$$\mu_d = \overline{X_d} \pm t_{n-1}(1-\alpha/2)\frac{S_d}{\sqrt{n}}$$

$$\mu_d = +0.083 \pm 2.201\frac{0.148}{\sqrt{12}} = +0.083 \pm 0.094$$

$$-0.011 < \mu_d < +0.177 \quad \underline{\textit{not significant}}$$

c. Use a t-test to compare the two groups.

$$t = \frac{\overline{X_d}}{\frac{S_d}{\sqrt{n}}}$$

$$t = \frac{0.083}{\frac{0.148}{\sqrt{12}}} = 1.94$$

Decision: With $t < 2.20$, we fail to reject H_0 and fail to show a significant difference between the two time periods.

3. Calculation of measures of central tendency and 95% confidence interval.
   Independent variable: 5 time periods (discrete)
   Dependent variable: percent active ingredient (continuous)
   Test statistic: one-sample t-test

	Time (minutes)				
Sample	10	20	30	45	60
1	60.3	95.7	97.6	98.6	98.7
2	53.9	95.6	97.5	98.6	98.7
3	70.4	95.1	96.8	97.9	98.0
4	61.7	95.3	97.2	98.0	98.2
5	64.4	92.8	95.0	95.8	96.0
6	59.3	96.3	98.3	99.1	99.2

Example of the first (10-minute) time period:

Sample mean

$$\overline{X} = \frac{\sum x}{n} = \frac{60.3 + 53.9 + 70.4 + 61.7 + 64.4 + 59.3}{6} = 61.67\%$$

Sample variance/standard deviation

$$S^2 = \frac{\sum (x_i - \overline{X})^2}{n-1} \quad \frac{(60.3 - 61.67)^2 + \ldots (59.3 - 61.67)^2}{5} = 30.305$$

$$S = \sqrt{S^2} = \sqrt{30.305} = 5.505\,\%$$

Relative standard deviation

$$C.V. = \frac{S}{\overline{X}} = \frac{5.505}{61.67} = 0.089267$$

$$RSD = C.V. x\,100 = 0.08927 x\,100 = 8.927\%$$

95% Confidence interval: $\overline{X}$ = 61.67, S = 5.505, n = 6

$$\mu = \overline{X} \pm t_{1-\alpha/2}\, x \frac{S}{\sqrt{n}}$$

$$\mu = 61.67 \pm 2.57 \cdot \frac{5.505}{\sqrt{6}} = 61.67 \pm 5.78$$

$$55.89 < \mu < 67.45 \quad 95\%\, C.I.$$

Results for all five time periods (Table D.3):

4. Comparison of results from a contract laboratory and manufacturer's quality control laboratory.
   Independent variable: manufacturer versus contract laboratory (discrete)
   Dependent variable: assay results (continuous)
   Statistical test: two-sample t-test
   a. What is $t_{(1-\alpha/2)}$ for $\alpha = 0.05$? Critical $t = t_{10}(.975) = 2.228$
   b. Construct a 95% confidence interval for the difference between population means.

Table D.3 Dissolution Data Results

	Time (minutes)				
Sample	10	20	30	45	60
1	60.3	95.7	97.6	98.6	98.7
2	53.9	95.6	97.5	98.6	98.7
3	70.4	95.1	96.8	97.9	98.0
4	61.7	95.3	97.2	98.0	98.2
5	64.4	92.8	95.0	95.8	96.0
6	59.3	96.3	98.3	99.1	99.2
Mean	61.67	95.13	97.07	98.00	98.13
SD	5.505	1.214	1.127	1.164	1.127
RSD	8.927	1.276	1.161	1.188	1.148
95% confidence interval					
Upper limit	67.45	96.40	98.25	99.22	99.31
Lower limit	55.89	93.86	95.89	96.78	96.95

Two-Sample T-Test and CI: Manufacturer, Contract Lab

```
Two-sample T for Manufacturer vs Contract Lab

              N   Mean  StDev  SE Mean
Manufacturer  6  99.83   1.11     0.45
Contract Lab  6  98.95   1.30     0.53

Difference = mu (Manufacturer) - mu (Contract Lab)
Estimate for difference:  0.883
95% CI for difference:  (-0.695, 2.462)
T-Test of difference = 0 (vs not =): T-Value = 1.27  P-Value = 0.237  DF = 9
```

Figure D.3 Minitab output from Problem 4, Chapter 9.

Zero falls within the confidence interval; therefore assume there is a significant difference between the results from the two laboratories.

c. Use a t-test to compare the two groups.
Decision Rule: $\alpha = 0.05$, reject H_0 if $t > 2.228$ or $t < -2.228$.
Results: Figure D.3
Decision: With $t < 2.228$, fail to reject H_0, fail to show a significant difference between the results from the two laboratories.

5. First-time-in-humans clinical trial.
Independent variable: volunteer assignment
Dependent variable: C_{max} (continuous)
Test statistic: one-sample t-test
Results: $\overline{X} = 718.5$ $S^2 = 114.6$ $S = 10.7$ $n = 20$
Calculation:

Paired T-Test and CI: New, Senior

```
Paired T for New - Senior

            N    Mean  StDev  SE Mean
New        10  99.730  0.867    0.274
Senior     10  99.670  0.896    0.283
Difference 10   0.060  0.462    0.146

95% CI for mean difference: (-0.271, 0.391)
T-Test of mean difference = 0 (vs not = 0): T-Value = 0.41  P-Value = 0.691
```

Figure D.4 Minitab output from Problem 6, Chapter 9.

$$\mu = \overline{X} \pm t_{(1-\alpha/2)} \frac{S}{\sqrt{n}}$$

$$\mu = 718.5 \pm 2.09 \frac{10.7}{\sqrt{20}} = 718.5 \pm 5.00$$

$$713.5 < \mu_{C\,max} < 723.5 \text{ ng/ml}$$

Conclusion, with 95% confidence, the true population C_{max} is between 713.5 and 723.5 ng/ml.

6. Comparisons between the analytical results of the newly trained chemist and senior chemist.
   Independent variable: two time periods (each sample serves as own control)
   Dependent variable: assay results (continuous)
   Test statistic: paired t-test – Table 9.5
   Hypotheses: H_0: $\mu_h = \mu_c$
   H_1: $\mu_h \neq \mu_c$
   Decision rule: With $\alpha = 0.05$, reject H_0 if $t > 2.26$ or $t < -2.26$.
   Results: Figure D.4
   Decision: With $t > -2.26$, fail to reject H_0, fail to show a significant difference assay results for the two scientists.

7. Evaluating cognitive knowledge between hospital and community pharmacists.
   Independent variable: hospital versus community (discrete)
   Dependent variable: knowledge score (continuous)
   Statistical test: two-sample t-test

	Hospital Pharmacists	Community Pharmacists
Mean Score	82.1	79.9
Variance	151.29	210.25
Respondents	129	142

   Hypotheses: H_0: $\mu_h = \mu_c$
   H_1: $\mu_h \neq \mu_c$

Test statistic:

$$t = \frac{\overline{X_h} - \overline{X_c}}{\sqrt{\frac{S_P^2}{n_h} + \frac{S_P^2}{n_c}}}$$

Decision rule: With $\alpha = 0.05$, reject H_0 if $t > t_{169}(0.025)$ or $< -t_{169}(0.025)$. With $\alpha = 0.05$, reject H_0 if $t > 1.96$ or $t < -1.96$.

Computation:

$$S_p^2 = \frac{128(151.29)^2 + 141(210.25)^2}{129 + 142 - 2} = \frac{9162262.6}{269} = 34061.94$$

$$t = \frac{82.1 - 79.9}{\sqrt{\frac{34061.94}{129} + \frac{34061.94}{142}}} = \frac{2.2}{22.45} = 0.098$$

Decision: With $t < 1.96$ and > -1.96, do not reject H_0, conclude that a significant difference between the populations of pharmacists could not be found.

8. Evaluating cost effectiveness of a new treatment for peritoneal adhesiolysis.
   Independent variable: treatment received (each pair serves as its own control)
   Dependent variable: costs (continuous)
   Test statistic: paired t-test (one-tailed) – Table 9.6
   Hypotheses: H_0: $\mu_d \neq 0$
   H_1: $\mu_d > 0$
   Decision Rule: With $\alpha = 0.05$, reject H_0 if $t > t_{11}(0.05) = 1.795$.
   Results: Figure D.5
   Decision: With $t > 1.795$, reject H_0, conclude that the new treatment is more cost effective than the conventional one.

9. Evaluation of average length of stay for kidney transplant patients in a particular hospital.
   Independent variable: hospital
   Dependent variable: length of stay (continuous)
   Test statistic: one-sample t-test (one-tailed)
   Hypotheses: H_0: $\mu_A \neq 21.6$
   H_1: $\mu_A < 21.6$
   Decision rule: With $\alpha = 0.05$, reject H_0 if $t < -t_{50}(0.95) = -1.675$.
   Computations:

$$t = \frac{\overline{X} - \mu_0}{\frac{S}{\sqrt{n}}} = \frac{18.2 - 21.6}{\frac{8.3}{\sqrt{51}}} = \frac{-3.4}{1.16} = -2.93$$

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	8850.5	10038.6667
Variance	9901109.36	9250944.61
Observations	12	12
Pearson Correlation	0.8266346	
Hypothesized Mean	0	
df	11	
t Stat	-2.2557093	
P(T<=t) one-tail	0.02271638	
t Critical one-tail	1.79588482	
P(T<=t) two-tail	0.04543275	
t Critical two-tail	2.20098516	

Figure D.5 Minitab output from Problem 8, Chapter 9.

Decision: With $t < -1.675$, reject H_0 and assume that the lengths of stay for kidney transplant patients at Hospital A is significantly less than the other facilities.

Creating a confidence interval (5% error to estimate the upper limits of the interval):

$$\mu_{upper\ limit} = \overline{X} + t_{n-1}(1-\alpha/2)\cdot\frac{S}{\sqrt{n}}$$

$$\mu_U = 18.2 + (1.675)\frac{8.3}{\sqrt{51}} = 18.2 + 1.95$$

$$\mu_U < 20.15$$

Decision: The mean for all the hospitals, 21.6 days, does not fall within the upper limits of the confidence interval; therefore, Hospital A is significantly different and its patients appear to have shorter length of stays.

Chapter 10 – One-Way ANOVA

1. Collaborative trial with assays from four laboratories (Table 10.6).
   Independent variable: laboratories (discrete, 4 levels)
   Dependent variable: assay results (continuous)
   Statistical test: ANOVA (example of the computational formula)
   Hypotheses: H_0: $\mu_A = \mu_B = \mu_C = \mu_D$
   H_1: H_0 is false
   Decision rule: With $\alpha = 0.05$, reject H_0 if $F > F_{3,36}(0.95) \approx 2.87$.
   Calculations:

$$I = \sum_{k=1}^{K}\sum_{i=1}^{n} x_{jk}^2 = (100)^2 + (99.8)^2 + \ldots (100.1)^2 = 398{,}207.04$$

$$II = \frac{\left[\sum_{k=1}^{K}\sum_{i=1}^{n} x_{jk}\right]^2}{N_K} = \frac{(3991)^2}{40} = 398{,}202.025$$

$$III = \sum_{k=1}^{K} \frac{\left[\sum_{i=1}^{n} x_{jk}\right]^2}{n_K} = \frac{(999.0)^2}{10} + \ldots \frac{(1000)^2}{10} = 398{,}203.462$$

$$SSB = III - II = 398{,}203.462 - 398{,}202.025 = 1.437$$

$$SSW = I - III = 398{,}207.04 - 398{,}203.462 = 3.578$$

$$SST = I - II = 398{,}207.04 - 398{,}202.025 = 5.015$$

ANOVA Table

Source	DF	SS	MS	F
Between	3	1.437	0.479	4.84
Within	36	3.578	0.099	
Total	39	5.015		

Decision: With $F > 2.87$, reject H_0, conclude that $\mu_A = \mu_B = \mu_C = \mu_D$ is not true.

2. Evaluation of homogeneity of variance (Table 10.6)
Hartley's F-max test: Critical value from Table B8 ($k = 4$, $n - 1 = 9$) = 6.31

$$F_{max} = \frac{S^2_{largest}}{S^2_{smallest}} = \frac{(0.42)^2}{(0.22)^2} = \frac{0.176}{0.048} = 3.67$$

Decision: Fail to reject H_0: $\sigma_1^2 = \sigma_2^2 = \sigma_3^2 = \sigma_4^2$; assume homogeneity.
Cochran's C test: Critical value from Table B9 ($k = 4$, $n - 1 = 9$) = 0.502

$$C = \frac{S^2_{largest}}{\Sigma S^2_k} = \frac{(0.42)^2}{(0.25)^2 + (0.42)^2 + (0.22)^2 + (0.34)^2} = \frac{0.176}{0.403} = 0.437$$

Decision: Fail to reject H_0: $\sigma_1^2 = \sigma_2^2 = \sigma_3^2 = \sigma_4^2$; assume homogeneity.

3. Comparison of a raw material at three different production sites (Table 10.7)
Independent variable: production site (discrete, 3 levels)
Dependent variable: oil viscosity (continuous)
Statistical test: ANOVA (example of the computational formula)
Hypotheses: H_0: $\mu_A = \mu_B = \mu_C$
H_1: H_0 is false
Decision rule: With $\alpha = 0.05$, reject H_0 if $F > F_{2,12}(0.95) \approx 3.70$.
Calculations:

$$I = \sum_{k=1}^{K}\sum_{i=1}^{n} x_{jk}^2 = (10.23)^2 + (10.33)^2 + \ldots (10.22)^2 = 1574.1182$$

$$II = \frac{\left[\sum_{k=1}^{K}\sum_{i=1}^{n} x_{jk}\right]^2}{N_k} = \frac{(153.66)^2}{15} = 1574.0930$$

$$III = \sum_{k=1}^{K} \frac{\left[\sum_{i=1}^{n} x_{jk}\right]^2}{n_K} = \frac{(51.41)^2}{5} + \frac{(51.19)^2}{5} + \frac{(51.06)^2}{5} = 1574.1056$$

$$SS_B = III - II = 1574.1056 - 1574.0930 = 0.0126$$
$$SS_W = I - III = 1574.1182 - 1574.1056 = 0.0126$$
$$SS_T = I - II = 1574.1182 - 1574.0930 = 0.0252$$

ANOVA table:

Source	DF	SS	MS	F
Between	2	0.0126	0.0063	6.30
Within	12	0.0126	0.0010	
Total	14	0.0252		

Decision: With $F > 2.83$, reject H_0, conclude that $\mu_A = \mu_B = \mu_C$ is not true.

4. Evaluation of two formulations compared to the reference standard (Table D.4).
   Independent variable: formulations (discrete, 3 levels)
   subjects (blocks)
   Dependent variable: plasma elimination half-life (continuous)
   Statistical design: randomized block design
   Hypotheses: H_0: $\mu_A = \mu_B = \mu_{RS}$
   H_1: H_0 is false
   Decision rule: With $\alpha = 0.05$, reject H_0 if $F > F_{2,11} \approx 3.98$
   Calculations:

$$I = \sum_{k=1}^{K}\sum_{j=1}^{J} x_{kj}^2$$

$$I = (206)^2 + (212)^2 + (203)^2 + \ldots (219)^2 = 1567969$$

$$II = \frac{\left[\sum_{k=1}^{K}\sum_{j=1}^{J} x_{kj}\right]^2}{KJ}$$

$$II = \frac{(7509)^2}{36} = 1566252.25$$

$$III_R = \frac{\sum_{k=1}^{K}\left[\sum_{j=1}^{J} x_{kj}\right]^2}{K}$$

$$III_R = \frac{(621)^2 + (647)^2 + \ldots (655)^2}{3} = 1567729$$

Table D.4 Plasma Elimination Half-Life (in minutes)

Blocks (Subjects)	Form. A	Form. B	Reference Standard	∑	Mean
001	206	207	208	621	207.0
002	212	218	217	647	215.7
003	203	199	204	606	202.0
004	211	210	213	634	211.3
005	205	209	209	623	207.7
006	209	205	209	623	207.7
007	217	213	225	655	218.3
008	197	203	196	596	198.7
009	208	207	212	627	209.0
010	199	195	202	596	198.7
011	208	208	210	626	208.7
012	214	222	219	655	218.3
∑	2489	2496	2524	7509	
Mean	207.4	208.0	210.3		

$$III_C = \frac{\sum_{j=1}^{J}\left[\sum_{k=1}^{K} x_{kj}\right]^2}{J}$$

$$III_C = \frac{(2489)^2 + (2496)^2 + (2524)^2}{12} = 1566309.417$$

$$SS_{Total} = SS_T = I - II$$

$$SS_{Total} = 1567969 - 1566252.25 = 1716.75$$

$$SS_{Blocks} = SS_B = III_R - II$$

$$SS_{Blocks} = 1567729 - 1566252.25 = 1476.75$$

$$SS_{Treatment} = SS_{Rx} = III_C - II$$

$$SS_{Treatment} = 1566309.417 - 1566252.25 = 57.167$$

$$SS_{Error} = SS_{Residual} = SS_T - SS_B - SS_{Rx}$$

$$SS_{Residual} = 1716.75 - 1476.75 - 57.167 = 182.833$$

ANOVA Table

Source	df	SS	MS	F
Treatment	2	57.167	28.58	3.44
Blocks	11	1476.75	134.25	
Residual	22	182.833	8.31	
Total	35	1716.75		

Decision: With $F < 3.98$, fail to reject H_0 and conclude that there is no significant difference among the three products.

5. Evaluation of length of stay for patients of three physicians.
   Independent variable: physicians (discrete, 3 levels)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Physician A	8	83	10.375	5.125
Physician B	8	73	9.125	3.267857
Physician C	8	86	10.75	7.071429

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	11.58333	2	5.791667	1.123557	0.343897	3.4668
Within Groups	108.25	21	5.154762			
Total	119.8333	23				

Figure D.6 Excel output for Problem 5, Chapter 10.

Dependent variable: lengths of patient stays (continuous)
Statistical test: one-way ANOVA
Hypothesis: H_0: $\mu_{\text{physician A}} = \mu_{\text{physician B}} = \mu_{\text{physician C}}$
H_1: H_0 is false
Decision rule: With $\alpha = 0.05$, reject H_0 if $F > F_{2,21}(0.95) \approx 3.48$.
Results: Table 10.9 and Figure D.6
Decision: With $F < 3.48$, do not reject H_0, conclude that there is no difference among the three physicians.

6. Use of benzodiazepines and responses to a computerized simulated driving test.
Independent variable: drugs or placebo (discrete, 4 levels)
Dependent variable: driving score (continuous)
Statistical test: one-way ANOVA
Hypotheses: H_0: $\mu_A = \mu_B = \mu_C = \mu_{\text{Placebo}}$
H_1: H_0 is false
Decision rule: With $\alpha = 0.05$, reject H_0 if $F > F_{3,44}(0.95) \approx 3.85$.
Results: Table 10.10 and Figure D.7
Decision: With $F > 2.85$, reject H_0, conclude that $\mu_A = \mu_B = \mu_C = \mu_{\text{Placebo}}$ is not true.

7. Evaluation of replicate assays.
Independent variable: replicates, first versus second (discrete, 2 levels)
batches (blocks)
Dependent variable: percent recovered (continuous)
Statistical test: complete randomized block design
Hypotheses: H_0: $\mu_1 = \mu_2$
H_1: $\mu_1 \neq \mu_2$
Decision rule: With $\alpha = 0.05$, reject H_0 if $F > F_{1,5} = 6.61$

One-way ANOVA: Response versus Source

Source	DF	SS	MS	F	P
Source	3	621.1	207.0	5.30	0.003
Error	44	1718.8	39.1		
Total	47	2339.8			

S = 6.25 R-Sq = 26.54% R-Sq(adj) = 21.53%

Level	N	Mean	StDev
Benzo(A)	12	55.250	5.242
Benzo(B)	12	60.083	7.465
Benzo(C)	12	52.333	5.662
Placebo	12	50.583	6.403

Individual 95% CIs For Mean Based on Pooled StDev

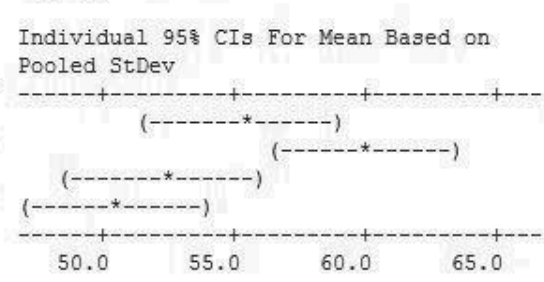


Figure D.7 Minitab output for Problem 6, Chapter 10.

	Treatment (% recovered)			
Blocks	Replicate 1	Replicate 2	∑	Mean
Batch A	93.502	92.319	185.821	92.911
Batch C	91.177	92.230	183.407	91.704
Batch D	87.304	87.496	174.800	87.400
Batch D2	81.275	80.564	161.839	80.920
Batch G	79.865	79.259	159.124	79.562
Batch G2	81.722	80.931	162.653	81.327
∑	514.845	512.799	1027.644	
Mean	85.808	85.467		

Calculations:

$$I = (93.502\ )^2 + (92.319\ )^2 + ...(80.931\ )^2 = 88347.4815$$

$$II = \frac{(1027.644\ )^2}{12} = 88004.3492$$

$$III_R = \frac{(185.821\ )^2 + (183.407\ )^2 + ...(162.653\ )^2}{2} = 88345.4597$$

$$III_C = \frac{(514.845\ )^2 + (512.799\ )^2}{6} = 88004.6980$$

$$SS_{Total} = 88347.4815 - 88004.3492 = 343.1323$$
$$SS_{Blocks} = 88345.4597 - 88004.3492 = 341.1105$$
$$SS_{Treatment} = 88004.6980 - 88004.3492 = 0.3488$$
$$SS_{Residual} = 343.1323 - 341.1105 - 0.3488 = 1.673$$

ANOVA Table:

Source	df	SS	MS	F
Treatment	1	0.3488	0.3488	1.0424
Blocks	5	341.1105	68.2221	
Residual	5	1.6730	0.3346	
Total	11	343.1323		

Decision: With $F < 6.61$, fail to reject H_0, conclude that there is no significant difference between the first and second replicates.

Chapter 11 – Multiple Comparisons

Possible examples for multiple comparison tests

1. Several different possible multiple comparisons.
   a. Scenario 1: Prior to the ANOVA the researcher decided on only two possible pairwise comparisons. There are three possible tests, multiple t-tests with adjusted α or Dunn's test.

   (1) Multiple t-tests with adjusted α: Keeping α constant at 0.05 and doing two separate t-test the $\alpha = 0.05/2 = 0.025$ and a reliability coefficient = 2.297 for $t_{0.9875,62}$ for A versus C and 2.298 for $t_{0.9875,61}$ for B versus C (using Microsoft® Excel, TINV(0.025,*df*)).

$$\mu_A - \mu_C = (\overline{X}_A - \overline{X}_C) \pm t_{n1+n2-2}(1-\alpha/2)\sqrt{\frac{s_p^2}{n_A} + \frac{s_p^2}{n_C}}$$

$$S_p^2 = \frac{(n_A - 1)S_A^2 + (n_C - 1)S_C^2}{n_A + n_C - 2}$$

$$S_p^2 = \frac{(30)(21.03)^2 + (32)(22.13)^2}{62} = 466.765$$

$$\mu_A - \mu_C = (14.13) \pm 2.298\sqrt{\frac{466.765}{31} + \frac{466.765}{33}}$$

$$+1.712 < \mu_A - \mu_C < +26.548$$

$$\mu_B - \mu_C = (12.72) \pm 2.298\sqrt{\frac{464.575}{30} + \frac{464.575}{33}}$$

$$+0.225 < \mu_B - \mu_C < +25.215$$

Results: Since zero was not within the interval in both cases, Drug C showed a significantly larger decrease in total cholesterol than either Drug A or Drug B.

(2) Dunn Test: The Dunn reliability coefficient for is $t'D_{.05;3;91} \approx 2.45$ (Table B11). Computation for $\overline{X}_A - \overline{X}_B$:

$$\mu_A - \mu_B = (\overline{X}_A - \overline{X}_B) \pm t' D_{\alpha/2;C;N-K}\sqrt{MS_E \cdot (\frac{1}{n_A} + \frac{1}{n_B})}$$

$$\mu_A - \mu_B = (1.41) \pm 2.45\sqrt{457.19 \cdot (\frac{1}{31} + \frac{1}{30})}$$

$$\mu_A - \mu_B = (1.41) \pm 13.416$$
$$-12.006 < \mu_A - \mu_B < +14.826$$

Results:

Pairing	Confidence Interval	Results
$\overline{X}_A - \overline{X}_B$	$-12.006 < \mu_A - \mu_B < +14.826$	
$\overline{X}_A - \overline{X}_C$	$+1.027 < \mu_A - \mu_C < +27.233$	Significant
$\overline{X}_B - \overline{X}_C$	$-0.495 < \mu_B - \mu_C < +25.935$	

b. Scenario 2: Prior to the ANOVA the researcher has decided on a control group and wishes to compare each alternative therapy to the control group. Only one possibility, the Dunnett test exists. In this case the means of the sample are ranked from smallest to largest:

$\overline{X}_C$	$\overline{X}_B$	$\overline{X}_A$
–21.39	–8.67	–7.26

Thus, the *p* and subsequent q-value for A versus C is 3 and 1.99, respectively, and for B versus C are 2 and 2.26 (Table B12).

$$\mu_C - \mu_A = \left(\overline{X}_C - \overline{X}_A\right) \pm q_{\alpha,p,N-k} \sqrt{MS_E \left(\frac{1}{n_C} + \frac{1}{n_A}\right)}$$

$$\mu_C - \mu_A = (14.13) \pm 1.99 \sqrt{457.19 \left(\frac{1}{33} + \frac{1}{31}\right)}$$

$$+3.487 < \mu_C - \mu_A < +24.773$$

$$\mu_C - \mu_B = (12.72) \pm 2.26 \sqrt{457.19 \left(\frac{1}{33} + \frac{1}{30}\right)}$$

$$+0.530 < \mu_C - \mu_B < +24.910$$

Results: In both cases zero did not fall within the interval. Thus, when Drug C is designated the control *a priori*, both pairwise comparisons were significant and Drug C showed a significantly larger decrease in total cholesterol than either Drug A or Drug B.

c. Scenario 3: *Post hoc* comparison is required. With unequal cell sizes and equal variances the possible tests are Tukey-Kramer, SNK, and Scheffé tests. (1) Tukey-Kramer test: With slightly different sample sizes the Tukey-Kramer test would be used over the Tukey test. In this case the *q*-value for the reliability coefficient would be the same for all three pairwise comparisons, $q_{.05,3,91} = 3.38$ (Table B10).

Computation for $\overline{X}_A - \overline{X}_B$:

$$\mu_A - \mu_B = (\overline{X}_A - \overline{X}_B) \pm (q_{\alpha,k,N-k}) \sqrt{\frac{MS_E}{2} \left(\frac{1}{n_A} + \frac{1}{n_B}\right)}$$

$$\mu_A - \mu_B = (1.41) \pm (3.38)\sqrt{\frac{457.19}{2}\left(\frac{1}{31}+\frac{1}{30}\right)}$$

$$\mu_A - \mu_B = (1.41) \pm (13.088)$$

$$-11.678 < \mu_A - \mu_B < +14.498$$

Results:

Pairing	Confidence Interval	Results
$\overline{X}_A - \overline{X}_B$	$-11.678 < \mu_A - \mu_B < +14.498$	
$\overline{X}_A - \overline{X}_C$	$+1.348 < \mu_A - \mu_C < +26.912$	Significant
$\overline{X}_B - \overline{X}_C$	$-0.171 < \mu_B - \mu_C < +25.611$	

(2) Student Newman-Keul: For unbalanced designs the Student Newman-Keul equation is exactly the same as the Tukey-Kramer and would give the exact same results, except that the *q*-statistic calculated as:

$$q = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{MS_E}{2}\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}}$$

would be compared to the critical value of 3.38. In these cases for:

$\mu_A - \mu_B$	$q = 0.364$	*not significant*
$\mu_A - \mu_C$	$q = 3.736$	*significant*
$\mu_B - \mu_C$	$q = 3.335$	*not significant*

Results: The only significant difference was that Drug C showed a significantly larger decrease in total cholesterol than either Drug A.

(3) Scheffé procedure: For all three possible pairwise comparisons, the Scheffe value (S^2) would equal $(k-1)(F_{k-1,N-k(1-\alpha)})$ = (2)(3.111) = 6.222. The error term is slightly different based on the pairing.

Computation for $\overline{X}_A - \overline{X}_B$:

$$var(\hat{\psi}_{AB}) = 457.19\left[\frac{(+1)^2}{31}+\frac{(-1)^2}{30}\right] = 29.988$$

$$\psi_{AB} = \hat{\psi}_{AB} \pm \sqrt{S^2 \cdot Var(\hat{\psi}_{AB})}$$

$$\psi_{AB} = 1.41 \pm \sqrt{(6.222)(29.988)}$$

$$\psi_{AB} = 1.41 \pm 13.659$$

$$-12.249 < \psi_{AB} < +15.069$$

Results:

Pairing	Confidence Interval	Results
$\overline{X}_A - \overline{X}_B$	$-12.249 < \mu_A - \mu_B < +15.069$	
$\overline{X}_A - \overline{X}_C$	$+0.790 < \mu_A - \mu_C < +27.470$	Significant
$\overline{X}_B - \overline{X}_C$	$-0.734 < \mu_B - \mu_C < +26.174$	

2. Three *post hoc* tests, discussed in this chapter, would be appropriate for results with equal sample sizes: 1) Tukey HSD; 2) Fisher LSD; and 3) Scheffe tests. Data comparing the site of raw materials and viscosity.

Sample Differences: $\overline{X}_A - \overline{X}_B = +0.04$

$\overline{X}_A - \overline{X}_C = +0.07$ $\quad MS_W = MS_E = 0.001$

$\overline{X}_B - \overline{X}_C = +0.03$

(1) Tukey HSD test: For the Tukey HSD test the reliability coefficient is $q_{.05;3;12} = 3.77$ (Table B10).

Computation for $\overline{X}_A - \overline{X}_B$:

$$\mu_A - \mu_B = (\overline{X}_A - \overline{X}_B) \pm (q_{\alpha,k,N-k}) \sqrt{\frac{MS_E}{n}}$$

$$\mu_A - \mu_B = (+0.04) \pm (3.77) \sqrt{\frac{0.001}{5}}$$

$$\mu_A - \mu_B = +0.04 \pm 0.053$$

$$-0.013 < \mu_A - \mu_B < +0.093$$

Results:

Pairing	Confidence Interval	Results
$\overline{X}_A - \overline{X}_B$	$-0.013 < \mu_A - \mu_B < +0.093$	
$\overline{X}_A - \overline{X}_C$	$+0.017 < \mu_A - \mu_C < +0.123$	Significant
$\overline{X}_B - \overline{X}_C$	$-0.023 < \mu_B - \mu_C < +0.083$	

(2) Fisher LSD test: For the Fisher LSD test the reliability coefficient is $t_{0.025;12} = 2.1788$ (Table B5).

Computation for $\overline{X}_A - \overline{X}_C$:

$$\mu_A - \mu_C = (\overline{X}_A - \overline{X}_C) \pm t_{1-\alpha/2,N-k} \sqrt{\frac{MS_E}{n_A} + \frac{MS_E}{n_C}}$$

$$\mu_A - \mu_C = (+0.07) \pm (2.1788) \sqrt{\frac{0.001}{5} + \frac{0.001}{5}}$$

$$\mu_A - \mu_c = (0.07) \pm 0.044$$

$$+0.026 < \mu_A - \mu_C < +0.114 \backslash$$

Results:

Pairing	Confidence Interval	Results
$\overline{X}_A - \overline{X}_B$	$-0.004 < \mu_A - \mu_B < +0.084$	
$\overline{X}_A - \overline{X}_C$	$+0.026 < \mu_A - \mu_C < +0.114$	Significant
$\overline{X}_B - \overline{X}_C$	$-0.014 < \mu_B - \mu_C < +0.074$	

(3) Scheffé procedure: Scheffé value is:

$$(Scheffe\ value)^2 = S^2 = (K-1)(F_{K\text{-}1,N\text{-}K}(1-\alpha)) = 2(3.70) = 7.40$$

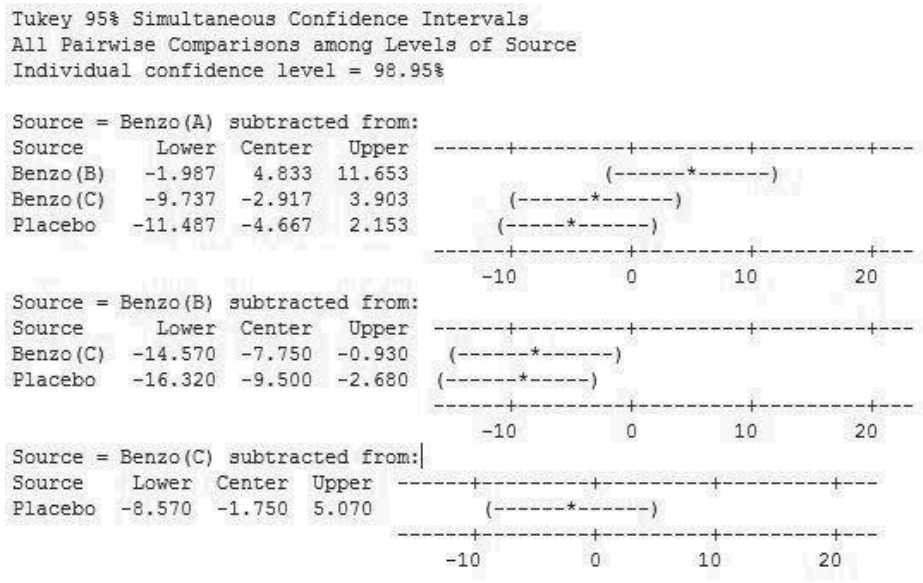


Figure D.8 Minitab for Tukey HSD output for Problem 4, Chapter 11.

Computation for $\overline{X}_B - \overline{X}_C$:

$$var(\hat{\psi}_3) = 0.001\left[\frac{(+1)^2}{5} + \frac{(-1)^2}{5}\right] = 0.0004$$

$$\psi_3 = +0.03 \pm \sqrt{(7.40)(0.0004)} = 0.03 \pm 0.054$$

$$-0.024 < \psi_3 < +0.084$$

Results:

Pairing	Confidence Interval	Results
$\overline{X}_A - \overline{X}_B$	$-0.014 < \mu_A - \mu_B < +0.094$	
$\overline{X}_A - \overline{X}_C$	$+0.016 < \mu_A - \mu_C < +0.124$	Significant
$\overline{X}_B - \overline{X}_C$	$-0.024 < \mu_B - \mu_C < +0.084$	

4. Use of benzodiazepines and responses to a computerized simulated driving test.
   Independent variable: drug or placebo (discrete, 4 levels)
   Dependent variable: driving score (continuous)
   ANOVA findings: Reject H_0: $\mu_A = \mu_B = \mu_C = \mu_{Placebo}$
   Results: Tukey HSD - Figure D.8
   Fisher LSD – Figure D.9
   Decision: Volunteers on Benzo(B) performed significantly worse on the driving scores than either Benzo(C) or placebo with both *post hoc* tests.

```
Fisher 95% Individual Confidence Intervals
All Pairwise Comparisons among Levels of Source
Simultaneous confidence level = 80.20%

Source = Benzo(A) subtracted from:
Source     Lower  Center  Upper  --------+---------+---------+---------+-
Benzo(B)  -0.309   4.833  9.976                   (-----*-----)
Benzo(C)  -8.059  -2.917  2.226          (-----*------)
Placebo   -9.809  -4.667  0.476        (-----*------)
                                --------+---------+---------+---------+-
                                   -8.0       0.0       8.0      16.0
Source = Benzo(B) subtracted from:
Source      Lower  Center   Upper  --------+---------+---------+---------+-
Benzo(C)  -12.892  -7.750  -2.608    (-----*------)
Placebo   -14.642  -9.500  -4.358  (-----*------)
                                --------+---------+---------+---------+-
                                      -8.0       0.0       8.0      16.0
Source = Benzo(C) subtracted from:
Source    Lower  Center  Upper  --------+---------+---------+---------+-
Placebo  -6.892  -1.750  3.392           (------*-----)
                                --------+---------+---------+---------+-
                                   -8.0       0.0       8.0      16.0
```

Figure D.9 Minitab for Fisher LSD output for Problem 4, Chapter 11.

5. Comparison of benzodiazepines with placebo considered the control substance with the computerized simulated driving test.
   Independent variable: drug or placebo (discrete, 4 levels), but placebo as the control
   Dependent variable: driving score (continuous)
   ANOVA findings: Reject H_0: $\mu_A = \mu_B = \mu_C = \mu_{Placebo}$
   Results: Dunnett's test - Figure D.10
   Decision: Only Benzo(B) was significantly different from the placebo control.

```
Dunnett's comparisons with a control
Family error rate = 0.05
Individual error rate = 0.0191

Critical value = 2.43
Control = level (Placebo) of Source

Intervals for treatment mean minus control mean
Level      Lower  Center   Upper  -------+---------+---------+---------+--
Benzo(A)  -1.541   4.667  10.874       (----------*---------)
Benzo(B)   3.292   9.500  15.708                 (----------*---------)
Benzo(C)  -4.458   1.750   7.958  (---------*---------)
                                  -------+---------+---------+---------+--
                                       0.0       6.0      12.0      18.0
```

Figure D.10 Minitab for Dunnett's output for Problems 5, Chapter 11.

Chapter 12 – Factorial Designs: An Introduction

1. Experiment with different fillers and various speeds on a tableting machine.

Hypotheses: H_{01}: $\mu_{\text{Speed 1}} = \mu_{\text{Speed 2}} = \mu_{\text{Speed 3}} = \mu_{\text{Speed 4}}$
H_{02}: $\mu_{\text{Filler 1}} = \mu_{\text{Filler 2}} = \mu_{\text{Filler 3}}$
H_{03}: No interaction between speed and filler

Hardness (kP)

Speed of Tableting Machine

Filler		80	100	120	180	
Lactose	$\sum =$	56	60	62	61	$\sum\sum = 239$
Microcrystalline Cellulose	$\sum =$	59	58	56	58	$\sum\sum = 231$
Dicalcium Phosphate	$\sum =$	51	49	47	50	$\sum\sum = 197$
$\sum\sum =$		166	167	165	169	$\sum\sum\sum = 667$

Decision rules: With $\alpha = 0.05$ and $n = 8$: reject H_{01} if $F > F_{3,84}(0.95) \approx 2.72$; reject H_{02} if $F > F_{2,84}(0.95) \approx 3.11$; and reject H_{03} if $F > F_{6,84}(0.95) \approx 2.21$.

Calculations:

$$I = \sum_{k=1}^{K}\sum_{j=1}^{J}\sum_{i=1}^{I} x_i^2$$

$$I = (7)^2 + (5)^2 + (8)^2 \ldots + (6)^2 + (6)^2 = 4809$$

$$II = \frac{\left[\sum_{k=1}^{K}\sum_{j=1}^{J}\sum_{i=1}^{I} x_i\right]^2}{N}$$

$$II = \frac{(667)^2}{96} = 4634.26$$

$$III_R = \frac{\sum_{k=1}^{K}\left[\sum_{j=1}^{J}\sum_{i=1}^{I} x_i\right]^2}{j \cdot n}$$

$$III_R = \frac{(239)^2 + (231)^2 + (197)^2}{(4)(8)} = \frac{149291}{32} = 4665.344$$

$$III_C = \frac{\sum_{j=1}^{J}\left[\sum_{k=1}^{K}\sum_{i=1}^{I} x_i\right]^2}{k \cdot n}$$

$$III_C = \frac{(166)^2 + (167)^2 + (165)^2 + (169)^2}{(3)(8)} = \frac{111231}{24} = 4634.625$$

$$IV = \frac{\sum_{k=1}^{K}\sum_{j=1}^{J}\left[\sum_{i=1}^{I} x_i\right]^2}{n}$$

$$IV = \frac{(56)^2 + (60)^2 \ldots + (47)^2 + (50)^2}{8} = \frac{37357}{8} = 4669.625$$

$$SS_R = III_R - II = 4{,}665.344 - 4{,}634.26 = 31.084$$

$$SS_C = III_C - II = 4{,}634.625 - 4{,}634.26 = 0.365$$

$$SS_{RC} = IV - III_R - III_C + II$$

$$SS_{RC} = 4{,}669.625 - 4{,}665.344 - 4{,}634.625 = 3.916$$

$$SS_E = I - IV = 4{,}809 - 4{,}669.625 = 139.375$$

$$SS_T = I - II = 4{,}809 - 4{,}634.26 = 174.74$$

ANOVA Table:

Source	df	SS	MS	F
Between				
Rows (filler)	2	31.084	15.542	9.368
Column (speed)	3	0.365	0.122	0.074
Interaction	6	3.916	0.653	0.394
Within (error):	84	139.375	1.659	
Total	95	174.740		

Decision: With α = 0.05, reject H_{01} and conclude that there is a significant difference between the three fillers used in the experiment, but there is no significant difference based on the speed of the tableting machine and no significant interaction between these two factors.

2. Experiment with quality of life indexes and various hospitals.

Hypotheses: H_{01}: $\mu_{\text{Index 1}} = \mu_{\text{Index 2}} = \mu_{\text{Index 3}}$

H_{02}: $\mu_{\text{Hospital A}} = \mu_{\text{Hospital B}} = \mu_{\text{Hospital C}}$

H_{03}: No interaction between index and hospital

Decision rules: With $\alpha = 0.05$: reject H_{01} if $F > F_{2,26}(0.95) \approx 3.39$; reject H_{02} if $F > F_{2,26}(0.95) \approx 3.39$; and reject H_{03} if $F > F_{4,26}(0.95) \approx 3.00$.

		Index 1	Index 2	Index 3	
Hospital A	$\sum$ =	270	344	380	$\sum\sum$ = 994
Hospital B	$\sum$ =	329	248	340	$\sum\sum$ = 917
Hospital C	$\sum$ =	325	317	325	$\sum\sum$ = 967
	$\sum\sum$ =	924	909	1045	$\sum\sum\sum$ = 2878

Calculations:

$$I = \sum_{k=1}^{K}\sum_{j=1}^{J}\sum_{i=1}^{I} x_i^2$$

$$I = (67)^2 + (73)^2 + (61)^2 \ldots + (82)^2 + (77)^2 = 238{,}646$$

$$II = \frac{\left[\sum_{k=1}^{K}\sum_{j=1}^{J}\sum_{i=1}^{I} x_i\right]^2}{N} = \frac{(2{,}878)^2}{35} = 236{,}653.83$$

$$III_R = \sum_{k=1}^{K} \frac{\left[\sum_{j=1}^{J}\sum_{i=1}^{I} x_i\right]^2}{N_R}$$

$$III_R = \frac{(994)^2}{12} + \frac{(917)^2}{11} + \frac{(967)^2}{12} = 236{,}704.87$$

$$III_C = \sum_{j=1}^{J} \frac{\left[\sum_{k=1}^{K}\sum_{i=1}^{I} x_i\right]^2}{N_C}$$

$$III_C = \frac{(924)^2}{12} + \frac{(909)^2}{11} + \frac{(1045)^2}{12} = 237{,}266.54$$

$$IV = \sum_{k=1}^{K}\sum_{j=1}^{J} \frac{\left[\sum_{i=1}^{I} x_i\right]^2}{N_i}$$

$$IV = \frac{(270)^2}{4} + \frac{(344)^2}{4} + \frac{(380)^2}{4} + \ldots \frac{(325)^2}{4} = 238{,}305.33$$

$$SS_R = III_R - II = 236{,}704.87 - 236{,}653.83 = 51.04$$

$$SS_C = III_C - II = 237{,}266.54 - 236{,}653.83 = 612.71$$

$$SS_{RC} = IV - III_R - III_C + II$$

$$SS_{RC} = 238{,}305.33 - 236{,}704.87 - 237{,}266.54 + 236{,}653.83 = 987.75$$

$$SS_E = I - IV = 238{,}646 - 238{,}305.33 = 340.67$$

$$SS_T = I - II = 238{,}646 - 236{,}653.83 = 1992.17$$

ANOVA Table:

Source	df	SS	MS	F	p
Between					
Rows (hospital)	2	51.04	25.52	1.95	0.153
Column (index)	2	612.71	306.36	23.39	<0.0005
Interaction	4	987.75	246.94	18.85	<0.0005
Within (error):	26	340.67	13.10		
Total	34	1992.17			

Decision: With $\alpha = 0.05$, reject H_{02} and conclude that there is a significant difference between the indexes used in this study. Reject H_{03},

conclude that a significant interaction exists between the two main factors, but there is no significant difference based on the hospital.

3. Repeatability and reproducibility among seven laboratories.
   H_{01}: $\mu_{L1} = \mu_{L2} = \mu_{L3} = \mu_{L4} = \mu_{L5} = \mu_{L6} = \mu_{L7}$ (Main effect of laboratories)
   H_{02}: $\mu_{S1} = \mu_{S2} = \mu_{S3}$ (Main effect of samples)
   H_{03}: $(\mu_{L1,S1} - \mu_{L1,S2}) = (\mu_{L2,S1} - \mu_{L2,S2}) =$ etc. (Interaction)

   Results: Two-way ANOVA – Figure D.11

   Application to repeatability and reproducibility where with 99% confidence, $Z_0 = 5.15$.

$$Repeatability = 5.15\sqrt{0.26} = 2.63$$

$$Reproducibility = 5.15\sqrt{\frac{0.42 - 0.36}{3(4)}} = 0.36$$

$$Interaction = 5.15\sqrt{\frac{0.36 - 0.26}{4}} = 0.81$$

$$R\,\&\,R = \sqrt{(2.63)^2 + (0.36)^2 + (0.81)^2} = 2.88$$

$$V_P = 5.15\sqrt{\frac{199.00 - 0.36}{(7)(4)}} = 13.72$$

$$V_T = \sqrt{(2.88)^2 + (13.72)^2} = 14.02$$

$$\%\ repeatibility = \left(\frac{2.63}{14.02}\right)^2 x100 = 3.52\%$$

$$\%\ reproducibility = \left(\frac{0.36}{14.02}\right)^2 x100 = 0.07\%$$

$$\%\ R\,\&\,R = \left(\frac{2.88}{14.02}\right)^2 x100 = 4.23\%$$

$$\%\ Sample = \left(\frac{13.72}{14.02}\right)^2 x\ 100 = 95.77\%$$

   Result: Good repeatability (<5%) and good reproducibility (<30%).

Chapter 13 – Correlation

1. Comparison of two different scales to measure patient anxiety levels.
   Variables: continuous (two measurement scales)
   a. Pearson product-moment
      Method A - variable *x* - mean = 57.7
      Method B - variable *y* - mean = 105.5
      Calculations (based on summary data in Table D.5):

Two-way ANOVA: Results versus Sample, Laboratory

Source	DF	SS	MS	F	P
Sample	2	398.006	199.003	752.53	0.000
Laboratory	6	2.503	0.417	1.58	0.168
Interaction	12	4.279	0.357	1.35	0.215
Error	63	16.660	0.264		
Total	83	421.448			

S = 0.5142 R-Sq = 96.05% R-Sq(adj) = 94.79%

Figure D.11 Minitab two-way ANOVA output for Problem 3, Chapter 12.

Table D.5 Data for Problem 1, Definitional Formula

x	y	$x-\overline{X}$	$y-\overline{Y}$	$(x-\overline{X})(y-\overline{Y})$	$(x-\overline{X})^2$	$(y-\overline{Y})^2$
55	90	–2.7	–15.5	41.85	7.29	240.25
66	117	8.3	11.5	95.45	68.89	132.25
46	94	–11.7	–11.5	134.55	136.89	132.25
77	124	19.3	18.5	357.05	372.49	342.25
57	105	–0.7	–0.5	0.35	0.49	0.25
59	115	1.3	9.5	12.35	1.69	90.25
70	125	12.3	19.5	239.85	151.29	380.25
57	97	–0.7	–8.5	5.95	0.49	72.25
52	97	–5.7	–8.5	48.45	32.49	72.25
36	78	–21.7	–27.5	596.75	470.89	756.25
44	84	–13.7	–21.5	294.55	187.69	462.25
55	112	–2.7	6.5	-17.55	7.29	42.25
53	102	–4.7	–3.5	16.45	22.09	12.25
67	112	9.3	6.5	60.45	86.49	42.25
72	130	14.3	24.5	350.35	204.49	600.25
				2236.85	1750.95	3377.75

$$r = \frac{\sum(x-\overline{X})(y-\overline{Y})}{\sqrt{\sum(x-\overline{X})^2(y-\overline{Y})^2}} = \frac{2236.85}{\sqrt{(1750.95)(3377.75)}} = 0.92$$

b. Computational formula (based on summary data in Table D.6):
Calculations:

$$r = \frac{15(93571)-(866)(1582)}{\sqrt{15(51748)-(866)^2}\sqrt{15(170226)-(1582)^2}}$$

$$r = \frac{1403565-1370012}{(162.06)(225.09)} = \frac{33553}{36478.08} = 0.92$$

Table D.6 Data for Problem 1, Computational Formula

	x	y	x^2	y^2	xy
	55	90	3025	8100	4950
	66	117	4356	13689	7722
	46	94	2116	8836	4324
	77	124	5929	15376	9548
	57	105	3249	11025	5985
	59	115	3481	13225	6785
	70	125	4900	15625	8750
	57	97	3249	9409	5529
	52	97	2704	9409	5044
	36	78	1296	6084	2808
	44	84	1936	7056	3696
	55	112	3025	12544	6160
	53	102	2809	10404	5406
	67	112	4489	12544	7504
	72	130	5184	16900	9360
$\sum =$	866	1582	51748	170226	93571

c. Conversion to *t*-statistic:
Hypothesis: H_0: $r_{xy} = 0$
H_1: $r_{xy} \neq 0$
Decision rule: With $\alpha = 0.05$, reject H_0 if $t > t_{13}(0.975) = 2.16$.
Calculations:

$$t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}} = \frac{.92\sqrt{15-2}}{\sqrt{1-(.92)^2}} = \frac{3.32}{0.39} = 8.51$$

Decision: With $t > 2.16$, reject H_0 and conclude there is a significant relationship between Method A and Method B.

2. Comparison of two drugs and length of stay at a specific hospital.
Variables: continuous (two measurement scales)
Calculation of the three paired correlations produced the following inter-correlation matrix:

Variables	LOS	Drug A	Drug B
LOS	...	–0.923	–0.184
Drug A	...	...	+0.195
Drug B	...	...	...

The partial correlation for length of stay versus Drug A is:

$$r_{la,b} = \frac{r_{la} - (r_{lb})(r_{ab})}{\sqrt{(1-r_{lb}^2)(1-r_{ab}^2)}} = \frac{-0.923 - (-0.184)(+0.195)}{\sqrt{(1-(-0.184)^2)(1-(0.195)^2)}} = +0.920$$

The partial correlation for length of stay versus Drug B is:

$$r_{lb,a} = \frac{r_{lb} - (r_{la})(r_{ab})}{\sqrt{(1 - r_{la}^2)(1 - r_{ab}^2)}} = \frac{-0.184 - (-0.923)(+0.195)}{\sqrt{(1 - (-0.923)^2)(1 - (0.195)^2)}} = -0.011$$

Evaluation of the partial correlation for length of stay versus Drug A:

Decision rule is with $\alpha = 0.05$, reject H_0 if $|t| > t_5(0.975) = 2.57$.

$$t_{la.b} = \frac{r_{la.b}\sqrt{n-k-1}}{\sqrt{1-(r_{la.b})^2}}$$

$$t_{yx.z} = \frac{(-0.92)\sqrt{8-2-1}}{\sqrt{1-(-0.92)^2}} = \frac{-2.057}{0.392} = -5.24$$

Decision: There is a strong correlation, statistically significant with 95% confidence, between the length of stay and administration of Drug A, but Drug B has very little influence on the length of stay.

3. Comparison of two analytical procedures on different concentrations of a drug.
   Variables: continuous (two measurement scales)
   Calculations (based on summary data in Table D.7):

$$r = \frac{n\sum xy - \sum x \sum y}{\sqrt{n\sum x^2 - (\sum x)^2}\sqrt{n\sum y^2 - (\sum y)^2}}$$

$$r = \frac{8(33,244.62) - (469.5)(471.8)}{\sqrt{8(33,138.09) - (469.5)^2}\sqrt{8(33,355.38) - (471.8)^2}}$$

$$r = \frac{265,956.96 - 221,510.1}{(211.36)(210.35)} = \frac{44,446.86}{44,459.58} = +0.9997$$

   Conclusion: A very strong correlation exists between methods GS and ALT.

4. Comparison of multiple test results:
   Variables: continuous (five measurement scales)
   Example of correlation coefficient for scores on law and pharmaceutical calculations sections (Table D.8).

Table D.7 Data for Problem 3, Computational Formula

Method GS	Method ALT			
x	y	x^2	y^2	xy
90.1	89.8	8,118.01	8,064.04	8,090.98
85.2	85.1	7,259.04	7,242.01	7,250.52
79.7	80.2	6,352.09	6,432.04	6,391.94
74.3	75.0	5,520.49	5,625.00	5,572.50
60.2	61.0	3,624.04	3,721.00	3,672.20
35.5	34.8	1,260.25	1,211.04	1,235.40
24.9	24.8	620.01	615.04	617.52
19.6	21.1	384.16	445.21	413.56
469.5	471.8	33,138.09	33,355.38	33,244.62

Table D.8 Data for Problem 4, Computational Formula

Law (x)	Calculations (y)	x^2	y^2	xy
23	18	529	324	414
22	20	484	400	440
25	21	625	441	525
20	19	400	361	380
24	23	576	529	552
23	22	529	484	506
24	20	576	400	480
20	17	400	289	340
22	19	484	361	418
24	21	576	441	504
23	20	529	400	460
21	21	441	441	441
271	241	6149	4871	5460

Calculations:

$$r = \frac{n\sum xy - \sum x \sum y}{\sqrt{n\sum x^2 - (\sum x)^2}\sqrt{n\sum y^2 - (\sum y)^2}}$$

$$r = \frac{12(5460) - (271)(241)}{\sqrt{12(6149) - (271)^2}\sqrt{12(4871) - (241)^2}} = \frac{209}{358.8} = +0.582$$

Conclusion: A moderate correlation between law and calculation scores.
Correlation Matrix: Figure D.12
Results: Strongest correlation between two sections is +0.943 between law and pharmacology.

```
             Law     Math  Pcology  Medchem
Math       0.582
           0.047

Pcology    0.943    0.678
           0.000    0.015

Medchem   -0.674   -0.591   -0.689
           0.016    0.043    0.013

Total      0.832    0.712    0.877   -0.324
           0.001    0.009    0.000    0.305

Cell Contents: Pearson correlation
               P-Value
```

Figure D.12 Minitab correlation matrix for Problem 3, Chapter 13.

Chapter 14 – Linear Regression

1. Comparison of content of a medication at different time periods.
   Variables: continuous independent variable (time in months)
   continuous dependent variable (amount of drug in milligrams)
   Hypothesis: H_0: Time (x) and assay results (y) are not linearly related
   H_1: Time and assay results are linearly related
   Decision Rule: With $\alpha = 0.05$, reject H_0 if $F > F_{1,4}(0.95) = 7.71$
   x = time (months) y = assay (mg)

	x	y	x^2	y^2	xy
	6	995	36	990025	5970
	12	984	144	968256	11808
	18	973	324	946729	17514
n = 6	24	960	576	921600	23040
	36	952	1296	906304	34272
	48	948	2304	898704	45504
$\sum$ =	144	5812	4680	5631618	138108

   Calculations:
   Slope and intercept:

$$b = \frac{n\sum xy - (\sum x)(\sum y)}{n\sum x^2 - (\sum x)^2} = \frac{6(138108) - (144)(5812)}{6(4680) - (144)^2} = -1.13$$

$$a = \frac{\sum y - b\sum x}{n} = \frac{5812 - (-1.13)(144)}{6} = 995.78$$

   Coefficient of determination:

$$SS_{explained} = b^2\left[\sum x^2 - \frac{(\sum x)^2}{n}\right] = (1.13)^2\left[4680 - \frac{(144)^2}{6}\right] = 1562.93$$

$$SS_{total} = \sum y^2 - \frac{(\sum y)^2}{n} = 5631618 - \frac{(5812)^2}{6} = 1727.33$$

$$SS_{unexplained} = SS_{total} - SS_{explained} = 1727.33 - 1562.93 = 164.40$$

$$r^2 = \frac{SS_{explained}}{SS_{total}} = \frac{1562.93}{1727.33} = 0.905$$

   ANOVA Table:

Source	SS	df	MS	F
Linear Regression	1562.93	1	1562.93	38.03
Residual	164.40	4	41.10	
Total	1727.33	5		

   Decision: With $F > 7.71$, reject H_0 and conclude that a linear relationship exists between the storage time and the assayed amount of drug.

   Slope of the population:
   Hypotheses: H_0: $\beta = 0$
   H_1: $\beta \neq 0$
   Decision Rule: With $\alpha = 0.05$, reject H_0 if $t > |t_4(1 - \alpha/2)| = 2.776$

Calculations:

$$S_b = \sqrt{\frac{MS_{residual}}{\sum x^2 - \frac{(\sum x)^2}{n}}}$$

$$S_b = \sqrt{\frac{41.10}{4680 - \frac{(144)^2}{6}}} = 0.183$$

$$t = \frac{b - 0}{S_b} = \frac{-1.13 - 0}{0.183} = -6.17$$

Decision: With $t < -2.776$, reject H_0, conclude that there is a slope and thus a relationship between time and assay results.

95% C.I. for the slope:

$$\beta = b \pm t_{n-1}(1-\alpha/2) \cdot S_b$$

$$\beta = -1.13 \pm 2.776(0.183) = -1.13 \pm 0.51$$

$$-1.64 < \beta < -0.62$$

Decision: Since zero does not fall within the confidence interval, reject H_0 and conclude that a relationship exists. With 95% confidence the slope of the line (β) is between −1.64 and −0.62.

Confidence interval around the regression line:

Example at 48 months, where $\overline{X} = 24$

$$\bar{y} = y_c \pm t_{n-2}(1-\alpha/2) \cdot \sqrt{MS_{residual}} \cdot \sqrt{\frac{1}{n} + \frac{(x_i - \overline{X})^2}{\sum x^2 - \frac{(\sum x)^2}{n}}}$$

$$\bar{y} = 941.54 \pm 2.776\sqrt{41.10}\,2 \sqrt{\frac{1}{6} + \frac{(48-24)^2}{4680 - \frac{(144)^2}{6}}}$$

$$\bar{y} = 941.54 \pm 14.20$$

$$927.34 < \bar{y} < 955.74$$

Results (Figure D.13):

Time (months)	Sample (mg)	y_c	95% Confidence Intervals Lower Limit	Upper Limit	Range
6	995	989.00	977.40	1000.60	23.20
12	984	982.22	972.84	991.60	18.76
18	973	975.44	967.69	983.19	15.50
24	960	968.66	961.54	975.78	14.24
36	952	955.10	945.72	964.48	18.75
48	948	941.54	927.34	955.74	28.40

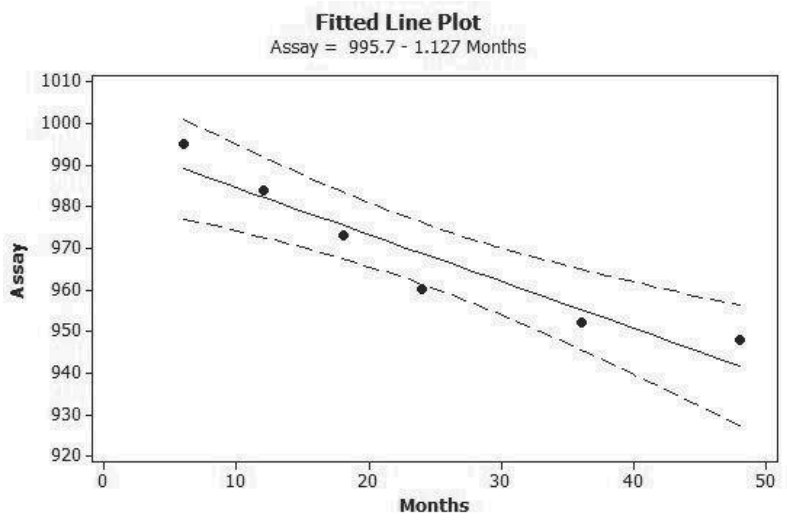


Figure D.13 Minitab confidence bands for results Problem 1, Chapter 14.

2. Comparison of various concentrations to effect on the optical density.
   Variables: continuous independent variable (concentration)
   continuous dependent variable (optical density)
   Hypotheses: H_0: Concentration and density are not linearly related
   H_1: Concentration and density are linearly related
   Decision Rule: With $\alpha = 0.05$, reject H_0 if $F > F_{1,2}(1 - \alpha) = 18.5$

	Concentration x	Density y	x^2	y^2	xy
	1	0.24	1	0.058	0.24
	2	0.66	4	0.436	1.32
n = 4	4	1.15	16	1.323	4.60
	8	2.34	64	5.476	18.72
∑ =	15	4.39	85	7.293	24.88

Calculations:

Slope and *y*-intercept:

$$b = \frac{4(24.88) - (15)(4.39)}{4(85) - (15)^2} = 0.293$$

$$a = \frac{4.39 - 0.293(15)}{4} = -0.00125$$

Coefficient of determination:

$$SS_{total} = \sum y^2 - \frac{(\sum y)^2}{n} = 7.293 - \frac{(4.39)^2}{4} = 2.47498$$

$$SS_{explained} = b^2 \cdot \left[\sum x^2 - \frac{(\sum x)^2}{n}\right] = (.293)^2 \left[85 - \frac{(15)^2}{4}\right] = 2.46816$$

$$SS_{unexplained} = SS_{total} - SS_{explained} = 2.47498 - 2.46816 = 0.00682$$

$$r^2 = \frac{SS_{explained}}{SS_{total}} = \frac{2.46816}{2.47498} = 0.997$$

ANOVA table:

Source of Variation	SS	df	MS	F
Linear Regression	2.46816	1	2.46816	723.80
Residual	0.00682	2	0.00341	
Total	2.47498	3		

Decision: With $F > 18.5$, reject H_0 and conclude that a linear relationship exists between the concentration and amount of optical density.

Slope of the population:

Hypotheses: H_0: $\beta = 0$

H_1: $\beta \neq 0$

Decision Rule: With $\alpha = 0.05$, reject H_0 if $t > |t_2(1 - \alpha/2)| = 4.302$

Calculations:

$$S_b = \sqrt{\frac{MS_{residual}}{\sum x^2 - \frac{(\sum x)^2}{n}}}$$

$$S_b = \sqrt{\frac{0.00341}{85 - \frac{(15)^2}{4}}} = 0.0109$$

$$t = \frac{b - 0}{S_b} = \frac{0.293 - 0}{0.0109} = 26.88$$

Decision: With $t > 4.302$, reject H_0, conclude that there is a slope and thus a relationship between concentration and density.

95% C.I. for the slope:

$$\beta = b \pm t_{n-1}(1 - \alpha/2) \cdot S_b$$

$$\beta = 0.23 \pm 4.302(0.0109) = 0.293 \pm 0.047$$

$$0.246 < \beta < 0.340$$

Decision: Since zero does not fall within the confidence interval, reject H_0 and conclude that a relationship exists. With 95% confidence the slope of the line (β) is between +0.246 and +0.340.

Confidence interval around the regression line:

Example at 4% concentration, where $\overline{X} = 3.75$

$$\overline{y} = y_c \pm t_{n-2}(1 - \alpha/2) \cdot \sqrt{MS_{residual}} \cdot \sqrt{\frac{1}{n} + \frac{(x_i - \overline{X})^2}{\sum x^2 - \frac{(\sum x)^2}{n}}}$$

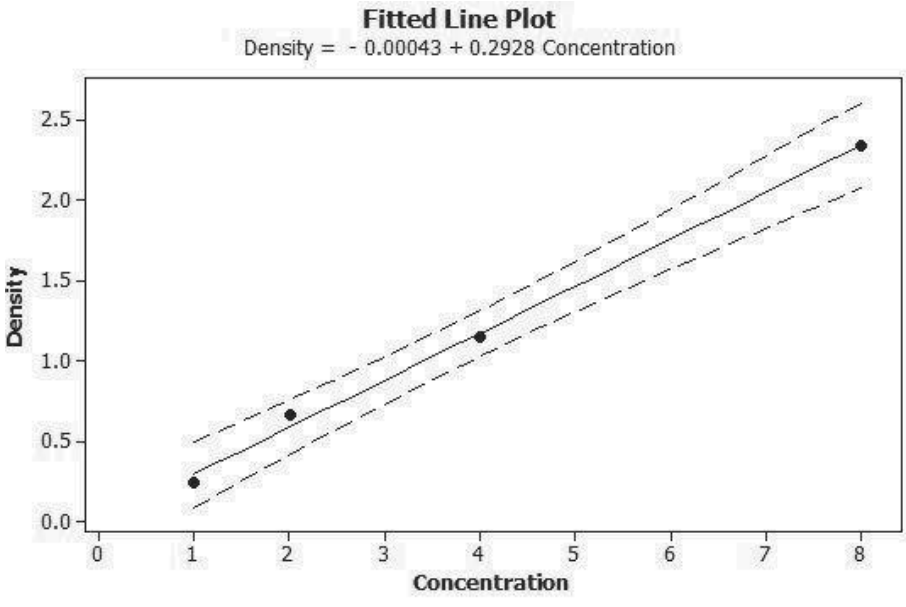


Figure D.14 Minitab confidence bands for results of Problem 2, Chapter 14.

$$\bar{y} = 1.17 \pm 4.302\sqrt{0.00341}\sqrt{\frac{1}{4} + \frac{(4-3.75)^2}{85 - \frac{(15)^2}{4}}}$$

$$\bar{y} = 1.17 \pm 0.13$$

$$1.04 < \bar{y} < 1.30$$

Results (Figure D.14):

		95% Confidence Intervals			
Concentration	Density	y_c	Lower Limit	Upper Limit	Range
1	0.24	0.29	0.11	0.47	0.36
2	0.66	0.58	0.43	0.73	0.30
4	1.15	1.17	1.04	1.30	0.26
8	2.34	2.34	2.11	2.57	0.46

3. Comparison of various concentrations to effect on the optical density for two solutions (test versus reference standard) and evaluating if the two results produce parallel lines.

   Variables: continuous independent variables (concentrations for the test solution and the reference standard)
   continuous dependent variable (optical density)

   Hypotheses: H_0: $\beta_T = \beta_S$
   H_1: $\beta_T \neq \beta_S$

   Decision Rule: With $\alpha = 0.05$, reject H_0 if $t > t_{4+4-4}(1 - \alpha/2) = 2.777$

Much of the information needed about the test solution was calculated in Problem 2 and will be presented below. Information needed for the reference standard solution is as follows:

	Concentration x	Density y	x^2	y^2	xy
	1	0.22	1	0.0484	0.22
	2	0.74	4	0.5476	1.48
n = 4	4	1.41	16	1.9881	5.64
	8	2.76	64	7.6176	22.08
∑ =	15	5.13	85	10.2017	29.42

Calculations:

Slope and y-intercept:

$$b = \frac{4(29.42) - (15)(5.13)}{4(85) - (15)^2} = 0.354$$

$$a = \frac{5.13 - 0.354(15)}{4} = -0.0450$$

Coefficient of determination:

$$SS_{total} = \sum y^2 - \frac{(\sum y)^2}{n} = 10.2017 - \frac{(5.13)^2}{4} = 3.62248$$

$$SS_{explained} = b^2 \cdot \left[\sum x^2 - \frac{(\sum x)^2}{n}\right] = (0.354)^2 \left[85 - \frac{(15)^2}{4}\right] = 3.60284$$

$$SS_{unexplained} = SS_{total} - SS_{explained} = 3.62248 - 3.60284 = 0.01964$$

ANOVA table:

Source of Variation	SS	df	MS	F
Linear Regression	3.60284	1	3.60284	366.89
Residual	0.01964	2	0.00982	
Total	3.62248	3		

Information required for a comparison of parallelism:

	Test	Standard
n	4	4
df	2	2
Σx	15	15
Σx^2	85	85
b	0.293	0.354
SS_R	0.00682	0.01964

$$(S^2_{x/y})_p = \frac{0.00682 + 0.01964}{4 + 4 - 4} = 0.00662$$

$$t = \frac{0.293 - 0.354}{\sqrt{\frac{0.00662}{85 - \frac{(15)^2}{4}} + \frac{0.00662}{85 - \frac{(15)^2}{4}}}} = \frac{-0.061}{0.0215} = -2.84$$

Decision: With $t < -2.777$, reject H_0, conclude that the data have slopes that are not parallel.

95% C.I. for the difference between the two slopes:

$$\beta_1 - \beta_2 = (0.293 - .0354) \pm (2.777) \sqrt{\frac{0.00662}{85 - \frac{(15)^2}{4}} + \frac{0.00662}{85 - \frac{(15)^2}{4}}}$$

$$\beta_1 - \beta_2 = (-0.061) \pm (0.0597)$$

$$-0.1207 < \beta_1 - \beta_2 < -0.0013$$

Decision: Since zero does not fall within the confidence interval, reject H_0 and conclude that two lines are not parallel. With 95% confidence the difference between the two slopes is between –0.121 and –0.001.

4. Comparison of percent of coating with rate of release

Variables: continuous independent variable (percent)
continuous dependent variable (rate of release)

Hypothesis: H_0: Percent (x) and rate of release (y) are not linearly related
H_1: Percent and rate of release are linearly related

and H_0: There is no lack of linear fit
H_1: There is lack of linear fit

```
The regression equation is
Rate = 3.85 - 0.0774 Percent

Predictor       Coef   SE Coef       T      P
Constant     3.85101   0.06841   56.30  0.000
Percent    -0.077412  0.002939  -26.34  0.000

S = 0.148425   R-Sq = 97.7%   R-Sq(adj) = 97.6%

Analysis of Variance
Source          DF      SS      MS       F      P
Regression       1  15.281  15.281  693.65  0.000
Residual Error  16   0.352   0.022
  Lack of Fit    4   0.046   0.011    0.45  0.771
  Pure Error    12   0.307   0.026
Total           17  15.634

No evidence of lack of fit (P >= 0.1).
```

Figure D.15 Minitab results from analysis Problem 4, Chapter 14.

Results: Figure D.15
Decision: The line drawn between the data points (y = 3.85 + 0.08); not shown in Figure D.15) represents 97.7% of the variability on the *y*-axis. There is a significant linear relationship between the percent of coating and rate of release ($p < 0.001$) and it was not possible to reject the null hypothesis of lack of fit.

5. As seen in the Minitab printout (Figure D.16), the slope is –0.004 and the intercept is 2.15. There is a significant linear relationship, $F = 240.96$, $p < 0.001$. The line represents 97.6% of all the variation on the *y*-axis. Figure D.17 presents the graphic representation of the data with 95% confidence bands. Note at the bottom of Figure D.16 the results were also calculated for quadratic and cubic relationships and the linear results were the best representations for the sample data.

Chapter 15 – z-Tests of Proportions

1. Production was run with an expected defect rate of 1.5%, but a rate of 5% for 100 tablets was found.
Hypotheses: H_0: $\hat{p} = 0.015$
H_1: $\hat{p} \neq 0.015$
Decision rule: With $\alpha = 0.05$, reject H_0, if $z > z_{(1-\alpha/2)} = 1.96$ or $z < -1.96$.
Data: $P_0 = 0.015$; $\hat{p} = 0.05$; $n = 100$.

Regression Analysis: Results versus Dilution

The regression equation is
Results = 2.15 - 0.00416 Dilution

S = 0.0627316 R-Sq = 97.6% R-Sq(adj) = 97.2%

Analysis of Variance

Source	DF	SS	MS	F	P
Regression	1	0.94825	0.94825	240.96	0.000
Residual Error	6	0.02361	0.00394		
Total	7	0.97186			

Sequential Analysis of Variance

Source	DF	SS	F	P
Linear	1	0.948252	240.96	0.000
Quadratic	1	0.016933	12.68	0.016
Cubic	1	0.004979	11.72	0.027

Figure D.16 Minitab output for Problem 5, Chapter 14.

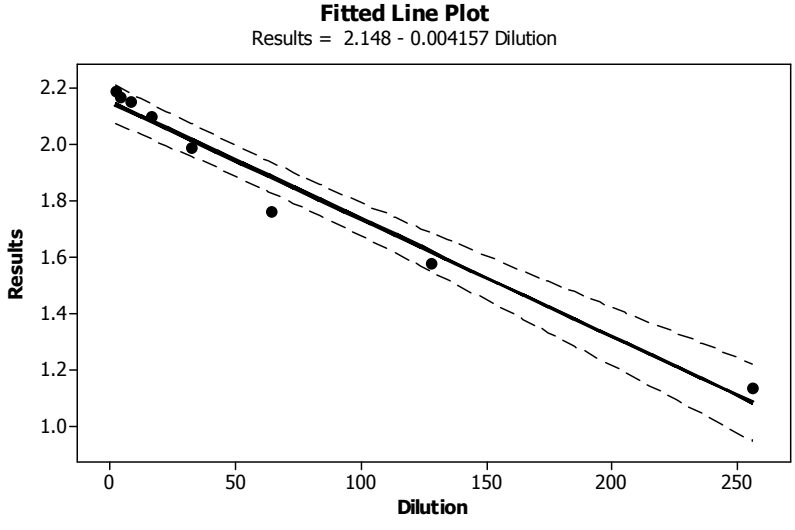


Figure D.17 Minitab confidence bands for results of Problem 5, Chapter 14.

Calculations:

$$z = \frac{\hat{p} - P_0}{\sqrt{\frac{P_0(1-P_0)}{n}}} = \frac{0.05 - 0.015}{\sqrt{\frac{(0.015)(0.985)}{100}}} = \frac{0.035}{0.012} = 2.92$$

Decision: With $z > 1.96$, reject H_0 and conclude that there is a significant difference between the sample and the expected proportion of defects.

Alternative confidence interval approach:

$$P_0 = \hat{p} \pm Z_{(1-\alpha/2)}\sqrt{\frac{P_0(1-P_0)}{n}}$$

$$P_0 = 0.05 \pm 1.96\sqrt{\frac{(0.015)(0.985)}{100}} = 0.05 \pm 0.024$$

$$+0.026 < P_0 < +0.074$$

Decision: The outcome 0.015 does not fall within the interval, therefore reject H_0.

2. Incidence of nausea and vomiting between two therapies.
   a. Two-tailed, not predicting direction:
      Hypotheses: H_0: $P_N = P_T$
      H_0: $P_N \neq P_T$
      Decision rule: With $\alpha = 0.05$, reject H_0, if $z > z_{(1-\alpha/2)} = 1.96$ or $z < -1.96$.

Calculations:

$$\hat{p}_0 = \frac{n_F \hat{N}_N + n_T \hat{p}_T}{n_N + n_T} = \frac{(190)(0.36) + (75)(0.55)}{190 + 75} = 0.413$$

$$1 - \hat{p}_0 = 1.00 - 0.413 = 0.587$$

$$z = \frac{0.36 - 0.55}{\sqrt{\frac{(0.413)(0.587)}{190} + \frac{(0.413)(0.587)}{75}}} = \frac{-0.19}{0.067} = -2.83$$

Decision: With $z < -1.96$ reject H_0, and conclude there is a significant difference between the incidence of nausea and vomiting between the new drug and traditional therapy.

b. One-tailed, predicting lower incidence with a new agent:

Hypotheses: H_0: $P_N \geq P_T$
H_0: $P_N < P_T$

Decision rule: With $\alpha = 0.05$, reject H_0, if $z < z_{(1-\alpha)} = -1.64$.

Decision: The computed z-value was –2.83. With $z < -1.64$ reject H_0, and conclude that the newer agent causes a significant decrease in the incidence of nausea and vomiting compared to traditional therapy.

3. Defects at two different speeds for a tablet press.

Speed	n	# of Defects
Low	500	11
High	460	17

$\hat{p}_L = 11/500 = 0.022$ $\hat{p}_H = 17/460 = 0.037$

Hypotheses: H_0: $P_L = P_H$ H_1: $P_L \neq P_H$

Decision rule: With $\alpha = 0.05$, reject H_0, if $z > z_{(1-\alpha/2)} = 1.96$ or $z < -1.96$.

Calculations:

$$\hat{p}_0 = \frac{n_L \hat{p}_L + n_H \hat{p}_H}{n_L + n_H} = \frac{(500)(0.022) + (460)(0.037)}{500 + 460} = 0.029$$

$$1 - \hat{p}_0 = 1.00 - 0.029 = 0.971$$

Test and CI for Two Proportions

```
Sample   X    N  Sample p
1       11  500  0.022000
2       17  460  0.036957

Difference = p (1) - p (2)
Estimate for difference:  -0.0149565
95% CI for difference:  (-0.0364629, 0.00654985)
Test for difference = 0 (vs not = 0):  Z = -1.36  P-Value = 0.173
```

Figure D.18 Minitab Z-test results for Problem 2, Chapter 15.

$$z = \frac{\hat{p}_L - \hat{p}_H}{\sqrt{\frac{\hat{p}_0(1-\hat{p}_0)}{n_L} + \frac{\hat{p}_0(1-\hat{p}_0)}{n_H}}}$$

$$z = \frac{0.022 - 0.037}{\sqrt{\frac{0.029(0.971)}{500} + \frac{0.029(0.971)}{460}}} = \frac{-0.015}{0.011} = -1.36$$

Decision: With the $z > -1.96$, fail to reject H_0, conclude that there is no significant difference in the defect rate based on the tablet press speed.

Yates' correction for continuity:

$$z = \frac{|\hat{p}_1 - \hat{p}_2| - \frac{1}{2}(\frac{1}{n_1} + \frac{1}{n_2})}{\sqrt{\frac{\hat{p}_0(1-\hat{p}_0)}{n_1} + \frac{\hat{p}_0(1-\hat{p}_0)}{n_2}}}$$

$$z = \frac{|0.022 - 0.037| - \frac{1}{2}(\frac{1}{500} + \frac{1}{460})}{\sqrt{\frac{0.029(0.971)}{500} + \frac{0.029(0.971)}{460}}} = \frac{0.015 - 0.002}{0.011} = \frac{0.013}{0.011} = 1.18$$

Decision: With the $z < 1.96$, fail to reject H_0.

Results: Figure D.18, Minitab does not use Yates' correction.

4. Incidence of a blood dyscrasia in a Phase IV clinical trial.

Hypotheses: H_0: $\hat{p} = 0.025$

H_1: $\hat{p} \neq 0.025$

Decision rule: With $\alpha = 0.05$, reject H_0, if $z > z_{(1-\alpha/2)} = 1.96$ or $z < -1.96$.

Data: $P_0 = 0.025$; $\hat{p} = 2/28 = 0.071$; n = 28

Calculations:

$$z = \frac{\hat{p} - p_0}{\sqrt{\frac{p_0(1-p_0)}{n}}} \frac{0.071 - 0.025}{\sqrt{\frac{(0.025)(0.975)}{28}}} = \frac{0.046}{0.029} = 1.59$$

Decision: With $z < 1.96$, fail to reject H_0 and not conclude that the sample results are different from what was found with the original clinical trials.

Alternative confidence interval approach:

$$P_0 = \hat{p} \pm Z_{(1-\alpha/2)}\sqrt{\frac{P_0(1-P_0)}{n}}$$

$$P_0 = 0.071 \pm 1.96\sqrt{\frac{(0.025)(0.975)}{28}} = 0.071 \pm 0.057$$

$$+0.014 < P_0 < +0.128$$

Decision: The outcome 0.025 falls within the interval; therefore H_0 cannot be rejected.

Chapter 16 – Chi Square Tests

1. Severe irritation to stomach mucosa compared with special tablet coatings.

	GI Irritation	
	Present (P_1)	Absent (P_2)
Formula A	10	40
Formula B	8	42
Formula C	7	43

Hypotheses: H_0: $P(P_1 | F_A) = P(P_1 | F_B) = P(P_1 | F_C) = P(P_1)$
$P(P_2 | F_A) = P(P_2 | F_B) = P(P_2 | F_C) = P(P_2)$
H_1: H_0 is false

Decision rule: With $\alpha = 0.05$, reject H_0 if $\chi^2 > \chi_2^2\,(0.05) = 5.99$

Observed			Expected		
10	40	50	8.33	41.67	50
8	42	50	8.33	41.67	50
7	43	50	8.33	41.67	50
25	125	150	25	125	150

Calculations:

$$\chi^2 = \Sigma \frac{(O-E)^2}{E}$$

$$\chi^2 = \frac{(10-8.33)^2}{8.33} + \frac{(40-41.67)^2}{41.67} + \ldots \frac{(43-41.67)^2}{41.67} = 0.66$$

Decision: With $\chi^2 < 5.99$, cannot reject H_0.

2. Above and below the median time needed for nurse surveyors to observe drug deliveries.

Hypotheses: H_0: P(2.5 or less|UD) = P(2.5 or less|Trad) = P(2.5 or less)
P(>2.5|UD) = P(>2.5|Trad) = P(>2.5)
(time required is not influenced by the delivery system)
H_1: H_0 is false

Decision rule: With $\alpha = 0.05$, reject H_0 if $\chi^2 > \chi_1^2\,(0.05) = 3.84$

Test statistic: (because of only one degree of freedom, use Yates' correction)

$$\chi^2_{corrected} = \frac{n(|ad-bc|-0.5n)^2}{(a+b)(b+d)(a+b)(c+d)}$$

Data:

	Unit Dose	Traditional	Total
2.5 hours or less	26	10	36
More than 2.5 hours	14	20	34
Total	40	30	70

```
Expected counts are printed below observed counts
Chi-Square contributions are printed below expected counts

        Unit Dose  Traditional  Total
1             26           10     36
           20.57        15.43
           1.433        1.910

2             14           20     34
           19.43        14.57
           1.517        2.022

Total         40           30     70

Chi-Sq = 6.882, DF = 1, P-Value = 0.009
```

Figure D.19 Minitab outcome for Problem 2, Chapter 16.

Calculations:

$$\chi^2_{corrected} = \frac{70(|(26)(20)-(14)(10)|-0.5(70))^2}{(40)(30)(36)(34)}$$

$$\chi^2_{corrected} = \frac{70(|520-140|-35)^2}{1468800} = \frac{70(345)^2}{1468800} = \frac{8331750}{1468800} = 5.67$$

Decision: With $\chi^2 > 3.84$, reject H_0 and conclude that the time required to do the nursing home surveys is dependent on the type of delivery system used in the facility.

Results: Figure D.19, Minitab does not make Yates' correction.

3. Paired comparison between technicians' evaluations at two times: McNemar's test.

Hypotheses: H_0: Experience did not influence opinion of equipment
H_1: H_0 is false

Decision rule: With $\alpha = 0.05$, reject H_0, if $\chi^2_{McNemar} > \chi^2_1(1-\alpha) = 3.84$.

Calculations:

		Preferred Method Before Experience: New	Old	
Preferred Method After Experience	New	12	8	20
	Old	3	7	10
		15	15	30

$$\chi^2_{McNemar} = \frac{(b-c)^2}{b+c} = \frac{(8-3)^2}{8+3} = \frac{25}{11} = 2.27$$

Correction of continuity:

$$\chi^2_{McNemar} = \frac{(|b-c|-1)^2}{b+c} = \frac{(|8-3|-1)^2}{8+3} = \frac{16}{11} = 1.45$$

Decision: Fail to reject H_0 and conclude there was no significant change in method preference over the six-month period.

4. Comparisons of two blister packs stored under different conditions.
Independent variable: Storage conditions (discrete)
Dependent variable: Type of blister pack (discrete)
Test statistic: One-tailed Fisher exact test (multiple cell with expected value <5)

	40 degrees 50% relative humidity	60 degrees 50% relative humidity	
Blister pack A	2	5	7
Blister pack B	6	6	12
	8	11	19

Hypothesis: H_0: Blister pack and storage conditions are independent
H_1: The two variables are not independent
Decision rule: With $\alpha = 0.05$, reject H_0 if $p(>2) > 0.05$.
Calculations:

a. $p(2)$ of two failures with blister pack A

$$p = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!a!b!c!d!} = \frac{7!12!8!11!}{19!2!5!6!6!} = 0.256$$

b. $p(1)$ of one failure with blister pack A

$$p = \frac{7!12!8!11!}{19!1!6!7!5!} = 0.073$$

c. $p(0)$ of no failures with blister pack A

$$p = \frac{7!12!8!11!}{19!0!7!8!4!} = 0.006$$

Decision: The probability of two or fewer failures with blister pack A under independent conditions is 0.335 (0.256 + 0.073 + 0.006); therefore we cannot reject H_0 and assume that the frequency of failures by blister pack is independent of the storage conditions.

5. Experiment with different amounts of torque and resulting moisture content in a pharmaceutical product.

	Torque (inch-pounds)				
Moisture	21	24	27	30	
<2000	26	31	36	45	138
≥2000	24	19	14	5	62
Total	50	50	50	50	200

Hypotheses:

H_0: $P(M_1|T_1) = P(M_1|T_2) = P(M_1|T_3) = P(M_1|T_4) = P(M_1)$
$P(M_2|T_1) = P(M_2|T_2) = P(M_2|T_3) = P(M_2|T_4) = P(M_2)$
H_1: H_0 is false

(The null hypothesis states that the moisture observed is independent of the torque placed upon the lid.)

Decision rule: With $\alpha = 0.05$, reject H_0 if $\chi^2 > \chi_3^2 (0.05) = 7.81$

Expected values:

	Torque (inch-pounds)				
Moisture	21	24	27	30	
<2000	34.5	34.5	34.5	34.5	138
≥2000	15.5	15.5	15.5	15.5	62
Total	50	50	50	50	200

Computation:

$$\chi^2 = \frac{(26 - 34.5)^2}{34.5} + \frac{(31 - 34.5)^2}{34.5} + \ldots \frac{(5 - 15.5)^2}{15.5}$$

$$\chi^2 = 18.43$$

Decision: With $\chi^2 > 7.81$ reject H_0 and conclude that there is a significant difference in moisture level based on the amount of torque applied during closure.

6. Comparison of three topical formulations: Cochran's Q.

Hypotheses: H_0: Development of erythema is independent of formulation used
H_1: H_0 is false

Decision rule: With $\alpha = 0.05$, reject H_0 if $Q > \chi^2_2(1 - \alpha) = 5.99$.

Data: Table D.9

Computations:

$$Q = \frac{(k-1)[(k\sum C^2) - (\sum R)^2]}{k(\sum R) - \sum R^2} = \frac{(2)[(3)(97) - (17)^2]}{(3)(17) - 35} = \frac{4}{16} = 0.25$$

Decision: With $Q < 5.99$, fail to reject H_0 and conclude that erythema is independent of the formulation.

7. Comparison of pass/fail rate with a piece of disintegration equipment at different temperatures, controlling for paddle speed: Mantel-Haenszel chi square.

Hypotheses: H_0: Temperature and failure rate are independent (controlling for paddle speed)
H_1: H_0 is false

Decision rule: With $\alpha = 0.05$, reject H_0 if $\chi^2_{MH} > \chi^2_1(1 - \alpha) = 3.84$.

Data: Disintegration test

		Test Results		
Speed of Paddle	Temperature	Pass	Fail	Totals
Fast	39°C	48	2	50
	35°C	47	3	50
		95	5	100
Slow	39°C	48	2	50
	35°C	45	5	50
		93	7	100

Table D.9 Data for Chapter 16, Problem 6

	Formulation (1 = erythema)				
Volunteer	A	B	C	R	R^2
001	0	1	0	1	1
002	1	0	1	2	4
003	0	0	0	0	0
004	0	0	0	0	0
005	0	1	1	2	4
006	0	0	0	0	0
007	0	0	0	0	0
008	0	0	0	0	0
009	0	0	0	0	0
010	1	1	0	2	4
011	0	0	1	1	1
012	0	0	0	0	0
013	1	0	1	2	4
014	0	0	0	0	0
015	0	0	0	0	0
016	0	0	0	0	0
017	1	1	0	2	4
018	0	0	0	0	0
019	1	0	1	2	4
020	1	1	1	3	9
C =	6	5	6		
C^2 =	36	25	36		
			$\sum R$ =	17	
	$\sum C^2$ = 97		$\sum R^2$ =		35

Intermediate steps for fast speed:

$$e_1 = \frac{(a_1+b_1)(a_1+c_1)}{n_1} = \frac{(50)(95)}{100} = 47.5$$

$$v_1 = \frac{(a_1+b_1)(c_1+d_1)(a_1+c_1)(b_1+d_1)}{n_1^2(n_1-1)} = \frac{(50)(50)(95)(5)}{100^2(99)} = 1.199$$

	Fast	Slow
a_i	48	48
e_i	47.5	46.5
v_i	1.2	1.6

Mantel-Haenszel chi square:

$$\chi^2_{MH} = \frac{[\sum(a_i - e_i)]^2}{\sum v_i} = \frac{[(48-47.5)+(48-46.5)]^2}{1.2+1.6} = 1.43$$

Decision: Fail to reject H_0 and conclude that the temperature and failure rates are independent.

Chapter 17 – Measures of Association

1. Measures of association between patient gender and outcome.

	Males	Females	
Success	45	30	75
Failure	5	20	25
	50	50	100

Several tests can be run on dichotomous data, including the *phi* statistic, Yule's Q, Yule's Y, and *tau-b*:

Preliminary information: $\chi^2 = 12.00$
$P = 900$ (45 × 20)
$Q = 150$ (5 × 30)
$X_0 = 825$ (45 × 5 + 30 × 20)
$Y_0 = 1450$ (45 × 30 + 5 × 20)

Phi statistic:

$$\phi = \sqrt{\frac{\chi^2}{n}} = \sqrt{\frac{12}{100}} = 0.346$$

Yule's Q:

$$Q = \frac{P - Q}{P + Q} = \frac{900 - 150}{900 + 150} = 0.714$$

Yule's Y:

$$Y = \frac{\sqrt{P} - \sqrt{Q}}{\sqrt{P} + \sqrt{Q}} = \frac{\sqrt{900} - \sqrt{150}}{\sqrt{900} + \sqrt{150}} = 0.420$$

Tau-b :

$$\tau_b = \frac{P - Q}{\sqrt{(P + Q + X_O)(P + Q + Y_O)}}$$

$$\tau_b = \frac{900 - 150}{\sqrt{(900 + 150 + 825)(900 + 150 + 1450)}} = 0.346$$

The most appropriate would be the *phi* statistic since both variables are nominal (see Table 17.6). However, if one considers success/failure an ordinal variable with success being better than failure, then Yule's Q could be used.

2. Measures of association comparing three therapies and three possible outcomes.

	Treatment A	Treatment B	Treatment C	
At goal	56	46	35	137
Not at goal	30	18	18	66
Discontinued	13	20	37	70
	99	84	90	273

Various tests could be run on this contingency table, including Pearson *C*, *C**, Tshuprow's *T*, Cramer's *V*, *lamdba*, *tau-c*, *tau-b*, Somer's *d*, and *gamma*.

Preliminary information: $\chi^2 = 20.44$ $X_0 = 7497$
$P = 10114$ $Y_0 = 9031$
$Q = 5641$

Pearson *C*:

$$C = \sqrt{\frac{\chi^2}{\chi^2 + N}} = \sqrt{\frac{20.44}{20.44 + 273}} = 0.264$$

Pearson *C**:

$$C^* = \frac{C}{\sqrt{\frac{k-1}{k}}} = \frac{0.264}{\sqrt{\frac{3-1}{3}}} = 0.323$$

Tshuprow's *T*:

$$T = \sqrt{\frac{\chi^2}{n\sqrt{(r-1)(c-1)}}} = \sqrt{\frac{20.44}{273\sqrt{(2)(2)}}} = 0.193$$

Cramer's *V*:

$$V = \sqrt{\frac{\chi^2}{Nm}} = \sqrt{\frac{20.44}{(273)(2)}} = 0.193$$

Note in a square table that T = V.

Lamdba:

$$\lambda = \frac{\sum f_i - f_d}{N - f_d} = \frac{(56 + 46 + 37) - 137}{273 - 137} = 0.015$$

Tau-c:

$$\tau_c = (P - Q)\left(\frac{2m}{n^2(m-1)}\right)$$

$$\tau_c = (10114 - 5641)\left(\frac{2(3)}{(273)^2 \cdot (2)}\right) = 0.180$$

Tau-b:

$$\tau_b = \frac{P - Q}{\sqrt{(P + Q + X_O)(P + Q + Y_O)}}$$

$$\tau_b = \frac{10114 - 5641}{\sqrt{(10114 + 5641 + 7497)(10114 + 5641 + 9031)}} = 0.186$$

Somer's *d*:

$$d_{yx} = \frac{(P - Q)}{(P + Q + Y_0)} = \frac{10114 - 5641}{10114 + 5641 + 9031} = 0.180$$

Gamma:

$$\Gamma = \frac{P - Q}{P + Q} = \frac{10114 - 5641}{10114 + 5641} = 0.284$$

Pearson *C* or *C** would seem appropriate since both variables are nominal and the table is square and smaller than a 5 × 5 configuration.

3. Association between practice setting and response on a Likert scale.

	Practice Setting			
Evaluation	Retail	Hospital	Long-Term Care	
5 "strongly agree"	10	2	4	16
4 "agree"	12	2	6	20
3 "uncertain"	24	12	14	50
2 "disagree"	36	20	28	84
1 "strongly disagree"	18	64	48	130
	100	100	100	300

Several tests can be run on dichotomous data, including the *phi* statistic, *tau-c*, *lambda*, *eta*, *omega*.

Preliminary information: $\chi^2 = 48.8$; $P = 14288$; $Q = 7368$

ANOVA Table:

Source	df	SS	MS	F
Between	2	54.43	27.21	23.13
Within	297	349.36	1.18	
Total	299	403.79		

Tau-c:

$$\tau_c = (P-Q)\left(\frac{2m}{n^2(m-1)}\right)$$

$$\tau_c = (14288-7368)\left(\frac{2(3)}{300^2(2)}\right) = 0.231$$

Lamdba:

$$\lambda = \frac{\sum f_i - f_d}{N - f_d} = \frac{(36+64+48)-130}{300-130} = 0.106$$

Eta:

$$E = \sqrt{\frac{SS_B}{SS_T}} = \sqrt{\frac{54.43}{403.79}} = 0.367$$

Omega:

$$\omega^2 = \frac{SS_B - (k-1)MS_W}{SS_T + MS_W} = \frac{54.43-(2)(1.18)}{403.79+1.18} = 0.129$$

Tau-c: Use if the researcher considers setting as an independent variable; if not, then use *lambda*. However, if the Likert scale is considered an interval scale, *eta* or *omega* could be used.

Chapter 18 – Odds Ratios and Relative Risk Ratios

1. Results of an odds ratio evaluation.

		Hypertension: Yes (+)	Hypertension: No (−)	
Met goal	Yes (+)	60	24	84
	No (−)	57	29	86
		117	53	170

The odds of meeting goal with hypertension:

$$a/c = 60/57 = 1.053$$

The odds of meeting goal without hypertension:

$$b/d = 24/29 = 0.828$$

The odds ratio for the factor of hypertension is:

$$OR = \frac{a/c}{b/d} = \frac{1.053}{0.828} = 1.272$$

Thus, a patient with hypertension is 1.272 times more likely to meet the established goal than one without hypertension. Is this statistically significant?

$$\ln\theta = \ln_{(OR)} \pm Z_{1-\alpha/2}\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}$$

$$\ln\theta = \ln(1.272) \pm 1.96\sqrt{\frac{1}{60}+\frac{1}{57}+\frac{1}{24}+\frac{1}{29}}$$

$$\ln\theta = 0.241 \pm 0.651$$

$$0 - 0.410 < \ln\theta < +0.892$$

$$e^{-0.410} = 0.664 \quad and \quad e^{+0.892} = 2.439$$

$$0.664 < OR_{Population} < 2.439$$

Since one is within the interval, we fail to reject the null hypothesis that OR = 1; therefore, even though hypertensive patients are 1.27 times more likely to meet their goal, this is not significantly better than nonhypertensive counterparts.

2. Survival ten years following radical mastectomy.
   a. Relative risk of death with positive node involvement:

		Nodal Involvement: Yes (+)	Nodal Involvement: No (−)	
Outcome in 10 years	Dead (+)	299	107	406
	Alive (−)	126	218	344
		425	325	750

$$RR = \frac{ab+ad}{ab+bc} = \frac{(299)(107)+(299)(218)}{(299)(107)+(107)(126)} = 2.136$$

$$Z_{RR} = Z_{1-\alpha/2}\sqrt{\frac{1}{a}-\frac{1}{a+c}+\frac{1}{b}-\frac{1}{b+d}}$$

$$Z_{RR} = 1.96\sqrt{\frac{1}{299}-\frac{1}{425}+\frac{1}{107}-\frac{1}{325}} = 0.264$$

$$RR_{Population} = RR_{Sample}\left(e^{\pm Z_{RR}}\right)$$

$$RR_{Population} = 2.136\left(e^{\pm 0.264}\right)$$

$$1.640 < RR_{Population} < 2.781$$

Decision: The risk of death is 2.136 times greater in patients with positive node involvement and this difference is significant since the calculated confidence interval for the population does not include the value one.

Chi square test of significance:

Hypotheses: H_0: RR = 1
H_1: RR ≠ 1

Decision rule: With $\alpha = 0.05$, reject H_0, if $\chi^2 > \chi^2_1(1-\alpha) = 3.84$.

Computations:

$$\chi^2 = \frac{n(|ad - bc| - 0.5n)^2}{(a+b)(c+d)(a+c)(b+d)}$$

$$\chi^2 = \frac{750[|(299)(218) - (126)(107)| - (0.5)(750)]^2}{(406)(344)(425)(325)} = 102.41$$

Decision: With $\chi^2 > 3.84$, reject H_0 and conclude there is a significant relationship between survival and presence or absence of positive nodes.

b. Relative risk of death with positive node involvement controlling for estrogen receptors:

$$RR_{MH} = \frac{\sum \frac{a_i(c_i+d_i)}{N_i}}{\sum \frac{c_i(a_i+b_i)}{N_i}}$$

$$RR_{MH} = \frac{\frac{179(100+148)}{453}+\frac{120(26+70)}{297}}{\frac{100(179+26)}{453}+\frac{26(120+81)}{297}} = \frac{136.783}{62.850} = 2.176$$

Significance of nodal involvement and death as outcomes controlling for the possible confounding factor of estrogen receptors.

Hypotheses: H_0: Nodal involvement and survival are independent (controlling for estrogen receptors)
H_1: H_0 is false

Decision rule: With $\alpha = 0.05$, reject H_0 if $\chi^2_{MH} > \chi^2_1(1-\alpha) = 3.84$.

Calculations:

$$\chi^2_{MH} = \frac{\left(\sum a_i - \Sigma \frac{a_i(c_i + d_i)}{N_i}\right)^2}{\Sigma \frac{(a_i + b_i)(c_i + d_i)(a_i + c_i)(b_i + d_i)}{N_i{}^2 (N_i - 1)}}$$

$$\chi^2_{MH} = \frac{(179 + 120) - \left(\frac{179 \cdot 248}{453} + \frac{120 \cdot 96}{297}\right)^2}{\left(\frac{205 \cdot 248 \cdot 279 \cdot 174}{(453)^2 \cdot 452}\right) + \left(\frac{201 \cdot 96 \cdot 146 \cdot 151}{(297)^2 \cdot 296}\right)} = \frac{26315.33}{42.906} = 613.33$$

Decision: Reject H_0 and conclude that survival and nodal involvement are related, controlling for estrogen receptors.

3. Logistic regression on four levels of torque:

Torque (inch-pounds):	Success (<2000)	Failure (≥2000)	
21	26	24	50
24	31	19	50
27	36	14	50
30	45	5	50
	138	62	200

Probabilities associated with each outcome:

Torque (inch-pounds):	Success (<2000)	Failure (≥2000)
21	0.130	0.120
24	0.155	0.095
27	0.180	0.070
30	0.225	0.025

Calculation of the logit for the 21 inch-pounds of pressure would be:

$$logit = ln \frac{\pi_{i1}}{\pi_{i2}}$$

$$logit(21) = ln \frac{0.130}{0.120} = ln(1.083) = 0.080$$

The logit for 30 inch-pounds would be:

$$logit(30) = ln \frac{0.225}{0.025} = ln(9.000) = 2.197$$

The results for all the logit calculations would be:

Torque (inch-pounds):	Success (<2000)	Failure (≥2000)	Logit
21	26	24	0.080
24	31	19	0.490
27	36	14	0.944
30	45	5	2.197

Based on the data available, it appears that there is an increasing likelihood of success as the torque increases during the sealing process.

4. Comparison of survival rated based on nutritional status during a cholera outbreak. This is retrospective data; therefore an odds ratio and confidence interval can be calculated.

Odds of surviving with good nutrition: a/c = 79/32 = 2.469
Odds of surviving with poor nutrition: b/d = 72/87 = 0.828
Odds ratio for nutritional status:

$$OR = \frac{a/c}{b/d} = \frac{2.469}{0.828} = 2.982$$

Thus, children with a good nutritional status were almost three times more likely to survive. The 95% confidence interval for the odds ratio is:

$$Ln_{(OR)} = ln(2.982) = 1.093$$

$$\hat{\sigma}_{ln(OR)} = \sqrt{\frac{1}{79} + \frac{1}{72} + \frac{1}{32} + \frac{1}{87}} = 0.263$$

$$ln\theta = ln_{(OR)} \pm Z_{1-\alpha/2}(\hat{\sigma}_{ln(OR)}) = 1.093 \pm (1.96)(0.263)$$

$$0.578 < ln\,\theta < 2.186$$

$$\theta = e^{ln\,\theta}$$

$$1.782 < \theta < 8.900$$

The odds ratio is significant since one does not fall within the interval and the odds of surviving with good nutrition is between 1.78 and 8.90 times greater than with poor nutrition.

Chapter 19 - Evidence-Based Practice: An Introduction

1. Sensitivity, specificity, and probability of a false negative result for a trial urine pregnancy test.

		Study Volunteers: Pregnant	Study Volunteers: Not Pregnant	
Test Results for Pregnancy	Positive	73	5	78
	Negative	2	70	72
		75	75	150

$$Sensitivity = \frac{a}{a+c} = \frac{73}{75} = 0.973$$

$$Specificity = \frac{d}{b+d} = \frac{70}{75} = .933$$

2. Probability of colorectal cancer in a 52-year-old male having a positive result on a fecal occult blood test.
   a. Bayesian approach
   Based on the information provided we know the prevalence (preprobability) and the probability of not having the disease:

$$p(D) = 0.0087 \qquad and \qquad p(\overline{D}) = 0.9913$$

   Additional information is available about true positives (sensitivity) and true negatives (specificity)

$$Sensitivity = p(T|D) = 0.52$$

$$Specificity = p(\overline{T}|\overline{D}) = 0.91$$

   The probabilities of false positive and false negative results:

$$p(\overline{T}|D) = 0.48 \quad and \quad p(T|\overline{D}) = 0.09$$

   The post probability of the disease given a positive test results is:

$$PVP = p(D|T) = \frac{p(T|D)p(D)}{p(T|D)p(D) + p(T|\overline{D})p(\overline{D})}$$

$$PVP = \frac{(0.52)(0.0087)}{(0.52)(0.0087) + (0.09)(0.9913)} = \frac{0.004524}{0.093741} = 0.0483$$

   The post probability of not having the disease given a negative test result is:

$$PVN = p(\overline{D}|\overline{T}) = \frac{p(\overline{T}|\overline{D})p(\overline{D})}{p(\overline{T}|\overline{D})p(\overline{D}) + p(\overline{T}|D)p(D)}$$

$$PVN = \frac{(0.91)(0.9913)}{(0.91)(0.9913) + (0.48)(0.0087)} = \frac{0.902083}{0.906259} = 0.9954$$

   b. Frequentist approach

$$prevalence = p(D) = 0.0087$$

$$LR^{+} = \frac{Sensitivity}{1 - Specificity} = \frac{0.52}{0.09} = 5.7778$$

$$odds_{pre} = \frac{p}{1-p} = \frac{0.0087}{0.9913} = 0.00878$$

$$odds_{post} = (odds_{pre})(LR^{+}) = (0.00878)(5.7778) = 0.0507$$

$$posttest\ probability = \frac{odds_{post}}{odds_{post} + 1} = \frac{0.0507}{1.0507} = 0.0483$$

$$LR^{-} = \frac{1 - Sensitivity}{Specificity} = \frac{0.48}{0.91} = 0.5275$$

$$odds(-)_{pre} = \frac{p}{1-p} = \frac{0.9913}{0.0087} = 113.8425$$

$$odds(-)_{post} = \frac{odds(-)_{pre}}{LR^{-}} = \frac{113.8425}{0.5275} = 216.0047$$

$$posttest\ \ probabilit\, y(-) = \frac{odds(-)_{post}}{odds(-)_{post} + 1} = \frac{216.0047}{217.0047} = 0.9954$$

3. Based on the information provided we know the following:

   $p(D)$ = prevalence = 0.15 ; the complement $p(\overline{D})$ = 0.85

   $p(T|D)$ = sensitivity = 0.75; the complement $p(\overline{T}|D)$ = 0.25

   $p(\overline{T}|\overline{D})$ = specificity = 0.80; the complement $p(T|\overline{D})$ = 0.20

   Using Bayes' theorem the probability of developing the disease given a positive test or the predictive value positive is:

$$PVP = \frac{(sensitivity)(prevalence)}{[(sensitivity)(prevalence)] + [(1-specificity)(1-prevalence)]}$$

$$PVP = \frac{(0.75)(0.15)}{(0.75)(0.15) + (0.20)(0.85)} = \frac{0.1125}{0.2825} = 0.3982$$

   Alternatively, the probability of not developing the disease given a negative test, or the predictive value negative, is:

$$PVN = \frac{(specificity)(1-prevalance)}{[(specificity)(1-prevalence)] + [(1-sensitivity)(prevalence)]}$$

$$PVN = \frac{(0.80)(0.85)}{(0.80)(0.85) + (0.25)(0.15)} = \frac{0.6800}{0.7175} = 0.9477$$

   Using the pretest probability of having the disease (0.15) and the LR^{+} the posttest probability would be:

$$LR^{+} = \frac{Sensitivity}{1-Specificity} = \frac{0.75}{0.20} = 3.75$$

$$odds_{pre} = \frac{p}{1-p} = \frac{0.15}{0.85} = 0.1765$$

$$odds_{post} = (odds_{pre})(LR^{+}) = (0.01765)(3.75) = 0.6619$$

$$posttest\ \ probabilit\, y = \frac{odds_{post}}{odds_{post} + 1} = \frac{0.6619}{1.6619} = 0.3982$$

   Using the pretest probability of not having the disease (0.85) and the LR^{-} the posttest probability would be:

$$LR^{-} = \frac{1-Sensitivity}{Specificity} = \frac{0.25}{0.80} = 0.3125$$

$$odds(-)_{pre} = \frac{p}{1-p} = \frac{0.85}{0.15} = 5.6667$$

$$odds(-)_{post} = \frac{odds(-)_{pre}}{LR^{-}} = \frac{5.6667}{0.3125} = 18.1334$$

$$posttest\ \ probabilit\,y(-) = \frac{odds(-)_{post}}{odds(-)_{post}+1} = \frac{18.1334}{19.1334} = 0.9477$$

Chapter 20 – Survival Statistics

1. Listed in Table D.10 and Table D.11 are the results from calculating the survival function and confidence interval using both the actuarial and Kaplan-Meier methods. Below are presented the actual calculations for test results 150 times to failure.
   Actuarial method:

$$n_i' = n_i - \frac{w_i}{2} = 83 - \frac{0}{2} = 83$$

$$q_i = \frac{d_i}{n_i'} = \frac{9}{83} = 0.108$$

$$p_i = 1 - q_i = 1 - 0.108 = 0.892$$

$$\hat{S}_i = \Pi(p_i) = (1.000 \cdot 0.989 \cdot \ldots \cdot 0.892) = 0.822$$

$$SE(\hat{S}_i) = \hat{S}_i \sqrt{\sum \frac{q_i}{n_i'(p_i)}}$$

$$SE(\hat{S}_i) = 0.822\sqrt{\frac{0}{90(1.000)} + \frac{0.011}{90(0.989)} + \ldots + \frac{0.108}{83(0.892)}} = 0.040$$

Table D.10 Actuarial Method for Determining $\hat{S}_i$ for Container Failures

Max. Times	n_i	d_i	w_i	q_i	p_i	s_i	SE (s_i)	Confidence Limits Lower	Confidence Limits Upper
25	90	0	0	0.000	1.000	1.000	0.000	1.000	1.000
50	90	1	0	0.011	0.989	0.989	0.011	0.967	1.000
75	89	1	0	0.011	0.989	0.978	0.016	0.947	1.000
100	88	2	0	0.023	0.977	0.956	0.022	0.913	0.998
125	86	3	0	0.035	0.965	0.922	0.028	0.867	0.978
150	83	9	0	0.108	0.892	0.822	0.040	0.744	0.900
175	74	16	0	0.216	0.784	0.644	0.051	0.546	0.743
200	58	36	22	0.766	0.234	0.151	0.042	0.070	0.232

$$S_i = \hat{S}_i \pm z_{1-\alpha/2} \cdot SE(\hat{S}_i) = 0.822 \pm 1.96(0.040)$$
$$0.744 < S_i < 0.900$$

Kaplan-Meier:

$$n_i = n_{i-1}' - w_i = 75 - 0 = 75$$
$$n_i' = n_i - d_i = 75 - 1 = 74$$
$$p_i = \frac{n_i'}{n_i} = \frac{74}{75} = 0.987$$
$$\hat{S}_i = \Pi(p_i) = (0.989 \cdot 0.989 \cdot \ldots \cdot 0.987) = 0.822$$
$$SE(\hat{S}_i) = \hat{S}_i \sqrt{\sum \frac{d_i}{n_i(n_i - d_i)}}$$
$$SE(\hat{S}_i) = \sqrt{\frac{1}{90(90-1)} + \frac{1}{89(89-1)} + \ldots + \frac{1}{75(75-1)}} = 0.040$$
$$S_i = \hat{S}_i \pm z_{1-\alpha/2} \cdot SE(\hat{S}_i) = 0.822 \pm 1.96(0.040)$$
$$0.744 < S_i < 0.900$$

The median survival function would be the point on the curve where it crosses 0.5. Looking at Tables D.10 and D.11, the first times point below 0.5 would be:

Actuarial method median survival = 200 times

Kaplan-Meier median survival = 186 times

Figure D.10 presents a graphic representation of the survival curve for the actuarial method. Kaplan-Meier method would product a similar curve, but with 48 intervals instead of just eight from the actuarial method.

2. Comparison on time-to-event comparing two antibiotics.

Hypotheses: H_0: Time-to-event (antibiotic A) = Time-to-event (antibiotic B)

H_1: Time-to-event (antibiotic A) ≠ Time-to-event (antibiotic B)

Decision rule: With $\alpha = 0.05$, reject H_0, if $z > z_{(1-\alpha/2)} = 1.96$ or $z < -1.96$ or if $\chi^2_{CMH} > \chi_1^2 = 3.84$.

Calculations (log-rank test):

Table D.12 contains the calculations for each U_L and S_{U_L}:

$$U_L = \sum (a_i - e_i) = -3.555$$
$$S_{U_L} = \sqrt{\sum \frac{(a_i + c_i)(b_i + d_i)(a_i + b_i)[n_i - (a_i + b_i)]}{n_i^2 (n_i - 1)}}$$
$$S_{U_L} = \sqrt{7.904} = 2.811$$
$$z = \frac{U_L}{S_{U_L}} = \frac{-3.555}{2.811} = -1.265$$

Decision: With z greater than −1.96, fail to reject the null hypothesis and assume there is no difference in the time to discharge between antibiotics A and B.

Table D.11 Kaplan-Meier Method for Determining $\hat{S}_i$ for Container Failures

Max. Time	n_{i-1}'	w_i	n_i	d_i	n_i'	p_i	s_i	SE (s_i)	Confidence Limits Lower	Confidence Limits Upper
36	90	0	90	1	89	0.989	0.989	0.011	0.967	1.000
65	89	0	89	1	88	0.989	0.978	0.016	0.947	1.000
81	88	0	88	1	87	0.989	0.967	0.019	0.930	1.000
97	87	0	87	1	86	0.989	0.956	0.022	0.913	0.998
107	86	0	86	1	85	0.988	0.944	0.024	0.897	0.992
115	85	0	85	1	84	0.988	0.933	0.026	0.882	0.985
121	84	0	84	1	83	0.988	0.922	0.028	0.867	0.978
128	83	0	83	1	82	0.988	0.911	0.030	0.852	0.970
132	82	0	82	1	81	0.988	0.900	0.032	0.838	0.962
134	81	0	81	1	80	0.988	0.889	0.033	0.824	0.954
136	80	0	80	1	79	0.988	0.878	0.035	0.810	0.945
139	79	0	79	1	78	0.987	0.867	0.036	0.796	0.937
142	78	0	78	1	77	0.987	0.856	0.037	0.783	0.928
146	77	0	77	1	76	0.987	0.844	0.038	0.770	0.919
148	76	0	76	1	75	0.987	0.833	0.039	0.756	0.910
150	75	0	75	1	74	0.987	0.822	0.040	0.744	0.900
154	74	0	74	2	72	0.973	0.800	0.042	0.717	0.883
156	72	0	72	1	71	0.986	0.789	0.043	0.705	0.873
157	71	0	71	1	70	0.985	0.778	0.044	0.692	0.864
159	70	0	70	2	68	0.971	0.756	0.045	0.667	0.844
162	68	0	68	2	66	0.971	0.733	0.047	0.642	0.825
163	66	0	66	1	65	0.985	0.722	0.047	0.628	0.815
165	65	0	65	1	64	0.985	0.711	0.048	0.618	0.805
166	64	0	64	1	63	0.984	0.700	0.048	0.605	0.795
169	63	0	63	1	62	0.984	0.689	0.049	0.593	0.785
172	62	0	62	2	60	0.968	0.667	0.050	0.569	0.764
174	60	0	60	1	59	0.983	0.656	0.050	0.557	0.754
175	59	0	59	1	58	0.983	0.644	0.051	0.546	0.743
178	58	0	58	1	57	0.983	0.633	0.051	0.534	0.733
179	57	0	57	1	56	0.983	0.622	0.051	0.522	0.722
180	56	0	56	2	54	0.964	0.600	0.052	0.499	0.701
181	54	0	54	1	53	0.982	0.589	0.052	0.487	0.691
182	53	0	53	3	50	0.943	0.556	0.052	0.453	0.658
184	50	0	50	1	49	0.980	0.544	0.053	0.442	0.647
185	49	0	49	3	46	0.939	0.511	0.053	0.408	0.614
186	46	0	46	2	44	0.957	0.489	0.053	0.386	0.592
187	44	0	44	2	42	0.955	0.467	0.053	0.364	0.570

continued

Table D.11 Kaplan-Meier Method for Determining $\hat{S}_i$ for Container Failures (continued)

Max. Time	n_{i-1}'	w_i	n_i	d_i	n_i'	p_i	s_i	SE (s_i)	Confidence Limits Lower	Upper
189	42	0	42	1	41	0.976	0.456	0.053	0.353	0.558
190	41	0	41	3	38	0.927	0.422	0.052	0.321	0.524
191	38	0	38	2	36	0.947	0.400	0.052	0.299	0.501
192	36	0	36	1	35	0.972	0.389	0.051	0.288	0.490
193	35	0	35	2	33	0.943	0.367	0.051	0.267	0.466
194	33	0	33	3	30	0.909	0.333	0.050	0.236	0.431
195	30	0	30	2	28	0.933	0.311	0.049	0.216	0.407
196	28	0	28	1	27	0.964	0.300	0.048	0.205	0.395
197	27	0	27	1	26	0.963	0.289	0.048	0.195	0.383
198	26	0	26	3	23	0.885	0.256	0.046	0.165	0.346
200	23	22	1	1	0	0.000	0.000	…	…	…

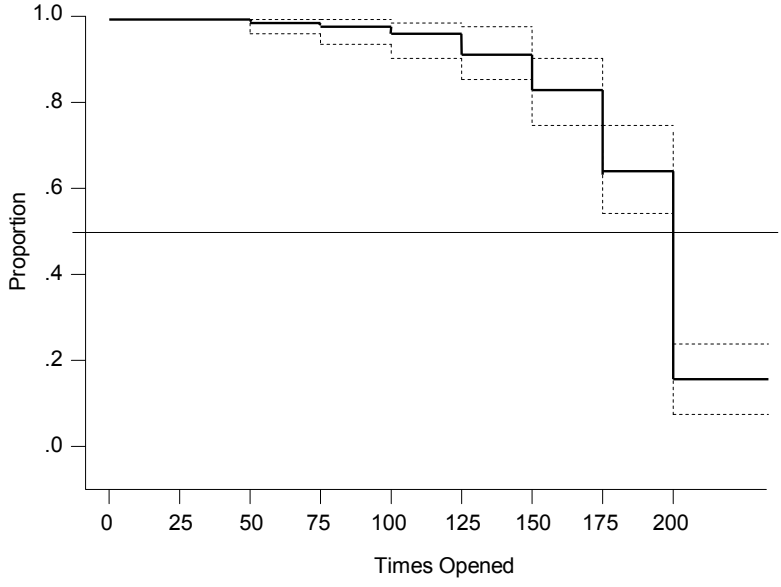


Figure D.20 Actuarial curve with 95% confidence bands for example problem.

Calculations (Cochran-Mantel-Haenszel test):

Table D.12 contains the calculations for each e_i and v_i:

$$e_i = \frac{(a_i + b_i)(a_i + c_i)}{n_i}$$

$$\sum (a_i - e_i) = -3.555$$

$$v_i = \frac{(a_i + b_i)(c_i + d_i)(a_i + c_i)(b_i + d_i)}{n_i^2 (n_i - 1)}$$

$$\sum v_i = 7.935$$

The calculation of the Cochran-Mantel-Haenszel chi square is as follows:

$$\chi^2_{CMH} = \frac{[\Sigma(a_i - e_i)]^2}{\Sigma v_i}$$

$$\chi^2_{CMH} = \frac{(-3.555)^2}{7.935} = 1.593$$

Decision: With χ^2_{CMH} less than 3.84, fail to reject the null hypothesis and assume there is no difference in the time to discharge between antibiotics A and B.

3. Study of two treatment approaches for stage III prostate cancer.
   Test: Kaplan-Meier
   Results: Figure D.21 and Figure D.22
   Decision: There is a statistically significant difference (Figure D.21) and a visual difference (Figure D.22) between the two treatments with the experimental treatment providing better results.

Chapter 21 - Nonparametrics

1. Comparison of two physical therapy regimens.
   Independent variable: two physical therapy regimens (discrete)
   Dependent variable: percent range of motion (ranked to ordinal scale)
   Statistical test: Mann-Whitney U test and median test
   a. Mann-Whitney U
      Hypotheses: H_0: Samples are from the same population
      H_1: Samples are drawn from different populations
      Decision rule: With $\alpha = 0.05$, reject H_0, if $|z| >$ critical $z_{(0.975)} = 1.96$
      Data: Table D.13
      Calculations:

$$U = n_1 n_2 + \frac{n_1(n_1 + 1)}{2} - \Sigma R_{ij}$$

$$U = (9)(11) + \frac{(9)(10)}{2} - 86.5 = 57.5$$

$$Z = \frac{U - \frac{n_1 n_2}{2}}{\sqrt{\frac{n_1 n_2 \cdot [n_1 + (n_2 + 1)]}{12}}}$$

Table D.12 Comparison of Two Antibiotics and Time-to-Event (Discharge)

Event (Hours)	a_i	b_i	c_i	d_i	n_i	e_i	$a_i - e_i$	v_i	SLU Int*
42	1	0	19	20	40	0.500	–0.500	0.250	0.250
43	0	1	19	19	39	0.487	0.513	0.250	0.250
57	1	0	18	19	38	0.500	–0.500	0.250	0.250
63	1	0	17	19	37	0.486	–0.486	0.250	0.250
65	0	1	17	18	36	0.472	0.528	0.249	0.249
88	0	2	17	16	35	0.971	1.029	0.485	0.485
90	0	1	17	15	33	0.515	0.485	0.250	0.250
92	0	1	17	14	32	0.531	0.469	0.249	0.249
98	1	0	16	14	31	0.548	–0.548	0.248	0.248
105	1	0	14	14	29	0.517	–0.517	0.250	0.250
106	0	1	14	13	28	0.500	0.500	0.250	0.250
108	0	1	14	12	27	0.519	0.481	0.250	0.250
112	0	1	14	11	26	0.538	0.462	0.249	0.249
116	0	1	14	9	24	0.583	0.417	0.243	0.243
120	0	1	14	8	23	0.609	0.391	0.238	0.238
127	0	1	13	8	22	0.591	0.409	0.242	0.242
130	0	1	13	7	21	0.619	0.381	0.236	0.236
132	3	0	10	7	20	1.950	–1.950	0.611	0.611
133	3	1	7	6	17	2.353	–1.353	0.787	0.787
135	0	1	7	5	13	0.538	0.462	0.249	0.249
139	1	0	7	4	12	0.667	–0.667	0.222	0.222
140	1	0	6	4	11	0.636	–0.636	0.231	0.231
146	0	1	6	2	9	0.667	0.333	0.222	0.222
161	1	0	6	2	8	0.875	–0.875	0.250	0.219
165	0	1	5	1	7	0.714	0.286	0.204	0.204
180	2	0	3	1	6	1.667	–1.667	0.222	0.222
195	2	0	1	1	4	1.500	–1.500	0.250	0.250
203	0	1	1	0	2	0.500	0.500	0.250	0.250
					$\Sigma =$	21.555	–3.555	7.935	7.904

* SLU intermediate = $[(a_i + c_i)(b_i + d_i)(a_i + b_i)(n_i - (a_i + b_i)]/[n_i^2(n_i - 1)]$.

$$Z = \frac{57.5 - \frac{(9)(11)}{2}}{\sqrt{\frac{(9)(11)\cdot[9+12)]}{12}}} = \frac{57.5 - 49.5}{13.16} = 0.61$$

Decision: With $z < 1.96$, fail to reject H_0 and fail to show a significant difference between the two types of physical therapy.

Distribution Analysis: Weeks by Treatment

Comparison of Survival Curves

Test Statistics

Method	Chi-Square	DF	P-Value
Log-Rank	8.79713	1	0.003
Wilcoxon	9.35384	1	0.002

Figure D.20 Survival comparisons for Problem 3, Chapter 20.

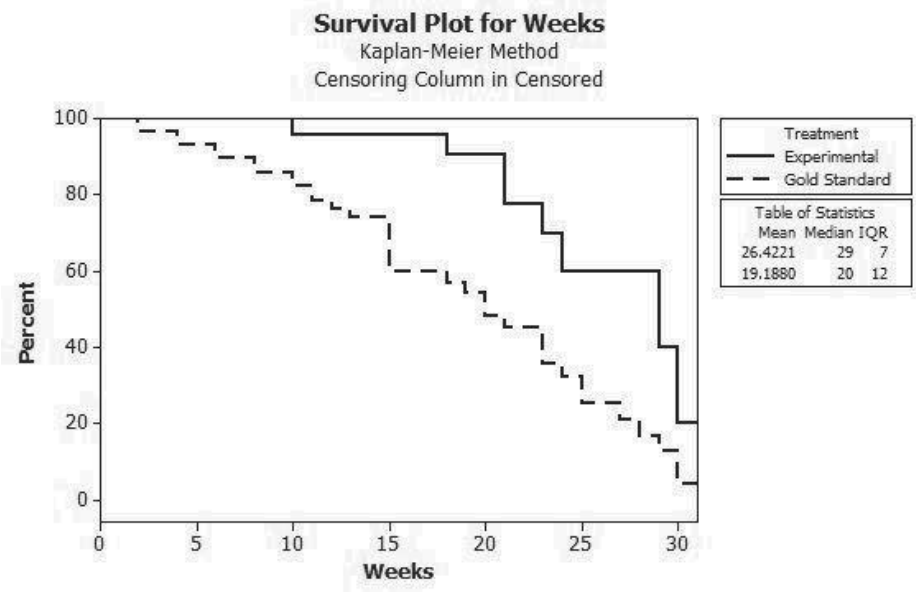


Figure D.21 Survival curves for Problem 3, Chapter 20.

b. Median test
Median for all the values in both groups:

$$Median = \frac{84+86}{2} = 85$$

	Group 1	Group 2
Above the median	4	6
Below the median	5	5

$$p = \frac{(a+b)!\,(c+d)!\,(a+c)!\,(b+d)!}{n!\,a!\,b!\,c!\,d!}$$

Table D.13 Data and Ranking Associated with Comparison of Two Groups of Physical Therapy Patients

Group 1	Ranks	Group 2	Ranks
78	5	75	3.5
87	13.5	88	15.5
75	3.5	93	20
88	15.5	86	11.5
91	18.5	84	10
82	9	71	2
87	13.5	91	18.5
65	1	79	6
80	7	81	8
		86	11.5
		89	17
∑R =	86.5	∑R =	123.5

$$p = \frac{10!\,10!\,9!\,11!}{20!\,4!\,6!\,5!\,5!} = \frac{3969}{12597} = 0.315$$

Decision: With $p > 0.05$, fail to reject H_0 and fail to show a significant difference between the two types of physical therapy.

2. Comparison of the analytical results of a newly trained and senior chemist.
Independent variable: two time periods (each sample serves as own control)
Dependent variable: assay results (ranked to ordinal scale)

Table D.14 Data and Ranking Associated with Comparison of Two Chemists for the Wilcoxon Matched-Pairs Test

Sample Batch	New Chemist	Senior Chemist	d	Rank d	Rank Associated with Least Frequent Sign
A,42	99.8	99.9	0.1	1.5	
A,43	99.6	99.8	0.2	4	
A,44	101.5	100.7	–0.8	9.5	9.5
B,96	99.5	100.1	0.6	8	
B,97	99.2	98.9	–0.3	6.5	6.5
C,112	100.8	101.0	0.2	4	
C,113	98.7	97.9	–0.8	9.5	9.5
D,21	100.1	99.9	–0.2	4	4
D,22	99.0	99.3	0.3	6.5	
D,23	99.1	99.2	0.1	1.5	
					T = ∑ = 29.5

Test statistic: Wilcoxon matched-pairs test, sign test, or Friedman two-way analysis of variance

Hypotheses: H_0: No difference between the two chemists
H_1: Difference exists between the two chemists

a. Wilcoxon matched-pairs test
Decision rule: With $\alpha = 0.05$, reject H_0 if $|z| > 1.96$.
Data: Table D.14
Calculations:

$$E(T) = \frac{n(n+1)}{4} = \frac{(10)(11)}{4} = 27.5$$

$$Z = \frac{T - E(T)}{\sqrt{\frac{n(n+1)(2n+1)}{24}}}$$

$$Z = \frac{29.5 - 27.5}{\sqrt{\frac{10(11)(21)}{24}}} = \frac{2}{\sqrt{96.25}} = 0.20$$

Decision: Using the Wilcoxon matched-pairs test, the result is $z < 1.96$. Thus we fail to reject H_0 and fail to show a significant difference in the assay results for the two scientists.

b. Sign test
H_0: $p(+) = 0.50$
H_1: $p(+) \neq 0.50$
Using the data presented in Table D21.2, number of negative results = 6

Table D.15 Data and Ranking Associated with Comparison of Two Chemists for the Friedman Two-Way Analysis of Variance

	New Chemist		Senior Chemist	
Sample, Batch	Data	Rank	Data	Rank
A,42	99.8	1	99.9	2
A,43	99.6	1	99.8	2
A,44	101.5	2	100.7	1
B,96	99.5	1	100.1	2
B,97	99.2	2	98.9	1
C,112	100.8	1	101.0	2
C,113	98.7	2	97.9	1
D,21	100.1	2	99.9	1
D,22	99.0	1	99.3	2
D,23	99.1	1	99.2	2
Σ =		14		16

Total number of events = 10

$$p(x) = \binom{n}{x} p^x q^{n-x}$$

$$p(6\ positives) = \binom{10}{6}(0.50)^6(0.50)^4 = 0.205$$

$$p(7\ positives) = \binom{10}{7}(0.50)^7(0.50)^3 = 0.117$$

$$p(8\ positives) = \binom{10}{8}(0.50)^8(0.50)^2 = 0.044$$

$$p(9\ positives) = \binom{10}{9}(0.50)^9(0.50)^1 = 0.0098$$

$$p(10\ positives) = \binom{10}{10}(0.50)^{10}(0.50)^0 = 0.00098$$

$$p(\geq 6\ positives) = \sum = 0.377$$

Fail to reject H_0 because $p > 0.05$.

c. Friedman two-way analysis of variance, data (Table D.15)
Decision rule: With $\alpha = 0.05$, reject $\chi_r^2 > \chi_1^2 = 3.84$.
Calculations:

$$\chi_r^2 = \frac{12}{nk(k+1)} \sum (R_j)^2 - 3n(k+1)$$

$$\chi_r^2 = \frac{12}{10(2)(3)}[(14)^2 + (16)^2] - 3(10)(3)$$

$$\chi_r^2 = (0.02)(452) - 90 = 0.40$$

Decision: Using the Friedman two-way analysis of variance, the result is a $\chi_1^2 < 3.84$. Thus we fail to reject H_0 and fail to show a significant difference in the assay results for the two scientists.

Table D.16 Absorption of Ultraviolet Light

Sample A	Rank A	Sample B	Rank B	Sample C	Rank C
7.256	8	7.227	2	7.287	15
7.237	4	7.240	5	7.288	14
7.229	3	7.257	9	7.271	12
7.245	7	7.241	6	7.269	11
7.223	1	7.267	10	7.282	13
Sums =	23		32		65

3. Comparison of ultraviolet data for three different samples.
   Independent variable: samples (discrete, 3 levels)
   Dependent variable: ultraviolet data (based on continuous scale)
   Statistical test: Kruskal-Wallis
   Hypothesis:
   H_0: All three samples are from the same population
   H_1: All three samples are not from the same population
   Decision rule: With $\alpha = 0.05$, reject H_0 if $H > \chi^2_2(0.95) = 5.99$.
   Data: Table D.16
   Calculations:

$$H = \frac{12}{N(N+1)}\left[\sum \frac{(\sum R_{ij})^2}{n_j}\right] - 3(N+1)$$

$$H = \frac{15}{15(16)}\left[\frac{(23)^2}{5} + \frac{(32)^2}{5} + \frac{(65)^2}{5}\right] - 3(16)$$

$$H = 0.0625(105.8 + 204.8 + 845.0) - 48 = 24.225$$

   Decision: With H > 5.99, reject H_0 and conclude that there is a significant difference among the three samples and that they are not from the same population.

Post hoc comparison for location(s) of significant difference(s):
Comparison of Samples A and B:

$$q = \frac{R_A - R_B}{\sqrt{\frac{n(nk)(nk+1)}{12}}}$$

Table D.17 Data and Ranking Associated with Comparison of Two Methods Using the Spearman *Rho* Test

	Method A		Method B			
Sample	x	Rank	y	Rank	d	d^2
1	66	6	67	6	0	0
2	77	10	75	10	0	0
3	57	3.5	57	3	–0.5	0.25
4	59	5	59	4.5	–0.5	0.25
5	70	8	69	8	0	0
6	57	3.5	59	4.5	+1	1.00
7	55	2	56	2	0	0
8	53	1	51	1	0	0
9	67	7	68	7	0	0
10	72	9	74	9	0	0
					$\sum$ =	1.50

$$q = \frac{23 - 33}{\sqrt{\frac{5(5 \cdot 3)[(5 \cdot 3) + 1]}{12}}} = \frac{-10}{10} = -1.0$$

Results for all three Kruskal-Wallis *post hoc* comparisons:

Pairing	q-statistic	Critical Value	Results
$R_A - R_B$	–1.0	3.73	
$R_A - R_C$	–4.1	3.73	Significant
$R_B - R_C$	–3.1	3.73	

Decision: The only significant difference was between Samples A and C.

4. Comparison of results from two analytical methods.
   Independent variable: Continuous (Method A)
   Dependent variable: Continuous (Method B)
   Statistical test: Spearman *rho* correlation
   Data: Table D.17
   Computation:

$$\rho = 1 - \frac{6(\sum d^2)}{n^3 - n}$$

$$\rho = 1 - \frac{6(9)}{10^3 - 10} = 1 - \frac{54}{990} = 0.945$$

   Decision: There is a very strong correlation between the two analytical methods. Plotting the data would show that the correlation is positive.

5. Comparison of various doses of an anticoagulant and prothrombin times.
   Independent variable: dosages (continuous)
   Dependent variable: prothrombin times (continuous)
   Statistical test: Theil's incomplete method

Subject	Dose (mg)	Prothrombin Time (seconds)
2(1)	180	18
3(2)	190	19
1(3)	200	20
5(4)	210	19
4(5)	220	21
6(6)	230	20

   Calculate the median slope:

$$b_{ij} = \frac{(y_j - y_i)}{(x_j - x_i)}$$

$$b_{14} = \frac{19 - 18}{210 - 180} = \frac{1}{30} = 0.033$$

$$b_{25} = \frac{21-19}{220-190} = \frac{2}{30} = 0.067$$

$$b_{36} = \frac{20-20}{230-200} = \frac{0}{30} = 0$$

$$Median\ b = 0.033$$

Calculation of the median intercept:

$$a = y - bx$$

$$a_1 = 18 - (0.033)(180) = 12.06$$

$$a_2 = 19 - (0.033)(190) = 12.73$$

$$a_3 = 20 - (0.033)(200) = 13.40$$

$$a_4 = 19 - (0.033)(210) = 12.07$$

$$a_5 = 21 - (0.033)(220) = 13.47$$

$$a_6 = 20 - (0.033)(230) = 12.41$$

$$Median\ \ intercept\ (a) = \frac{(12.41) + (12.73)}{2} = 12.57$$

Line of best fit:

$$y = a + bx = 12.57 + 0.033x$$

6. Runs test to determine if random sampling of volunteers was successful (Table 21.12). The volunteers will be recoded as E (experimental) and C (control) base on the sequence in which they volunteered for the study.

CECCEECCECCECCEECEECCEEEEEECCC

Hypotheses: H_0: The pattern of occurrence is determined by a random process

H_1: The pattern of occurrences is not random

Decision rule: Table B18, with $n_1 = 15$ and $n_2 = 15$, reject H_0 if r is < 11 or > 21.

Spacing the sequence in runs, there are 15 runs.

C E CC EE CC E CC E CC EE C EE CC EEEEEE CCC

Therefore, we fail to reject H_0 and assume that the randomization process was successful

Wilcoxon Signed Rank Test: Delta

```
Test of median = 0.000000 versus median not = 0.000000

           N for   Wilcoxon         Estimated
      N    Test  Statistic      P     Median
Delta 12     10       44.5  0.093     0.1000
```

Figure D.23 Minitab outcome for repeat of Problem 2, Chapter 9.

7. Clinical trial to evaluate the effectiveness of a new bronchodilator.
   Independent variable: two time periods (patient serves as own control)
   Dependent variable: forced expiratory volume (continuous)
   Test: Wilcoxon signed rank test on the difference for each person (delta)
   Results: Figure D.23
   Decision: Similar results as the paired t-test, fail to reject H_0. With the paired t-test $p = 0.108$.

8. Comparison of results from a contract laboratory and manufacturer's quality control laboratory.
   Independent variable: manufacturer versus contract laboratory (discrete)
   Dependent variable: assay results (continuous)
   Statistical test: Mann-Whitney test
   Results: Figure D.24
   Decision: Similar results tovhe two-sample t-test, fail to reject H_0. With the two-sample t-test $p = 0.237$.

9. Comparison of a raw material at three different production sites.
   Independent variable: production site (discrete, 3 levels)
   Dependent variable: oil viscosity (continuous)
   Statistical test: Kruskal-Wallis Test
   Results: Figure D.25
   Decision: Similar results to the one-way ANOVA, reject H_0 and conclude there is a difference in the viscosity from the different facilities. With the one-way ANOVA $p = 0.013$.

10. Comparison of two analytical procedures on different concentrations of a drug.
    Variables: continuous (two measurement scales)
    Statistical test: Spearman *rho* test
    Results: Figure D.26
    Decision: Similar results to the correlation coefficient, a very strong correlation between methods GS and ALT.

Mann-Whitney Test and CI: Manufacturer, Contract Lab

```
               N  Median
Manufacturer   6  99.800
Contract Lab   6  98.900


Point estimate for ETA1-ETA2 is 1.150
95.5 Percent CI for ETA1-ETA2 is (-0.499,2.801)
W = 45.0
Test of ETA1 = ETA2 vs ETA1 not = ETA2 is significant at 0.3785
The test is significant at 0.3768 (adjusted for ties)
```

Figure D.24 Minitab outcome for repeat of Problem 4, Chapter 9.

Kruskal-Wallis Test: Viscosity versus Batch

Kruskal-Wallis Test on Viscosity

Batch	N	Median	Ave Rank	Z
A	5	10.28	11.9	2.39
B	5	10.24	7.5	-0.31
C	5	10.21	4.6	-2.08
Overall	15		8.0	

H = 6.76 DF = 2 P = 0.034
H = 6.79 DF = 2 P = 0.034 (adjusted for ties)

Figure D.25 Minitab outcome for repeat of Problem 3, Chapter 10.

Tabulated statistics: GC, ALT

Rows: GC Columns: ALT

Pearson's r	1
Spearman's rho	1

Figure D.26 Minitab outcome for repeat of Problem 3, Chapter 13.

Chapter 22 – Statistical Tests for Equivalence

1. Clinical trial data comparing a new generic product to an innovator's branded drug. Is there less than a 10% difference between the products?

	Innovator	Generic
Mean =	289.7	271.6
Standard Deviation =	18.1	20.4
n =	24	23

10% difference = 28.97 (δ = 289.7 × 0.10)
Difference observed = 18.1 (289.7 – 271.6)
Pooled variance:

$$S_p^2 = \frac{(n_1-1)S_1^2+(n_2-1)S_2^2}{n_1+n_2-2} = \frac{23(18.1)^2+22(20.4)^2}{24+23-2} = 370.9$$

Standard error portion of the equations:

$$\sqrt{\frac{S_P^2}{n_1}+\frac{S_P^2}{n_2}} = \sqrt{\frac{370.9}{24}+\frac{370.9}{23}} = 5.62$$

a. Confidence interval

$$Lower\ Limit = \frac{(d - SE) + \overline{X}_R}{\overline{X}_R} \times 100\%$$

$$Lower\ Limit = \frac{(18.1 - 5.62) + 289.7}{289.7} \times 100\% = 104.3\%$$

$$Upper\ Limit = \frac{(d + SE) + \overline{X}_R}{\overline{X}_R} \times 100\%$$

$$Lower\ Limit = \frac{(18.1 + 5.62) + 289.7}{289.7} \times 100\% = 108.2\%$$

The limits of our estimated interval are:

$$Lower\ Limit < \frac{\mu_T}{\mu_R} < Upper\ Limit$$

$$104.3\% < \frac{\mu_T}{\mu_R} < 108.2\%$$

Therefore, we are 95% confident that we have equivalence because the difference is well within our criteria of ±10% and the true population ratio is somewhere between 104.3 and 108.2%.

b. Two one-tailed t-tests

Hypotheses: $H_{01}: \mu_T - \mu_R \leq 10\%$
$H_{11}: \mu_T - \mu_R > 10\%$
$H_{02}: \mu_T - \mu_R \geq 10\%$
$H_{12}: \mu_T - \mu_R < 10\%$

Decision rule: With $\alpha = 0.05$, reject H_{01} or H_{02} if $|t| > t_{45}(.95) \approx 1.679$

$$t_1 = \frac{(\overline{X}_T - \overline{X}_R) - \delta_1}{\sqrt{\frac{2S_p^2}{n}}} = \frac{18.1 - (-28.97)}{5.62} = 8.38$$

$$t_2 = \frac{\delta_2 - (\overline{X}_T - \overline{X}_R)}{\sqrt{\frac{2S_p^2}{n}}} = \frac{28.97 - 18.1}{5.62} = 1.93$$

Decision: Reject H_{01} and H_{02}, and conclude that the difference between the two populations is less than 10%.

2. To compare Country A and the "standard" original facility, the first step is to calculate the difference term in the denominator:

$$\Sigma(R_t - T_t)^2 = (49.8 - 57.3)^2 \ \ldots\ + (93.6 - 84.6) = 453.95$$

The calculation of the remainder of the f_2 formula is as follows:

$$f_2 = 50 \cdot \log\left[\frac{1}{\sqrt{1+\frac{1}{n}\Sigma(R_t - T_t)^2}} \times 100\right]$$

$$f_2 = 50 \cdot \log\left[\frac{1}{\sqrt{1+\frac{1}{6}(453.95)}} \times 100\right]$$

$$f_2 = 50 \cdot \log(11.421) = 50 \cdot (1.058) = 52.9$$

Decision: With $f_2 > 50$ conclude that the two dissolution profiles are the same and that there is no significant difference between the product produced in Country A and product from the manufacturer's original production site.

To compare Country B and the "standard" original facility, the same process is used:

$$\Sigma(R_t - T_t)^2 = (49.8 - 54.1)^2 \ \ldots \ + (93.6 - 88.8) = 137.56$$

$$f_2 = 50 \cdot \log\left[\frac{1}{\sqrt{1+\frac{1}{6}(137.56)}} \times 100\right]$$

$$f_2 = 50 \cdot \log(20.444) = 50 \cdot (1.311) = 65.55$$

Decision: With $f_2 > 50$ conclude that the two dissolution profiles are the same and that there is no significant difference between the product produced in Country A and product from the manufacturer's original production site.

Note that Country B produced a higher f_2 and that confirms the visual assessment that the dissolution profile for Country B was closer to that of the original product.

Chapter 23 – Outlier Tests

1. Outlier tests to evaluate 12.9:
   a. Rank order of data: 12.0, 12.3, 12.4, 12.5, 12.9
   b. Mean and standard deviation:

Without 12.9:	$\overline{X}$ = 12.3	S = 0.22
With 12.9 included:	$\overline{X}$ = 12.42	S = 0.33

   c. Rule for huge error

$$M = \frac{|x_i - \overline{X}|}{S} = \frac{|12.3 - 12.9|}{0.22} = \frac{0.6}{0.22} = 2.73$$

Decision with 2.73 < 4.00, do not reject 12.9 as an outlier.

d. Grubbs' test – critical value with $n = 5$ and $\alpha = 0.05$ is 1.672.

$$T = \frac{X_n - \overline{X}}{S} = \frac{12.9 - 12.42}{0.33} = \frac{0.48}{0.33} = 1.45$$

Decision with 1.45 < 1.672, do not reject 12.9 as an outlier.

e. Dixon test – with $n = 5$ and $\alpha = 0.05$, critical $\tau = 0.642$.

$$\frac{(x_n - x_{n-1})}{(x_n - x_1)} = \frac{12.9 - 12.5}{12.9 - 12.0} = \frac{0.4}{0.9} = 0.44$$

Decision with 0.44 < 0.642, do not reject 12.9 as an outlier.

f. Hampel's rule

The results for the calculations appear in Table D.18. The median for the five values is 12.4. For 12.9 the AD_i is:

$$AD_i = |x_i - Md| = |12.9 - 12.4| = 0.5$$

The median AD_i is 0.1 and the MAD is 0.1(1.483) = 0.1483. For 12.9 the NAD_i is:

$$NAD_i = \frac{|Md - x_i|}{MAD_i} = \frac{|12.4 - 12.9|}{0.1483} = 3.37$$

With the NAD_i less than 3.5, do not reject 12.9 as an outlier.

2. Outlier tests to determine if 94.673% is an outlier.

a. Rank order of data:

87.765, 88.975, 89.096, 89.204, 89.470, 89.578
89.711, 89.954, 90.122, 91.738, 91.993, 94.673

b. Mean/standard deviation:

Without 94.673:	$\overline{X} = 89.69$	S = 1.07
With 94.673 included:	$\overline{X} = 90.11$	S = 1.77

c. Rule for huge error

$$M = \frac{|x_i - \overline{X}|}{S} = \frac{|89.69 - 94.673|}{1.07} = \frac{4.983}{1.07} = 4.66$$

Decision with 4.66 < 4.00, reject 94.673 as an outlier.

Table D.18 Hampel's Rule Applied to First Example Problem

	Data	Absolute Deviations (AD_i)	Absolute Normalized Deviations (NAD_i)
	12.0	0.4	2.697
	12.3	0.1	0.674
	12.4	0.0	0.000
	12.5	0.1	–0.674
	12.9	0.5	–3.372
	12.0	0.4	2.697
Median =	12.4	0.1	
MAD =		0.1483	

d. Grubbs' test – critical value with $n = 12$ and $\alpha = 0.05$ is 2.27.

$$T = \frac{x_n - \overline{X}}{S} = \frac{94.673 - 90.11}{1.77} = \frac{4.563}{1.77} = 2.58$$

Decision with 2.58 < 2.27, reject 94.673 as an outlier.

e. Dixon test – with $n = 12$ and $\alpha = 0.05$, critical $\tau = 0.546$.

$$\frac{(x_n - x_{n-2})}{(x_n - x_2)} = \frac{94.673 - 90.738}{94.673 - 89.096} = \frac{3.935}{5.577} = 0.705$$

Decision with 0.705 < 0.546, reject 94.673 as an outlier.

f. Hampel's rule

The results for the calculations appear in Table D.19. The median for the twelve values is 89.645%. For 94.673 the AD_i is:

$$AD_i = |x_i - Md| = |94.673 - 89.645| = 5.028$$

The median AD_i is 0.513 and the MAD is 0.513(1.483) = 0.761. For 94.673 the NAD_i is:

$$NAD_i = \frac{|Md - x_i|}{MAD_i} = \frac{|89.645 - 94.673|}{0.761} = 6.610$$

With the NAD_i greater than 3.5, reject 94.673 as an outlier.

3. Evaluation of HPLC analysis to determine if 50% is a possible outlier. Listed below are the results of the typical regression analysis table and the calculated slope and *y*-intercept for all the data, and the data excluding the potential outlier.

Table D.19 Hampel's Rule Applied to Second Example Problem

	Data	Absolute Deviations (AD_i)	Absolute Normalized Deviations (NAD_i)
	89.470	0.175	0.229
	94.673	5.028	6.610
	89.578	0.067	0.087
	89.096	0.549	0.721
	88.975	0.670	0.880
	89.204	0.441	0.579
	87.765	1.880	2.470
	91.993	2.348	3.087
	89.954	0.309	0.407
	90.738	1.093	1.437
	90.122	0.477	0.628
	89.711	0.066	0.087
Median =	89.645	0.513	
MAD =		0.761	

Outcomes:	With the potential outlier included	With the potential outlier excluded
n =	10	9
$\sum x$ =	750	700
$\sum y$ =	742.1	700.1
$\sum x^2$ =	64,500	62,000
$\sum y^2$ =	63,713.21	61,949.21
$\sum xy$ =	64,072	61,972
b =	+1.02	+0.99
a =	–2.29	+0.79

As can be seen, the proposed outlier does affect the slope and intercept point, but is this effect significant and should the 50% response be considered an outlier? Figure D.27 shows a scatter plot for the HPLC data and the line of best fit. The results of the linear regression analysis would be as follows:

Source	SS	df	MS	F
Linear Regression	8583.30	1	8583.30	1170.98
Residual	58.67	8	7.33	
Total	8641.97	9		

The values on the line of best fit can be calculated using the formula $y_c=a+bx$. These values and the residuals associated with the difference between the data (y) and y_c are presented in Table D.20. If the residuals are ranked from the lowest to the highest we find the following:

x	y_i	r	x	y_i	r
50	42.0	–6.71	100	100.1	+0.39
120	119.4	–0.71	40	39.7	+1.19
110	109.7	–0.21	70	70.8	+1.69
90	89.3	–0.21	30	30.4	+2.09
60	59.1	+0.19	80	81.6	+2.29

A box-and-whisker plot can be created with the median of +0.29 (average of fifth and sixth ranks), 25th percentile of –0.21 (third rank) and 75th percentile of +1.69 (eighth rank). In this case the whiskers would extend to –2.56 and +3.14. Clearly the value of –6.71 would be an outlier because it is located beyond the lower whisker. A studentized residuals plot can be created for each HPLC outcome. For example the value at 100% would be:

$$t = \frac{y_i - y_c}{\sqrt{MS_E}} = \frac{100.1 - 99.71}{\sqrt{7.33}} = 0.144$$

Each of the studentized residuals are plotted and the critical t-value is $t_{n-1}(1 - \alpha/2)$, which is $t_9(0.975)$ or 2.26.

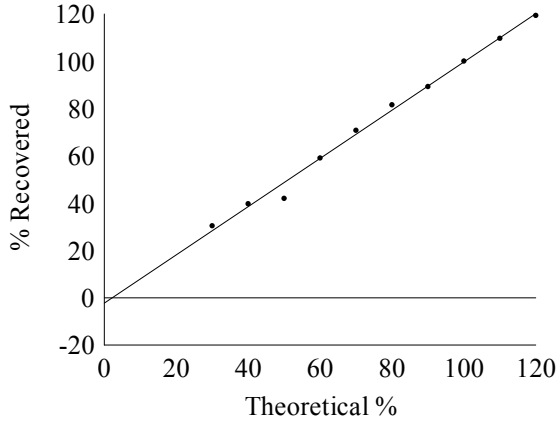


Figure D.27 Scatter plot of HPLC outcomes.

Table D.20 Residuals Presented in Figure D.27

x	y_i	y_c	r
30	30.4	28.31	+2.09
40	39.7	38.51	+1.19
50	42.0	48.71	–6.71
60	59.1	58.91	+0.19
70	70.8	69.11	+1.69
80	81.6	79.31	+2.29
90	89.3	89.51	–0.21
100	100.1	99.71	+0.39
110	109.7	109.91	–0.21
120	119.4	120.11	–0.71
			$\sum = 0.000$

4. Using a box plot (Figure D.28) the value 0.85 appears to be an outlier. Using the tests for outliers, this assumption is proven, with Group 1 having a mean and standard deviation of 3.632 + 0.926 (n = 15) with potential outlier and 3.831 + 0.533 (n = 14) without the potential outlier.
   a. Rule for huge error

$$M = \frac{|x_i - \overline{X}|}{S} = \frac{|3.83 - 0.85|}{0.53} = \frac{2.98}{0.53} = 5.62$$

   Decision with 5.62 < 4.00, reject 0.85 as an outlier.
   b. Grubbs' test – critical value with n = 15 and α = 0.05 is 2.409.

$$T = \frac{\overline{X} - n_1}{S} = \frac{3.63 - 0.85}{0.93} = \frac{2.78}{0.93} = 2.98$$

Decision with 2.98 < 2.409, reject 0.85 as an outlier.

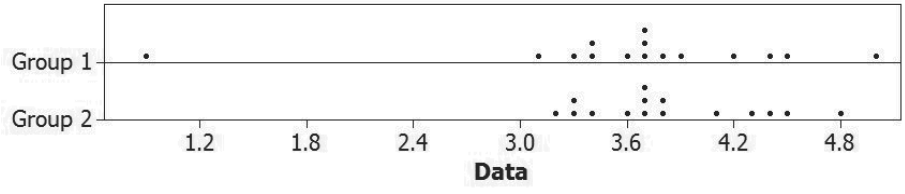


Figure D.28 Dot diagram of data in Problem 4, Chapter 23.

c. Dixon test – with $n = 15$ and $\alpha = 0.05$, critical $\tau = 0.525$.

$$\frac{(x_3 - x_1)}{(x_{n-2} - x_1)} = \frac{3.25 - 0.85}{4.41 - 0.85} = \frac{2.40}{3.56} = 0.674$$

Decision with 0.674 < 0.525, reject 0.85 as an outlier.

However, with the relative large sample size of 30 points total, the result of a two-sample t-test, with or without the outlier would be the same: a failure to reject the null hypotheses ($\mu_1=\mu_2$) where the critical *t*-value is 2.04 with $\alpha = 0.05$.
The mean and standard deviation for Group 2 is 3.828 ± 0.479.
With the outlier:

$$S_p^2 = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2} = \frac{14(0.926)^2 + 14(0.479)^2}{15 + 15 - 2} = \frac{15.217}{28} = 0.543$$

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{S_P^2}{n_1} + \frac{S_P^2}{n_2}}} = \frac{3.632 - 3.828}{\sqrt{\frac{0.543}{15} + \frac{0.543}{15}}} = \frac{-0.196}{0.269} = -0.728$$

Without the outlier:

$$S_p^2 = \frac{(n_1 - 1)S_1^2 + (n_2 - 1)S_2^2}{n_1 + n_2 - 2} = \frac{13(0.533)^2 + 14(0.479)^2}{14 + 15 - 2} = \frac{6.905}{27} = 0.256$$

$$t = \frac{\overline{X}_1 - \overline{X}_2}{\sqrt{\frac{S_P^2}{n_1} + \frac{S_P^2}{n_2}}} = \frac{3.831 - 3.828}{\sqrt{\frac{0.256}{14} + \frac{0.256}{15}}} = \frac{0.003}{0.188} = 0.016$$

In this case it would make no difference if the outlier was removed or not.

Statistics

BASIC STATISTICS AND PHARMACEUTICAL STATISTICAL APPLICATIONS

THIRD EDITION

Building on its best-selling predecessors, **Basic Statistics and Pharmaceutical Statistical Applications, Third Edition** covers statistical topics most relevant to those in the pharmaceutical industry and pharmacy practice. It focuses on the fundamentals required to understand descriptive and inferential statistics for problem solving. Incorporating new material in virtually every chapter, this third edition now provides information on software applications to assist with evaluating data.

New to the Third Edition

- Use of Excel® and Minitab® for performing statistical analysis
- Discussions of nonprobability sampling procedures, determining if data is normally distributed, evaluation of covariances, and testing for precision equivalence
- Expanded sections on regression analysis, chi square tests, tests for trends with ordinal data, and tests related to survival statistics
- Additional nonparametric procedures, including the one-sided sign test, Wilcoxon signed-ranks test, and Mood's median test

With the help of flow charts and tables, the author dispels some of the anxiety associated with using basic statistical tests in the pharmacy profession and helps readers correctly interpret their results using statistical software. Through the text's worked-out examples, readers better understand how the mathematics works, the logic behind many of the equations, and the tests' outcomes.

CRC Press
Taylor & Francis Group
an informa business
www.crcpress.com

6000 Broken Sound Parkway, NW
Suite 300, Boca Raton, FL 33487
711 Third Avenue
New York, NY 10017
2 Park Square, Milton Park
Abingdon, Oxon OX14 4RN, UK

K20792
ISBN: 978-1-4665-9673-3
90000
9 781466 596733