SEROLOGY OF INFECTIOUS DISEASE SYNDROMES

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Virion Edition

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Edwin H. Lennette
Mirko Jung
Franciska Jung


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Virion Edition

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Preface

From its very inception, the laboratory diagnosis of infectious diseases has been based on the isolation and identification of the causal organism, and to this concept there was later added, as the methods became available, immunologic (serologic) support for the diagnosis. Laboratory diagnosis of microbial infections – viral, bacterial, fungal and parasitic – thus came to rest on two firm pillars, namely microbial culture and identification and the immune (generally humoral) response. Over the years these guiding principles, for a variety of reasons, have to some extent been eroded, as reflected in the differences in approach to diagnosis in bacteriology and virology. Thus, because of the relative rapidity and ease with which bacterial agents can be cultivated *in vitro* (usually hours, sometimes days), serology has come to play a subordinate role. Conversely, isolation of viral agents is a slow (days, sometimes weeks) and complicated process, and hence the serologic approach to diagnosis has always played a major role in establishing the cause of a presumed viral illness. Then, too, with an increasing number of immuno-compromised individuals in the population, there has been a blurring of the distinction between pathogen and saprophyte, with the concomitant difficulty of identifying the offending invader.

Unlike chemical tests which can promptly give the clinician answers to the questions he has raised, biological tests are influenced by temporal factors that are difficult or impossible to circumvent. In consequence, diagnostic microbiology has been (and in large part still is) what Tilton (1985) refers to as a »confirmatory science», meaning that laboratory findings or data may be so delayed as to lose some of their relevance to the clinical management of the patient's illness.

Dissatisfaction with the time lag in diagnosis employing the usual or traditional (or even classic) methods has led to searches for new procedures or techniques that are not only more rapid, but also more precise, more sensitive, more specific, highly versatile, more foolproof, cheaper, etc. Witness the recent introduction of enzyme immunoassays, Western blot, DNA probes, the polymerase chain reaction and, on the horizon, the Q-beta replicase reaction.

All of these new methods have their virtues (generally highly touted by the manufacturer) and their failings (mentioned little or not at all), as do the older one. Despite all the glowing encomiums, before we undertake replacement of older methods with the newer, we should know and thoroughly understand the shortcomings, pitfalls and limitations of what we are adopting. Thus, as Tenover (1988) points out, «the goal of DNA technology is to eliminate routine cultures, whether they be bacterial, viral or fungal». Many laboratories are already using probes, as for stool cultures, and while answers might be forthcoming within 48 hours, say, with a *Salmonella* probe, routine cultures still are required to determine the species, the serotype and the antimicrobial sensitivity of the organism. Cultures are required, too, for the detection of other pathogens, for which probes are not available. The question of sensitivity and specificity of DNA probes arises, and we might consider whether we are ready to sound the death knell of the Petri dish and the cell culture tube for a technique that is less sensitive and less specific than microbial cultures (Tenover, 1988). Excellent summaries of the current situation surrounding the newer methods introduced in the recent past are given by Tenover (1988), Ritchie (1986) and Tilton (1985), to whom the reader is referred.

Two diagnostic methods which have won widespread acceptance are immunofluorescence assays and enzyme immunoassays, reflecting part of the drive for more rapid diagnostic procedures. Although aimed primarily at antibody detection, these methods also have, as does probe technology, a long-term objective of replacing cultures methods with direct detection of microbial antigens in clinical specimens. The significance of the presence of such antigens, especially in the case of long-persisting agents (cytomegalovirus, varicella-zoster, herpes simplex, Epstein-Barr virus, and possibly others) is difficult to assess, and points up the potential role of serology in the evaluation of the findings. Negative «rapid detection methods» do not rule out infection with that agent – too little specific microbial antigenic material may be present to permit detection, and amplification of the agent or its antigens may be necessary, which means *in vitro* cultivation or the use of the polymerase chain reaction to multiply the specific genomic sequences for probe identification attempts.

This is not to gainsay the usefulness of a new method, whose sensitivity, specificity and simplicity can be developmentally improved with time, but we should keep in mind that newer is not necessarily better. Thus, there is no good alternative to the complement-fixation test (available commercially) for the identification of adenoviral infections, since this test reacts with a group antigen shared by all of the 40-some human strains of adenovirus. Another example is the adoption of the hemagglutination-inhibition test as a virtual replacement for the CF test for influenza, the latter being group-antigen specific and thus able to detect infection with any type A influenza virus, whereas the former is strain specific and requires the use of test strains antigenically appropriate to the current outbreak.

Analogies exist in the bacterial field. Although antibodies to *Campylobacter jejuni*, an enteric pathogen, are detectable by several methods, the antigenic heterogeneity of the bacterial strains is considered to make serologic diagnosis impractical in sporadic cases (cf Morris and Patton, 1985); in the case of outbreaks, it is recommended (Morris and Patton, 1985) that the diagnostic antigen be prepared from a fresh isolate derived from a patient in that outbreak, thus avoiding the problem of strain heterogeneity. This problem is easier and better avoided in the complement-fixation test, in which an antigen prepared from a single strain (but preferably from a pool of several) will detect antibodies to *C. jejuni* in patients from any geographic area. A multi-strain CF antigen is available commercially, and makes the CF test the only routine diagnostic test available for *C. jejuni* on the biological market.

Another example is the serodiagnosis of *Mycoplasma pneumoniae* infections. The ELISA tests developed so far are of inadequate sensitivity (Kenny, 1985) and since the antigen, like that for the CF test is a lipid extract, false positive reactions are occasionally encountered, chiefly in cases of bacterial meningitis. The CF test for diagnosis of atypical pneumonia has been in use for more than two decades, utilizes a lipid extract antigen, and is of known high sensitivity for the detection of mycoplasmal pneumonias; the nonspecific reactions encountered are few and generally readily differentiated. The procedure is the most generally used method for the serodiagnosis of *M. pneumoniae* infections (Swack, 1988), and is the only serodiagnostic test commercially available for this purpose.

In essence, therefore, the medical microbiologist has at his disposal a variety of diagnostic tests, whose virtues, or lack thereof, are known and so make possible the selection of a procedure(s) suitable for the

purpose at hand, and compatible with the needs of the laboratory.

Finally, why this monograph? Nearly a century has passed since Bordet (1895) enunciated the principles on which the complement-fixation test is based (Bordet 1895, 1898) and Sternberg (1898) introduced the virus neutralization test. Over the intervening years, there has been a considerable accumulation of information on the development of the immune system and on its role in defense against infectious diseases. This information has come from disparate sources, has appeared in a broad range of journals, and is even difficult of access. Forming part of this information pool are facets or data relevant to laboratory diagnosis of microbial infections, and we have tried, in a general way, to retrieve and tie together for the reader those aspects of immunology and microbiology which have a direct bearing on the practice of diagnostic serology.

Introduction

It is generally accepted that the most solid basis for the diagnosis of a microbial infection rests on the isolation and identification of the causal agent, together with assessment of its pathogenetic role in terms of the patient's clinical, serologic and epidemiologic background. However, isolation of the offending agent may not always be feasible or possible for any of a number of reasons. In the case of bacterial diseases, for example, attempts to isolate the responsible pathogen may prove negative because of prior antibiotic therapy or cultures may be negative because the clinical specimen was taken too late in the illness, and bacterial shedding has ceased. Also, negative cultures are not unusual in complications or the subacute phase of a disease. Conversely, the significance of positive cultures may be difficult to assess in healthy carriers, who may shed bacteria over prolonged intervals after convalescence, and in asymptomatic or clinically atypical cases. Similarly, in cases with a culture showing a mixed bacterial flora, it may be difficult to incriminate any one organism as the responsible pathogen, a situation which has become increasingly difficult in recent years with the blurring of the demarcation between pathogen and saprophyte engendered by the increase of immunocompromised hosts in the general population.

Much the same situation obtains with respect to viral agents. Isolation attempts may prove negative because the agent is not cultivable *in vitro* or because an insusceptible cell strains or line was used, or because the clinical sample for isolation attempts was taken too late in the illness, when viral shedding no longer occurs. As is true of positive bacterial cultures, the significance of a viral isolate must be judged in relation to the patient's clinical findings and epidemiologic history. It has long been known that viruses (cytomegalovirus, herpes simplex, varicella-zoster, Epstein-Barr, human immunodeficiency virus and, probably, some others) may persist in the host organism over prolonged periods, if not for life. The detection of a virus, therefore, does not necessarily correlate with the diagnosis of an acute disease. This is an important limitation of the diagnostic usefulness of viral isolation or antigen detection regardless of the rapidity and effectiveness of the test itself.

As is true of bacterial and viral (and also rickettsial) infections, laboratory diagnosis of a mycosis cannot always be achieved by cytopathologic examination of lesion material or by cultivation of the fungus. Diagnosis in such cases becomes feasible or possible only through immunologic methods, especially serologic methods. A serum reacting specifically at a titer of 1:32 or greater is considered positive and in some cases may be diagnostically meaningful even if only a single blood is tested. Titers less than this may represent residual antibody from a previous infection, antibody being formed in response to a current infection, or perhaps a cross-reaction with a related organism. To resolve this dilemma, multiple serum specimens should be tested for detection of possible rises in antibody titer, and tests should be done against a battery of antigens, especially those from antigenically-related pathogens. Here, too, meticulous analysis of the signs and symptoms of the patient's illness and his epidemiologic background in relation to the serologic findings is a requisite.

With respect to parasitology, serologic tests find a wide application, since detection of the parasite is not always simple, and especially in

such occult infections as echinococcosis, cysticercosis, visceral larva migrans, etc. According to Kagan (1986), the broad variety of serodiagnostic tests currently in use cover at least 24 infections caused by protozoan, trematode, cestode or nematode parasites. Serology is thus obviously an important means of achieving a definitive diagnosis of a parasitic infection.

While the direct approach to diagnosis, i.e., isolation and identification of the causal microbe, may represent the ideal, it is not always applicable or possible for the several reasons just stated above. In such cases recourse is had to indirect methods, viz., serological methods. These, rather than being methods of last resort, possess clear advantages of their own in diagnostic microbiology. Thus, the results of serological tests (except in unusual instances of bacterial infections) are little, if at all, influenced by previous chemotherapy in the patient; the tests are relatively simple and less labor-intensive than isolation of the causal agent; retrospective diagnosis is feasible; rapid detection of outbreaks is frequently realizable; etc. *

It has been estimated (Nichols, 1982) that in bacteriological diagnostic laboratories the use of cultures for isolation of the causal agent exceeds the use of serodiagnostic procedures by a ratio of 50 to one. This is quite different from the situation in virology, where the proportion of direct to indirect diagnostic tests appears to be in a ratio of one to nine. This may be a reflection of the fact that isolation and identification of viruses, because of its complicated nature, has remained essentially within the domain of the reference laboratory.

Whatever the reasons, microbial serology, except for virology, appears on the whole to have been under-utilized, although the recognition in recent years of new bacterial species, and the need to meet their special nutrient conditions and atmospheric ambience, has rekindled interest in serodiagnostic methods. Simple, sensitive and specific serologic tests should prove a useful adjunct to the direct diagnostic approaches of the laboratory dealing with bacteria, fungi and parasites.

This brings up the matter of the comparisons and evaluations made, and value judgments rendered on the several tests considered herein. Assessments were made virtually entirely on those diagnostic methods for which the required reagents are available commercially, either as individual items or in the form of kits, and which are part of the normal, routine daily operation of the laboratory. Methods suitable only for a specialized reference or research laboratory were not included, even if the components of the given test are on the market, because the book is not aimed at this segment of the medical community but to staff of hospital-based or privately operated microbiological diagnostic laboratories directly concerned with patient management.

Perhaps one final point merits mention here, namely, the matter of the sensitivity of a test. In some quarters, sensitivity is equated, reasonably and logically so, with the height of the antibody titer the test can detect. This might perhaps be better defined, in a broader sense, as reactivity, since neither the severity of the patient's illness nor the intensity of his immune response enters into statistical determination of the sensitivity of a diagnostic test, nor on the efficiency of that test. To quote

* The discovery of adenoviruses in 1954 and the concomittant development of serologic methods, permitted detection of an adenovirus epidemic among U.S. soldiers which occurred on Hawaii during 1944 (10 years earlier); this was possible because samples of sera from patients had been lyophilized and thus were available for subsequent testing.

from Galen (1986), «sensitivity, expressed as a percentage, indicates the frequency of positive test results in patients with a particular disease». Note that it is the frequency of occurrence of positive tests, not the intensity of the patho-physiological response, on which sensitivity criteria are based. The efficiency of a test represents the percentage of patients correctly classified as to whether they have that particular disease or do not have it. *In toto,* sensitivity is defined in terms of the presence, not quantity, of antibody, i.e., «yes» or »no», and any test which gives a yes answer (provided it is specific) is a sensitive test. (See Galen (1986) for an excellent exposition of this subject).

Serologic Tests for the Detection of Antibodies

Following the first, or primary, exposure to an infectious agent (bacterial, rickettsial, viral, fungal or parasitic), the host responds with specific antibody formation to the invading pathogen. A number of methods are available for the detection of the presence or appearance of antibodies, each with its own advantages and disadvantages, or even unique characteristics. Among these (in not necessarily a complete listing) are: Precipitation, agglutination direct or passive (latex or erythrocytes), hemagglutination, hemagglutination-inhibition, immune adherence hemagglutination, counter immunoelectrophoresis, radial hemolysis, complement-fixation,immunofluorescent antibody test, radio immunoassay, enzyme immunoassay, immuno-peroxidase assay, neutralization test, etc.

However desirable it might be to review all of the extant methods or procedures, it would seem preferable here to focus attention on those tests which are in current (and generally wide-spread) use in the clinical microbiology laboratory. No one test method is sufficiently versatile to provide a diagnostic answer in every disease or under any circumstances, hence the variety of methods, from which a selection or an appropriate procedure can be made.

The results of traditional serologic test, e.g., complement-fixation or hemagglutination, are generally expressed as the reciprocal of the highest dilution of test serum which reacts in the test. This does not apply to the newer «immuno-chemical» methods, such as enzyme immunoassays, where the results are recorded as positive or negative depending upon whether they fall above or below an arbitrarily established so-called «cut-off» point. Obviously, the former permits simple quantitation of antibody content, the latter is primarily a qualitative read-out.

Serologically, initial or primary infections are characterized by a change from an antibody-negative to an antibody-positive state, the so-called «serologic conversion», during which antibodies appear in the blood and increase in titer with progression of the disease. This is readily demonstrable if the blood samples for comparison are taken at appropriate intervals post-onset of illness. If the first blood sample is taken sufficiently early in the disease (acute phase), it will be devoid of antibody, whereas a second specimen of blood, taken late in the disease (convalescent phase), e.g., 10-14 days after the first sample, will be antibody positive. If there is a delay in collecting the first serum sample, the specimen may already contain antibody; in these instances diagnostic significance can be attached only where there is a four-fold or greater rise in antibody titer between the first and second serum specimens. If collection of the first blood specimen is unduly prolonged beyond the onset of the illness, antibody formation may have reached its peak, and the first and second blood samples may show equivalent titers of antibody. One must then resort to another serologic test, or to determination of the presence of specific IgM class antibodies. These practical considerations point to the unquestioned importance of collecting the first serum sample for serologic examination as early after the onset of illness as possible.

Serologic conversions can be detected by most diagnostic methods, but significant rises in titer are difficult to demonstrate by methods characterized by a high reactivity, such as enzyme- or radio-immunoassay. The

complement-fixation test, with a low reactivity (and, perhaps, in some cases the indirect immuno-fluorescence assay) is a better means of picking up seroconversions.

Interpretation of serodiagnostic tests in patients undergoing reinfection with the same virus, or suffering from a reactivation of a latent virus, is also beset with difficulties. Because of the boostering effect of reinfection or of viral reactivation, the first serum specimen, even when taken quite early, is likely to contain specific antibodies in high titer. Examination for IgM antibodies may give equivocal results, or results difficult to interpret.

Serologic testing has also been applied to determination of the immune status of individuals and the prevalence of antibodies in a given population. Such tests provide the basis for decisions as to whether immunization should be carried out in the individual (or in the population at large). Thus, the presence of antibodies in the pregnant woman to any of a number of congenitally-transmitted viruses not only rules out the need of immunization to that agent, but also militates against the probability of primary infection with that agent during the early period of pregnancy, the interval when the danger of intra-uterine transmission of infection is greatest. Tests in the early stages of pregnancy are of especial importance if the individual has had contact with others (especially children) presenting with signs and symptoms of any disease known to be trans-placentally transmitted. The tests most frequently performed are for antibodies to *Toxoplasma gondii,* rubella, cytomegalovirus and herpes simplex (often collectively referred to as TORCH). Tests for antibodies to HIV-1, the AIDS virus, will undoubtedly be included in this battery of tests in the future.

Over the past several years, much attention has been focused on serologic testing of presumably healthy blood donors, the objective being to avoid transmission of infection to the blood recipient. It has been generally assumed that the absence of antibodies essentially excludes infection with a given agent, although there is always the possibility that the donor may be in the very early stages of an infection and thus still antibody negative. Donors have most frequently been tested for hepatitis B, cytomegalovirus and human immunodeficiency (HIV-1) viruses. Tests for syphilis have been discontinued in many countries because blood is infective only when specific antibody is absent, i.e., if antibodies are detectable, transmission of infection appears improbable.

For the determination of immune status, or any similar tests for prophylactic and/or epidemiological purposes, methods with a highest possible sensitivity level should be employed. These primarily include enzyme- and radio-immunoassays, but indirect immunofluorescent antibody tests and passive hemagglutination have also not infrequently been used. The use of complement-fixation tests for such purposes has been confined, so far, to cytomegalovirus, herpes simplex and *Toxoplasma gondii.* In all these cases the titer of specific antibody appears of less importance; a yes or no answer is satisfactory.

Tests for antigen detection would obviously be a more practical approach to revealing the potential infectivity of blood donors. However, the only test routinely employed at present is for the presence of hepatitis B antigen; tests for cytomegalovirus and HIV-1 antigens are at present infeasible because of the unknown rate of false negative results. If direct antigen detection in blood and blood-products can be achieved, this would replace the need for antibody determinations.

Cross-Reactions in Serologic Tests

In the introduction, the sensitivity of a test was defined as the frequency with which it gives positive results in patients with that disease. The other important essential, with which we are concerned here, is specificity, which indicates the frequency with which the test is negative in patients without that disease. It thus becomes necessary to know whether, or how often and to what extent, the antigen will react with antibodies to other infectious agents. Such information on the cross-reactivity of an antigen can be obtained by examining it against an array of sera from patients with other, known diseases, and/or against hyperimmune sera produced in animals.

Table 1. Serological Evidence of Cross-Reactions Among Bacterial Antigens. Tested against Homologous and Heterologous Sera of Human Origin by Complement-Fixation (CF) (from Jung, M., Serologic Diagnosis of Bacterial Infections, 1983).

Antigens \ Antisera	Brucella abortus	Campylobacter jejuni	Campylobacter intestinalis	Listeria monocytogenes	Neisseria gonorrhoeae	Neisseria meningitidis	Shigella boyidii	Shigella dysenteriae	Shigella flexneri	Shigella sonnei	Yersinia enterocolitica 3	Yersinia enterocolitica 9	Yersinia pseudotuberculosis
Brucella abortus	+	0	0	0	0	0	0	0	0	0	0	+	0
Campylobacter jejuni	0	+	0	0	0	0	0	0	0	0	0	0	0
Campylobacter intestinalis	0	0	+	0	0	0	0	0	0	0	0	0	0
Listeria monocytogenes	0	0	0	+	0	0	0	0	0	0	0	0	0
Neisseria gonorrhoeae	0	0	0	0	+	+	0	0	0	0	0	0	0
Neisseria meningitidis	0	0	0	0	+	+	0	0	0	0	0	0	0
Shigella boyidii	0	0	0	0	0	0	+	+	+	+	0	0	0
Shigella dysenteriae	0	0	0	0	0	0	+	+	+	+	0	0	0
Shigella flexneri	0	0	0	0	0	0	+	+	+	+	0	0	0
Shigella sonnei	0	0	0	0	0	0	+	+	+	+	0	0	0
Yersinia enterocolitica 3	0	0	0	0	0	0	0	0	0	0	+	0	0
Yersinia enterocolitica 9	+	0	0	0	0	0	0	0	0	0	0	+	0
Yersinia pseudotuberculosis	0	0	0	0	0	0	0	0	0	0	0	0	+

A classic approach to this problem of specificity is illustrated in *Table 1*, which summarizes the results of cross complement-fixation tests using 13 bacterial antigens and human sera containing antibodies to one or another of the bacterial antigens. It will be seen that few cross-reactions were encountered and these, as might be expected, were chiefly among *Shigella sp.* and among *Neisseria sp.;* in one instance there was reciprocal crossing between *Brucella abortus* and *Yersinia enterocolitica 9.* Tests with these antigens are thus highly specific, and the knowledgeable serologist will be aware of the cross-reactions that might be expected and the extent of the crossing.

Another example of cross-reactivity tests, this one employing animal hyperimmune mouse antisera, is provided in *Table 2.* Six coxsackie B virus immunotypes were tested against antisera to all six types and the findings of the cross CF tests clearly show the specificity of the sera used; only three cross-reactions were encountered, viz., B1 virus with B4 antibodies, B5 virus with B6 antibodies and B6 virus with B3 antibodies, all of minor degree. It should be emphasized, however, that such clear-cut test results cannot be expected when human sera are examined except, perhaps, to a varying extent, among child populations, whose exposure to these viruses has been minimal. In adults, cross-reactions between viral immunotypes in the CF test are common because of prior exposures to these viruses, and so may not permit of definitive identification of the infecting viral immunotype; however, the antibody titer to the homologous (infecting) viral type may be significantly higher than to the heterologous type. A picornavirus group antigen, prepared by pooling several echovirus types with type A and type B coxsackie viruses, has been used for diagnosis of picornavirus infections by CF (Alexander, 1982).

Table 2. Cross-reactivities of Coxackie B1,2,3,4,5 and 6 Antigens/Antisera Tested by Complement-Fixation.

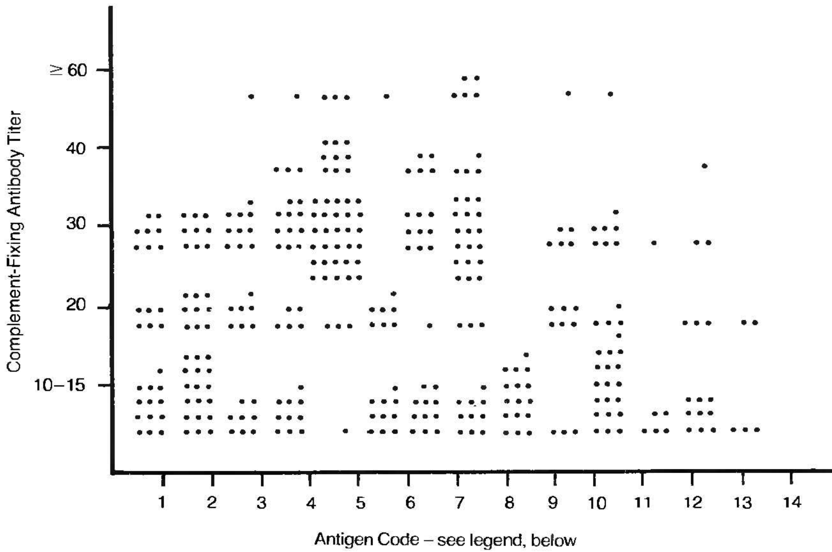
Antiserum (mouse	Complement-Fixing Antigens					
	B1	B2	B3	B4	B5	B6
B-1	**60**[1]	neg[2]	neg	neg	neg	neg
B-2	neg	**30**	neg	neg	neg	neg
B-3	neg	neg	**60**	neg	neg	10
B-4	5	neg	neg	**60**	neg	neg
B-5	neg	neg	neg	neg	**120**	neg
B-6	neg	neg	neg	neg	10	**40**

(1) reciprocal of the highest serum dilution fixing complement in the presence of specific antigen.
(2) antibody titer less than 1 : 5.

In another assay, sera from 335 blood donors carrying antibodies from prior infection with one or more of a variety of agents were examined for the presence of antibodies to HIV-1 (human immunodeficiency virus) by the ELISA technique and further checked by Western blot. None of these sera, containing antibodies to a viral, toxoplasmal, chlamydial or bacterial agent reacted with the HIV-1 antigens, so that no false positive results due to cross-reactivities were encountered *(Figure 1).*

The sensitivity and specificity (and the efficiency and predictive values) of a new test can also be evaluated by comparison with one or more existing tests whose characteristics in this respect are already known. An example of such a comparison appears in *Table 3*, in which the results of two enzyme-immunoassays and Western blot tests are compared, the tests of one manufacturer (A) being the recognized, accepted method, and hence used as a baseline for comparison, and those of the second manufacturer (B) being essentially untried and under evaluation. It is immediately obvious that the tests brought out by manufacturer B are comparable to those of manufacturer A and so are equally suitable for diagnostic purposes. Antigenic relationship and cross-reactivities may also be studied

Figure 1. Sera of 335 healthy blood donors found to contain complement-fixing antibodies against Chlamydiae, Toxoplasma and 12 viral agents.



Antigen Code: 1 = Influenza A; 2 = Influenza B; 3 = Picorna group; 4 = Measles; 5 = Herpes simplex; 6 = Varicella/Zoster; 7 = Epstein-Barr; 8 = Cytomegalovirus; 9 = Resp. syncytial virus; 10 = Toxoplasma gondii; 11 = Parainfluenza 1–3; 12 = Chlamydiae; 13 = Rota and 14 = Polyoma (human) virus.
All sera were negative for HIV-1 by Enzyme-Immuno-Assay and Western Blot.

by absorption experiments. The test serum should be absorbed by homologous and heterologous antigens, preferably prepared by a method other than the one used for the antigen in the assay procedure, e.g., enzyme immunoassay-complement fixing antigens in this case. An example of such an absorption is illustrated in *Figure 2*.

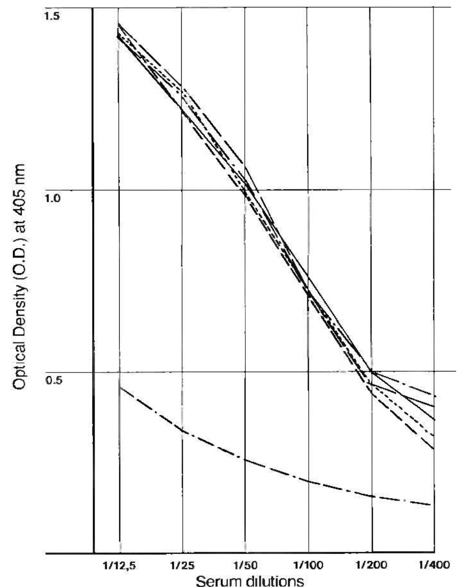


Figure 2. Patient's serum contains antibodies to the four human herpesviruses. Prior to the test the serum was absorbed with complement-fixing antigens for cytomegalovirus (————), Epstein-Barr virus (—·—·—·—), herpes simplex type 1 virus (-------) and varicella-zoster virus (—·—·—·—) as well as with antigen prepared from uninfected cells (— — —). The serum was also tested without previous absorption (————). The wells of a microplate were coated with varicella-zoster EIA antigen and the test was performed using an antihuman IgG labelled with alkaline phosphatase. Only absorption by the homologous antigen reduced the EIA reactivity of the serum.

Table 3. Comparison of Enzyme Immunoassay (EIA) and Western Blot from Two Different Sources Kits for the Detection of Antibody to Human Immunodeficiency 1 Virus (HIV-1).

Enzyme Immunoassay	Enzyme immunoassay		Western blot		Percent sensitivity	Percent specificity
	Results	No. of cases	Positive	Negative		
Manufacturer A	Positive	149	148	1	99.4 (148/148 + 1)	
	Negative	187	4	183		97.9 (183/183 + 4)
Manufacturer B	Positive	135	134	1	99.3 (134/134 + 1)	
	Negative	187	3	184		98.4 (184/184−3)

Immunoglobulins and the Classes of Antibodies*

Immunoglobulins in a normal serum are a heterogeneous collection of molecules of different biological and physico-chemical properties, and whose most important biological activity is to function as antibody. The physical and chemical properties of the globulins, on the other hand, provide a basis for arranging the immunoglobulins into classes and subclasses.

All immunoglobulins are made up of one or more basic units, each comprised of two pairs of polypeptide chains, each chain of the pair identical with the other. Since one pair of identical chains contains nearly twice as many amino acids as the other pair (and thus has approximately two times the molecular weight), it bears the label of heavy (or H) chain, the lighter version bearing the label light (or L) chain. Each chain, in turn, can be divided, on the basis of its amino acid sequence, into a variable (V) region (the amino terminal portion of the chain), and constant (C) region (the carboxyl terminal part). There is one variable and one constant region in the light chain, and one variable and three constant regions in the heavy chain of the IgG molecule (see *Fig. 3)*.

Based on antigenic differences in the H chains, five classes of immunoglobulins have been distinguished in man; the antigenically different H chains are labelled γ, μ, α, δ, ε, and the corresponding immunoglobulin classes are designated IgG, IgM, IgA, IgD and IgE respectively. These classes have been further subdivided in the case of IgG and IgA, and possibly of the IgM into subclasses,

IgG = IgG1, IgG2, IgG3 and IgG 4
IgA = IgA1 and IgA2
IgM = IgM1 and IgM2 (Biological differences between these two subclasses not yet defined (Dick and Kirkwood, 1984 **)

Infection with microbial agents leads to the elaboration of specific antibodies of the IgG, IgM and IgA classes. To what extent, if any, antibodies to foreign proteins are produced by the IgD class is largely unknown. IgE class is a domain of antibodies to allergens. Both, IgD and IgE immunoglobulins are present in normal serum in only trace or miniscule amounts: IgD 0.2% of total serum immunoglobulin, IgE 0.004% of total serum immunoglobulin.

IgG Antibody

The IgG immunoglobulin fraction makes up approximately 75% of the total normal serum immunoglobulins. This fraction is comprised of four subclasses, namely, IgG1 60-70%; IgG2 14-20%; IgG3 4-8%; and IgG4 2-6% (Morell and Skvaril, 1972; Wang, 1976); the percentages reflect individual variations in the population.

* Revised by Professor František Skvaril, Immunochemical Laboratory, Berne, Switzerland.

** Using monoclonal antibodies to IgM and Protein A, Harboe and Følling (1974) recognized two kinds of IgM, and observed that the distinction corresponds to an IgM subclass rather than to an allotype. Similar observations were also made by Lind, Harboe and Følling (1975). Salvedt and Harboe (1976) proposed naming the two IgM subclasses IgM1 and IgM2, the latter defined by its ability to react with Protein A. Also, that binding of some IgM from rabbit serum to Protein A may occur was described by Goding in 1976. All these differences in Protein A reactivity point to an isotype or subclass deviation of human IgM. However, the existence of two subclasses of IgM is still a point at issue.

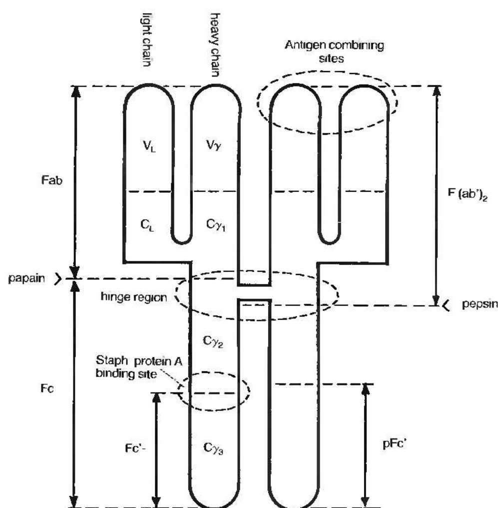


Figure 3. Molecular structure of the IgG immunoglobulin. It consists of two pairs of identical polypeptide chains (two light and two heavy). The heavy chains comprise four domains, one «variable» (Vγ) and three «constant» (Cγ1, Cγ2, and Cγ3), the light chains two domains, one «variable» (VL) and one «constant» (CL). Both parts are held together by disulphide bridges in the so-called «Hinge region».
IgG molecule can be split by proteolytic enzymes. Papain splits the molecule into two identical fragments called «Fab» and one «Fc» fragment. Pepsin splits the molecule in one «F(ab')$_2$ and one «pFc'» fragments: the remaining portion of the molecule (C γ 2) is digested into small dialysable peptides. In the Fab and F(ab')$_2$ fragments antigen combining sites, i.e. the antibody activity is preserved; two domains at the N-terminal end of the molecule (VL and Vγ) are involved in this activity. The fragment Fc (crystallizable) possesses various biological (effector) functions of the molecule, like C_1q fixation, transplacental transfer, binding to receptors on cells and binding to RF, Protein A etc. The IgG class-specific determinant groups (isotypic) are localized mainly on the Fc portion of IgG.

Antibodies of the IgG class appear following infection or administration of vaccine, and appear almost simultaneously with serum IgM antibodies, or within a day or two afterward. The IgG class antibodies thereafter increase in titer and remain high during convalescence and for some time afterward, whereas IgM antibodies decline with convalescence.

Diagnostic serology of microbial diseases is based virtually entirely on the appearance, or changes in concentration, of specific IgG and/ or IgM antibodies. As already mentioned, the presence of serum IgM antibodies, or a significant rise in antibody concentration between two serum specimens, one taken during the acute phase of the illness and the second taken 10-14 days later (convalescent phase), is regarded as evidence of current or recent infection. However, since certain tests (e.g., passive hemagglutination, hemagglutination-inhibition, complement-fixation), when not performed with class specific reagents, do not discriminate between IgM and IgG antibodies but measure both with equal facility, the may fail to reveal the occurrence of a significant change in IgG antibody. Thus, in the first blood specimen (acute phase), the concentration of IgM antibody may be high, and of IgG antibody, low. The reverse may hold in the second blood (convalescent phase), which may have a high IgG antibody content, and a low IgM antibody content. The resultant readings would show no change in antibody titer, i.e., a stationary antibody titer. Re-examination of the sera by an IgG (or IgM) antibody specific method should resolve the dilemma, and confirm whether the infection is of current origin (rise in antibody titer), or the stationary titer represents a prior infection.

IgM Antibody

The IgM component comprises some 10% of the total normal serum immunoglobulin. The earliest humoral antibody response to microbial infection is IgM antibody, which generally is detectable within several days after the onset of illness, reaches a peak by the 7th – 10th day, and then diminishes and disappears over the next months.
IgM antibodies, together with those of the IgG class, play a major role in such classic

immune reactions as agglutination, precipitation, complement-fixation, etc.

The occurrence of IgM antibodies in a patient's serum is generally regarded as evidence of a current or recent infection, albeit exceptions are encountered. Thus, IgM antibodies in the pregnant woman may be found throughout the entire gestational period and some months post partum, making it difficult to distinguish between a recent or past infection, a primary from a recurrent infection, or to predict whether congenital transmission may occur, or has occurred.

The presence of IgM antibody in cord blood or in the blood of the new-born infant points to congenital infection; since maternal IgM does not cross the placenta, the antibody must be of fetal origin in response to active infection.

Viral re-activation, accompanied by a serum IgM response has been reported (Cremer, 1985) for several viruses, e.g., cytomegalovirus, varicella and Epstein-Barr, and after administration of live virus vaccines (rubella, poliomyelitis [Cremer, 1985]).

IgA Antibody

The information available on IgA antibodies suggests that they may be markers of recent infection (as is IgM antibody). Like IgM antibodies, IgA antibodies are of short duration, as shown by indirect immunofluorescence for antibodies to mumps virus (Brown, Baublis and O'Leary, 1970) and rubella virus (Craddock-Watson, Bourne and Vandervelde, 1972). Levy and Sarov (1980) found specific IgA antibodies to cytomegalovirus to correlate well with the presence of IgM antibodies in primary infections as detected by enzyme immunoassay. The occurrence of IgA antibodies to respiratory syncytial virus (Anderson, 1988) and Chlamydiae (Csango, Sarov, Schiotz et al, 1988; Osborne, Hecht, Gorsline et al., 1988) has also been reported. Tests for IgA and sIgA (secretory) antibodies are not generally offered by diagnostic laboratories.

IgM Class Antibodies in Serodiagnosis

It is now well known that the first immunoglobulins to appear after infection are of the IgM class, and this serves as a useful indicator in assessing the development of the immune response in the host. These antibodies, appearing just before, or simultaneously with, IgG antibodies, are short-lived and are the first to disappear with resolution of the infectious process (see Figure 4, (which is self explanatory) for the nature of an antibody response, in this case to *Toxoplasma gondii).*

The presence of IgM antibodies in the serum is therefore of diagnostic significance, pointing, in general, to recent infection. It is of particular importance in the detection of possible maternal infection after exposure to teratogenic viruses such as rubella or cytomegalovirus. Since IgM antibodies do not cross the placenta, their presence in the cord blood or the neonate indicates a congenital infection.

There are several techniques available for the demonstration of IgM antibodies in serum. On the whole, these procedures can be categorized under four headings:

1. Comparison of the antibody titer in a serologic test before and after chemical degradation of the IgM molecule in patient's serum (2-mercaptoethanol, 2-ME).

2. Separation of IgM from other serum immunoglobulins by physico-chemical methods (density gradient ultracentrifugation, column exclusion chromatography) or the absorption/elimination of IgG from the serum (absorption of serum IgG by staphylococcal Protein A, absorption of IgG by antiserum to IgG); sera so treated must then be examined in a serologic test in parallel with the untreated sample.

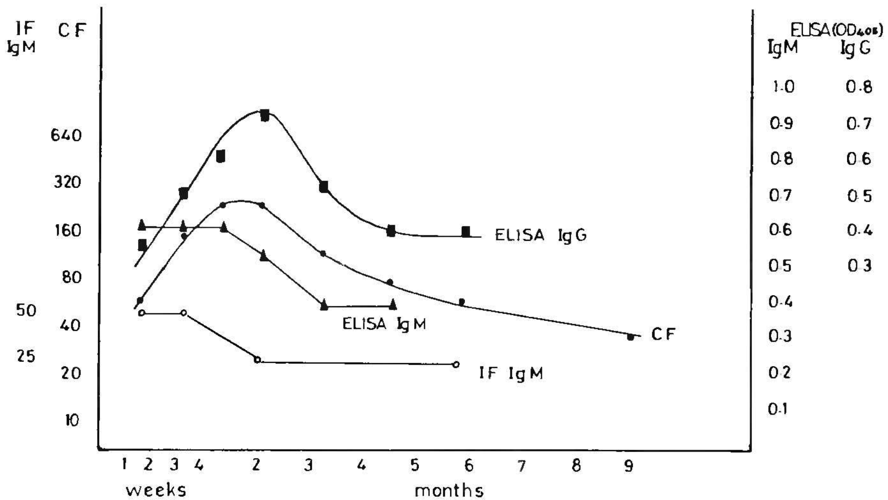


Figure 4. Comparison of IgG- and IgM class antibody responses to *Toxoplasma gondii.* IgG and IgM antibodies determined by Enzyme Immunoassay (ELISA); IgM antibodies by indirect immunofluorescence; total antibody (IgG + IgM) by complement-fixation.

3. Solid phase indirect immunoassays using labeled antihuman IgM.

4. Solid phase IgM capture assay.

There are several other assay methods available, but those listed have been the major ones employed.

Treatment of serum with 2-mercaptoethanol (2-ME) or with dithiothreitol (DTT) (Deutsch and Morton, 1957) disrupts the disulfide bond linkage between the polypeptide chains of the IgM, with a resultant destruction of the antigenic function of the molecule. When this phenomenon was first reported, it seemed a readily applicable and valuable tool in diagnostic serology, but experience soon revealed that the procedure, while quite simple, is too insensitive to detect the necessary differences in titer between treated and untreated serial blood specimens (Forghani, Schmidt and Lennette, 1973).

Staphylococcal Protein A. This is a simple and rapid screening-type procedure for the reduction of IgG immunoglobulins in a patient's serum. This protein, which occurs in the cell wall of most strains of *Staphylococcus aureus,* has the interesting property of binding to the immunoglobulins of most but not of all mammalian species. The protein molecule is bound to the cell wall through the C-terminus, and the N-terminal portion possesses four binding sites to which the gamma globulin moieties can attach. It is the Fc portion of the immunoglobulin that is bound; the Fab portion is unaffected, and retains its ability to bind to antigen.

Because of the great affinity of Protein A for IgG, attention turned to the possibility that it could be used to remove IgG from sera, so that any remaining immunologic activity would ostensibly be attributable to the IgM component left behind. Unfortunately, this hope did not fully materialize. Thus, absorption of serum with Protein A will serve to remove subclasses IgG1, IgG2 and IgG4, but IgG3 is essentially untouched because it possesses no binding sites for Protein A. Ordinarily, this has little effect on the results, since subclass IgG3 represents about 5% of the total IgG. If, however, the IgG3 subclass immunoglobulin concentration should be elevated or it bears antibodies under testing, a false positive reading may ensue. It is therefore mandatory that any test positive for IgM after Protein A absorption be confirmed by another method, such as a sucrose density gradient sedimentation (which is labor intensive and time consuming, and hence a technique for the highly specialized microbiological reference laboratory or the research laboratory). However, the Protein A absorption method is simple, can be performed without special equipment and serves to eliminate IgM negative samples.

Even though Protein A absorbs 20 to 40% of the native IgM immunoglobulins as well as IgG, false negative results for specific IgM antibodies are unlikely (Chantler, Devries, Allen et al., 1976; Ankerst, Christensen, Kjellén et al., 1974). Since Protein A differentiates IgM immunoglobulins into two subgroups/subclasses (Harboe and Følling, 1974; Lind, Harboe and Følling, 1975; Salvedt and Harboe, 1976) it might be that the subgroup/subclass absorbed by Protein A does not contain IgM antibody specific to the suspected agent. This may also explain the presence of comparable specific IgM antibody titers before and after absorption with Protein A (Chantler, Devries, Allen et al., 1976; Leinikki, Shekarchi, Dorsett et al., 1978).

Protein G. Bjorek and Kronvall (1984) described a novel IgG-binding reagent from Streptococcus of the Lancefield group G. This cell-wall protein, analogous to Protein A, also binds to the constant domains (both

Fc and Fab) of IgG immunoglobulins. It reacts with all IgG subclasses (1-4), and its reactivity is strictly limited to IgG. The preparation of a genetically engineered recombinant Protein G has been published recently (Fahnestock, 1989). However, its diagnostic usefulness has still to be determined.

Antihuman IgG is an antibody to IgG prepared in animals, usually sheep, goat or rabbit, and available commercially. Although its intended use was to eliminate IgG from the patient's serum before testing for IgM antibodies (Gispen, Nagel, Brand-Saathof et al., 1975), it is now almost exclusively used to remove rheumatoid factor (RF) from the serum. The antihuman IgG precipitates IgG from the serum forming immune complexes to which RF of the IgM class is bound. Competition between specific IgG and IgM which may be of importance, especially in the indirect immunofluorescent antibody (IFA) test, is also minimized.

Rheumatoid Factor (RF) which is an antibody to IgG immunoglobulins, constitutes a potential source of error in tests for IgM antibodies (Salonen, Vaheri, Suni et al., 1980). It is of considerable importance if present in the IgM class of immunoglobulins, whereas its presence in IgG and IgA immunoglobulins is relatively unimportant. RF of the IgM class reacts with specific viral IgG antibody, just as it does with any other IgG molecules, to form an immune complex; this complex is then recognized by the labelled antihuman IgM conjugates specific for μ chains. This results in a false positive reaction for IgM antibody. It is apparent, however, that in the absence of specific IgG antibodies, false positive results for specific IgM cannot occur.

In a larger sense, RF is a misnomer, since it also occurs in non-rheumatoidal diseases and even, although very rarely, in apparently healthy individuals. Patients with acute viral infections (such as viral pneumonia, rubella, cytomegalovirus, Epstein-Barr virus, herpes simplex, varicella-zoster virus) may have RF of the IgM class in their serum. The same is true of neonates congenitally-infected with *Treponema pallidum, Toxoplasma gondii,* cytomegalovirus, rubella. In neonates, the RF response may be evoked by the allotypic antigens (the Gm antigens) of the maternal IgG.

To obviate the problems engendered by the presence of RF in the serum, all specimens which give a positive reading in tests for IgM antibody should be tested for RF. A commercially available latex agglutination (Singer and Plotz, 1956) and an EIA test both of comparable sensitivity are available for this purpose. Since a serum may contain RF, yet fail to react in tests for its presence, we consider all sera to be potentially RF positive, and prior to testing treat them to remove RF.

Several procedures have been described for the removal of RF from serum. It may be absorbed from the specimen by adding to it foreign IgG insolubilized by heating, or by reacting it with a cross-linking agent such as glutaraldehyde (glutaraldehyde may give a false negative test result by absorption of non-RF IgM). Treatment of the serum with Protein A or, more effectively, with antihuman IgG prepared in animals, will also serve to eliminate the problem.

Reagents for the removal, or inactivation, of RF are commercially available. The manufacturer's instructions must be carefully followed, and all test runs should include adequate controls.

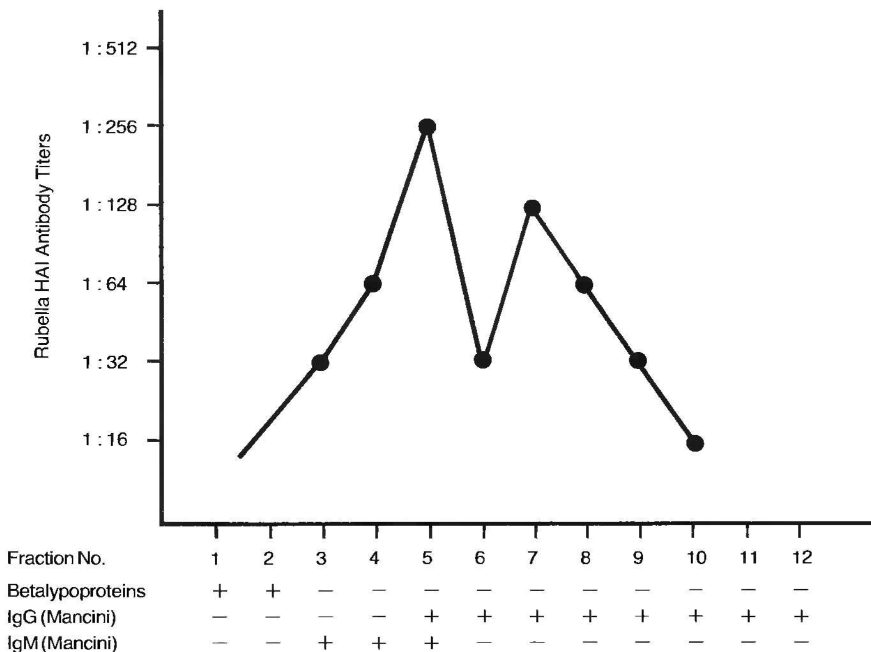
Methods for the detection of IgM antibodies. Any of several methods may be used for the detection of specific IgM antibodies in serum. It might be stated at the outset, however, that the complement-fixation test will not do for

this purpose, since it does not discriminate between IgG and IgM, and separation of immunoglobulin classes into fractions usable in this test is a tedious, labor-intensive and impractical procedure.

The hemagglutination-inhibition (HAI) test for the detection of specific IgM antibodies to rubella virus is prototypic, and represents the classic approach to this problem. IgM can be separated by column exclusion chromatography (gel filtration) as described by Bürgin-Wolf, Fernandez and Just (1971) or by sucrose density gradient centrifugation (Vesikari, 1968). Prior to the test, sera must be absorbed with erythrocytes of the same species as those to be used for the hemagglutination test itself. If column chromatography (gel filtration) is used for immunoglobulin separation, non-specific inhibitors of hemagglutination must also be removed. Immunoglobulin fractions obtained with such procedures should be tested for purity in agar gel by the Mancini technique (1965) against reference antisera to various immunoglobulins. Known antisera to human IgG and IgM are tested in agar gel against 10-12 fractions from the sucrose gradient. Reaction between the antigen (in this case the immunoglobulin fraction) and its homologous antiserum (here the reference serum) results in a visible precipitation line (ring). Such tests are commercially available in the form of Petri-dishes with agar containing antiserum to either IgG or IgM immunoglobulin, and with series of numbered perforations in the agar to hold various immunoglobulin fractions from the sucrose gradient.

A typical example is illustrated in *Figure 5*.

Figure 5. Sucrose density gradient experiment. Fractions were tested for purity by agar gel diffusion (Mancini). Rubella HAI test.



However, even with these methods, RF may be present and produce erroneous results, since there might be no clear, distinct or sharp separation between the IgG and IgM classes of antibody.

Patients' sera can also be treated with Protein A (or Protein A linked to Sepharose). The treated sample as well as an untreated sample are then titrated in parallel by HAI. If there is no inhibition of hemagglutination by the treated serum (IgG removed), the specimen does not contain IgM antibodies to rubella virus; inhibition of hemagglutination indicates the presence of specific IgM antibodies to the virus. Such a test is illustrated in *Figures 6 and 7.*

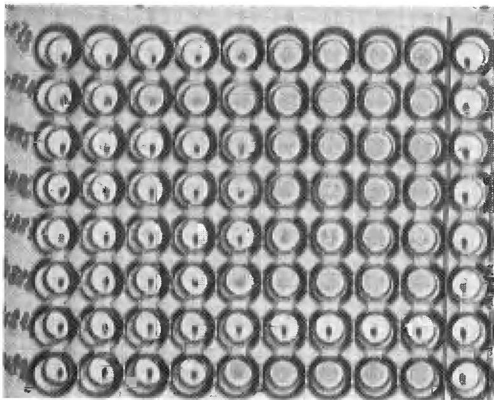


Figure 6. HAI for antibodies to rubella virus. Antibodies demonstrable at various titer heights with all eight specimens tested.

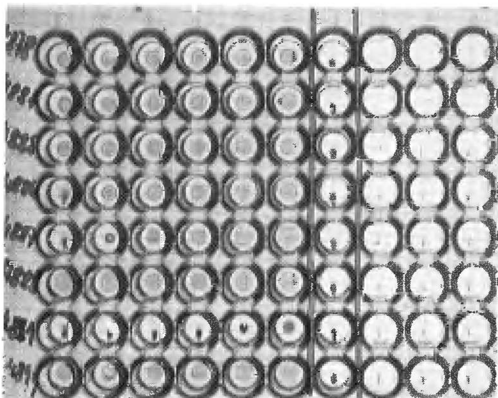


Figure 7. HAI with sera absorbed with Protein A (IgG eliminated, viz. reduced 90%). Specimen 4841 positive for specific IgM antibody.

As mentionned previously, any result positive for IgM by Protein A absorption must be confirmed using another method of separation, such as sucrose density gradient centrifugation.

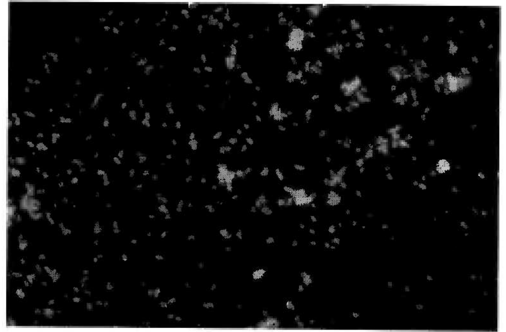


Figure 8. Indirect immunofluorescence test for specific IgM antibodies to *Bordetella pertussis*. Protein A-treated human serum stained with a FITC-labelled antihuman IgM.

The **indirect immunofluorescence test** is an excellent assay for the detection of specific IgM antibodies. For this purpose, affinity-purified FITC-conjugated antihuman IgM is used instead of IgG. The patient's serum in its native state, however, is not suitable for direct testing for IgM by indirect immunofluorescence because of a phenomenon known as «competitive inhibition» (Cohens, Norins and Julian, 1967). Because of their relatively small size (M.W.150,000, s.c. 6-7S) compared to IgM (M.W. 900,000, s. c. 19S) the IgG molecules rapidly attach to the antigen on the solid support (here the slide), and thus preempt or reduce the binding sites available to the larger IgM molecules. It is thus necessary to eliminate, or significantly reduce, the amount of IgG present by absorption with

Protein A (or Protein A linked to Sepharose) or by antihuman IgG serum produced in animals. Although IgG3 subclass immunoglobulin is not absorbed by Protein A, this does not affect the final results significantly since this subclass represents only about 5% of the total IgG. Two steps in the treatment merit mention. First, antigen-antibody incubation should be carried out for at least 3 hours at 37° C, and secondly, fixation of the antigen with pure acetone should be replaced by fixation with absolute methanol or at least by a mixture of acetone (one part) and methanol (three parts) in order to retain cell membrane permeability for the large IgM antibody molecules. Since RF may also cause false positive results, attention should be paid to its presence, viz. its absorption. However, treatment by Protein A greatly reduces the unwanted effect of RF present in the patient's serum.

The **enzyme immunoassay (EIA)** may also be applied to the detection of IgM antibodies, and require the use of the appropriate antihuman IgM conjugated to either alkaline phosphatase or horseradish peroxidase. Both methods require precise standardization of reagents and strict adherence to the test procedure or protocol. These complicated tests do not fully meet the needs of the small diagnostic laboratory, which is seldom called upon the test for IgM antibodies on a large scale.

Although absorption or elimination of IgG from test serum specimens is not mandatory, on the basis of our experience it is highly recommended. Antihuman IgG, produced in animals, is best suited for this purpose. This absorption procedure also simultaneously eliminates RF, if present in the IgM class and complexed with IgG. Thus, false positive results for IgM antibodies are avoided. Protein A absorption may also be used (Leinikki, Shekarchi, Dorsett et al., 1978).

The **reverse EIA technique**, or reverse IgM antibody capture method (Duermeyer and van der Vee, 1978), avoids the problems of non-specific reactivity and by-passes competitive interference. This solid phase test for IgM is highly specific and sensitive, and gives significantly better results than the two approaches just mentioned. The solid phase is coated with an antihuman IgM antibody, which serves to «capture» and hold the IgM antibodies in the test serum. IgG and any immune complexes present are removed with the rinse-water step, specific viral antigen is added followed by an appropriately labelled antiviral antibody.

Table 4. Methods Frequently Employed and (Mostly) Commercially Available for the Detection of Specific IgM-Class Antibodies in Human Sera*

Diaease agents	Test Methods		
	IFA	EIA	HAI
Viruses	Epstein-Barr (VCA) Cytomegalovirus	Epstein-Barr (VCA) Cytomegalovirus Rubella Herpes simplex Varicella-Zoster Mumps Measles Hepatitis A and B	Rubella
Protozoa and bacteria	Toxoplasma gondii Treponema pallidum Borrelia burgdorferi Bordetella pertussis	Toxoplasma gondii Chlamydia	

* IFA tests have also been developed for the detection of IgM-class antibodies to Measles, Mumps, Rubella, Herpes simplex and some other viruses but their use has been mostly confined to resarch laboratories; their commercial availability, if any, has been very limited.

Because IgM is captured and bound to the solid phase at the very outset of the test procedure, and IgG is left behind, there is no competitive interference. However, RF, which is as mentioned an anti-IgG of the IgM class, may be captured on the solid phase and thus bind to antiviral IgG antibodies. RF interference can be circumvented by employing aggregated IgG in the serum sample diluent to pre-empt the binding sites for RF. Naot, Barnett and Remington (1981) proposed the use of F(ab')2 fragments of IgG antibodies as enzyme conjugates to avoid false positive results in sera positive for both RF and antinuclear antibodies in either the conventional or in double sandwich IgM enzyme-linked immunosorbent assay.

Finally, tests which have been generally accepted for diagnostic purposes, are listed in *Table 4.*

Enzyme Immunoassay (EIA, ELISA)

These tests take advantage of the fact that one molecule of an enzyme can split many molecules of a substrate, thereby giving rise to, and an accumulation of, a large number of end-product molecules. These breakdown products of the substrate can be identified visually or microscopically, or can be measured radiometrically, colorimetrically, fluorometrically or by luminescence (see, for example, Engvall and Perlman 1971, 1972; Benjamin, 1979; Forghani, 1985).

While a number of enzymes have been tested in EIA applications, alkaline phosphatase and horseradish peroxidase have emerged as the two most versatile and applicable to diagnostic microbiology.

In the EIA, either of these two enzymes may be used as a label for the immunoreactant, usually IgG, IgM or IgA. The antigen (viral, bacterial, fungal, parasitic) is fixed to a solid support, usually polystyrene in the form of microplates (96 wells) or strips with 8 indentations. Next, the patient's serum is added, and if it contains antibodies specific to the antigen, it will bind to the antigen to form antigen-antibody complexes. This is the first phase of the test. In the second, enzyme-labelled antihuman globulin (IgG, IgM, IgA) is added, which reacts with the human globulin in the complex. To detect and measure the occurrence of such a specific union, the appropriate substrate is added – para-nitrophenyl phosphate for alkaline phosphatase, ortho-phenylene-diamin for horseradish peroxidase. A positive test is heralded by the development of a color change resulting from the accumulation of the end-products of the enzymatic reaction. Although the color change (especially if horseradish peroxidase is used) is readily visible, the test should be read with a photometer to determine the optical density (O.D.) of the absorbence which, within limits, correlates with the amount of antibody bound to the antigen.

The basic principles of the enzyme immunoassay methods for the detection of antibodies are outlined diagrammatically in *Figure 9.*

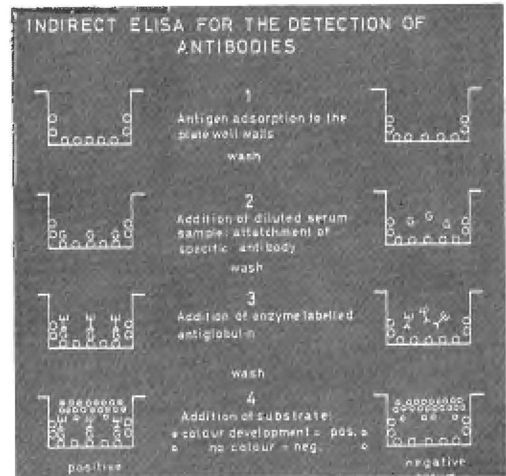


Figure 9. Indirect Elisa for the detection of antibodies.

The first step is attachment of the antigen to a solid state support, here the walls and bottom of the wells in a microplate. This is followed by the addition of the test serum sample, a step which permits attachment of specific antibody to the immobilized antigen. The third step is the addition of enzyme-labelled anti-immunoglobulin (either IgG or IgM), and the fourth is the addition of the substrate, which provides the means for detecting the end-reaction. If a color reaction develops, it indicates that the labelled antihuman immunoglobulin attached to the antibody moiety and thus was able to generate the colored

end-products of the enzymatic reaction (unbound reactants are eliminated during the several wash steps). The intensity of the color reaction is read photometrically, as mentioned above.

In using the enzyme methods, the usual routine in the serologic laboratory is to test a single dilution of the patient's serum; quantitation of antibody is seldom undertaken (cf Booth, Hannington, Bakir et al., 1982), and the test is essentially regarded as a qualitative, not a quantitative, indicator of an immune response. Should quantitation be undertaken, it should be kept in mind that the high reactivities of these methods necessitate the use of extremely high dilutions of the serum under test, and that in making a series of such dilutions, the smallest error is unduly magnified, giving results difficult to reproduce in replicate testing.

Figure 10 is included here to give an idea of the color change induced when alkaline phos-

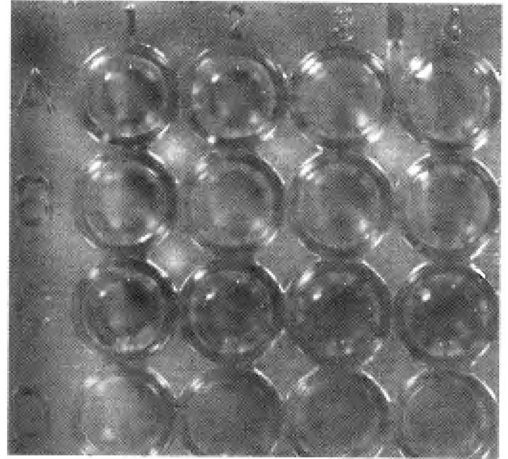


Figure 10. EIA results with three different sera.

Serum	IgG conjugate (in duplicate)		IgM conjugate (in duplicate)	
	1	2	1	2
A	+	+	0	0
B	0	0	0	0
C	+	+	+	+

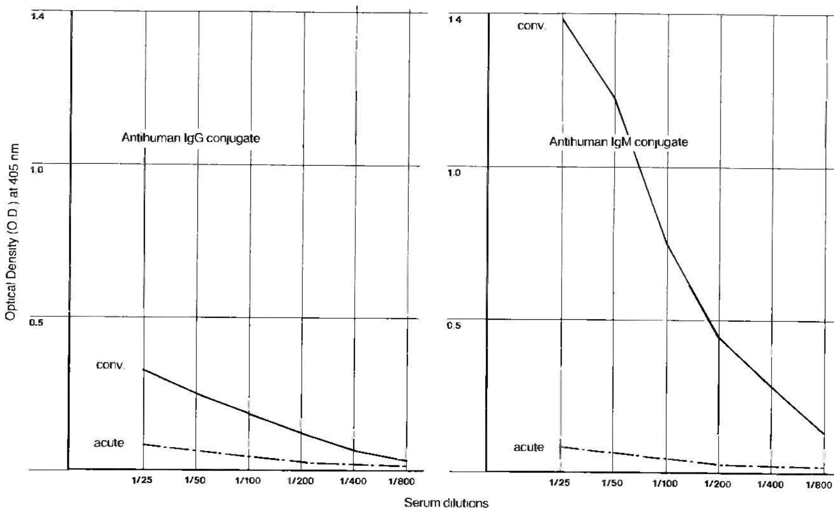


Figure 11. Optical densities (O.D.) of paired sera taken 10 days apart. Serial dilutions of the sera were tested against Epstein-Barr virus (antigen-coated microplate wells) and antihuman IgG and IgM conjugated with alkaline phospbatase.

phatase-labelled conjugates are used (paranitrophenyl phosphate is the substrate). This system produces yellow-staining end-products of the enzymatic reaction, as shown in the figure. Three sera were tested in duplicate, one set against the IgG conjugate, another against the IgM conjugate. The test results shown in the figure are clearcut and unequivocal. Reading from left to right, the first two wells in the top row (A) show a positive reaction with IgG antibody, the next two a negative reaction with IgM class antibodies. Serum B, in the four wells of the second·row (B), was devoid of demonstrable antibodies, no color change having occurred. Serum C, represented by four wells in row C, was positive in all four instances, reacting against the both IgG and IgM conjugates.

Figure 11 shows the results of a titration of paired serum specimens for antibodies against Epstein-Barr virus, using the ELISA method. It will be noted that at 10 days after the onset of the illness, antibody of the IgG class was present, but in low concentration. When the same paired sera were tested with an IgM conjugate, the titration showed that considerable amounts of specific antibody were present in the IgM immunoglobulin, and ostensibly were induced by the current illness.

Laboratories interested in conducting EIA tests should adhere closely to the working instruction provided by the manufacturer. Reference sera of human origin must be used to standardize the reaction, and the «cut-off» value for a positive test, expressed as an O.D. value, should be established from the controls of each run (not before). An EIA test is as good as its standardization, and false negative results may be expected if the manufacturer's recommendations are not scrupulously followed.

Indirect Immunofluorescent Antibody (IFA) Test

Immunofluorescence techniques as diagnostic tools were introduced by Coons (1941), who originated the method. Riggs, Seiwald, Burckhalter et al. (1958) replaced the Coon's unstable fluorescein isocyanate by fluorescein isothiocyanate, which was a stable compound. For a variety of reasons, the technique did not receive the recognition it merited, and though diagnostically applied for at least 20 years, only in recent years has it come into extensive use.

Virus- (or rickettsia-) infected cells, bacteria, fungi or parasites serve as the antigen, and are fixed to a solid glass support, in this case a microscope slide. Specific antibodies, if present in the patient's serum, bind to the corresponding (homologous) antigen. The resultant antigen-antibody complexes so formed are not visually discernible, but are made readily visible by the addition of antihuman immunoglobulins (IgG, IgM or IgA) labelled with a fluorochrome (fluorescent dye) such as rhodamine or, most commonly, fluorescein isothiocyanate (FITC). The tests are read by means of a fluorescence microscope system, using incident light illumination if possible, as its advantages have made the transmitted light system essentially passé. Specific fluorescence is seen as an apple-green fluorescent color.

The test is based on the fact that some dyes, here the fluorochromes, become «excited» when exposed to short wave length light in the violet-blue end of the spectrum. When returning to their quiescent state, the dye molecules emit light which is in the longer wave length spectrum and visible. The light source recommended is a 75 W Xenon arc, which has a spectral sensitivity close to daylight and matches the requirements of the FITC spectral curve. For a number of reasons, this lamp has essentially replaced high-pressure mercury arc lamps.

The band of light at 470-490 nm wave length, obtained by the use of an appropriate filter, serves to excite the fluorescein near its optimum of 495 nm. Ideally suited for this purpose are interference filters (Krech and Jung, 1972) as produced by Wild and Leitz. A barrier, or suppression, filter of 515 nm allows the passage only of light above this wave length, which is in the visible spectrum. Water or saline objectives are preferred instead of oil immersion objectives for three reasons: (a) cleanliness, (b) mounting of the preparation in buffered glycerol and use of a cover glass is unnecessary, and (c) preparations may be stored at 4-8° C or at −20° C for several weeks without significant loss of specific fluorescence.

The apple-green color seen in a positive test reflects the total amount of antibody immunoglobulins in the patient's serum, as the test *per se* does not differentiate between the several classes of immunoglobulins (i.e., IgG, IgM or IgA). Specific differentiation of antibodies of these three classes is possible, however, through the use of appropriate class-specific antihuman immunoglobulin conjugates (fluorochrome labelled).

For the detection of IgM antibodies, usually (but not always) associated with recent infection, it is necessary to reduce (total elimination is not required), the concentration of IgG to avoid the so-called competition phenomenon (Cohen, Norins and Julian, 1967). It will be recalled that IgG represents approximately 75% of the immunoglobulin content of normal human serum as compared to 10%

for IgM. One simple way to do this is through absorption with Protein A (Chantler, Devries, Allen and Hurn 1976; Skaug and Gaarder, 1978), which binds to the IgG immunoglobulins of most mammalian species. The binding is with the Fc portion of the immunoglobulins and does not interfere with the antigen binding capacity of the Fab moiety. Such absorption has no significant influence on the detection of IgM class antibodies, and serves to minimize false negative reactions (Chantler, Devries, Allen and Hurn, 1976).

The presence of rheumatoid factor (RF) of the IgM class may also interfere with the determination of specific IgM antibodies by indirect immunofluorescence (Fraser, Shirodaria and Stanford, 1971) resulting in false positive tests; these occur, however, only if specific IgG antibodies are also present in the patient's serum (Shirodaria, Fraser and Stanford, 1973). In this respect the situation resembles that of other solid-phase immunoassays.

RF of the IgM class can be removed, as indicated in the section on tests for the determination of IgM antibodies, by the addition of aggregated IgG, or antihuman IgG produced in animals. Protein A can also be used but is somewhat less effective. A preliminary absorption of the patient's serum is an essential step in surveying sera for specific IgM antibodies; testing for RF without such absorption is an inadequate procedure. The existence of a hidden RF, with specificity for native gamma-globulins (IgG), not detectable in native serum, has been described (Allen and Kunkel, 1966).

It will recalled here that FITC may be replaced (although not in all cases, for example minute organisms such as spirochets etc.) by horseradish peroxidase enzyme; an adequate substrate is needed to visualize the reaction and ordinary light microscopes can be used to read the test results.

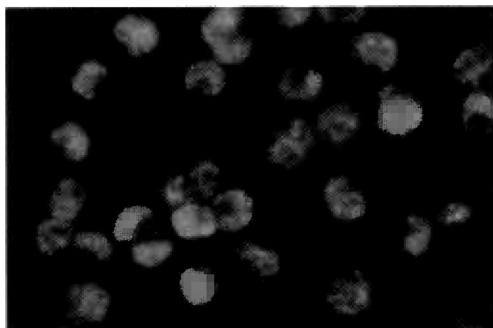


Figure 13. Raji cells (B-lymphocytes). Indirect immunofluorescent antibody test (IFA) with FITC for Epstein-Barr virus nuclear antigen (EBNA).

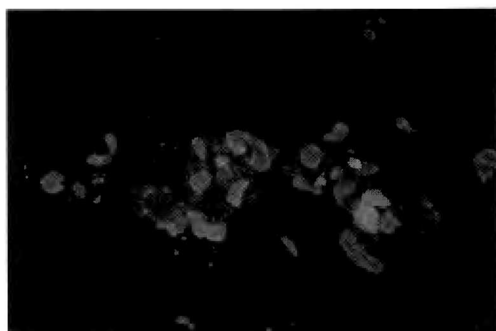


Figure 12. Characteristic focus of degeneration by human cytomegalovirus (Davis) in cultured fibroblasts. Indirect immunofluorescence (IFA) with FITC.

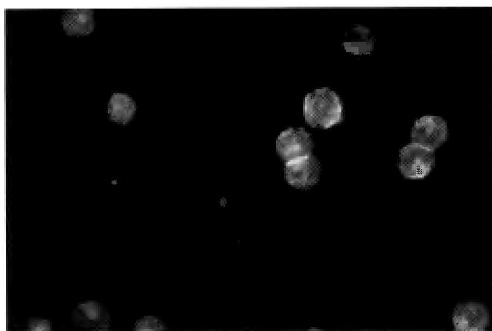


Figure 14. Human T-Leukemia Virus-1 (HTLV-1) in human T-lymphocytes. Indirect Immunofluorescence (IFA) with FITC.

Examples of viral antigens stained by the indirect fluorescent antibody method are shown in *Figures 12, 13* and *14. Figure 12* is presented to show the foci of cellular degeneration produced by human cytomegalovirus in a fibroblast culture, and the over-all pattern of apple-green fluorescence reveals the accumulation of viral material in the infected area. *Figure 13* shows the typical anticomplement indirect immunofluorescence staining pattern of Epstein-Barr virus nuclear antigen (EBNA) in Raji cells (B-lymphocytes). EBNA reacts with homologous antibodies, if present in the patient's serum, and thus forms an antigen-antibody complex. This complex bind the complement (usually added in the form of fresh or lyophilized guinea pig serum), and the reaction is rendered visible by appropriate fluorescein-tagged anti-C3 serum. The staining pattern is nuclear and should be present in all cells.

The next figure, *Figure 15* is illustrative of the indirect immunofluorescence test for IgG antibody to Borrelia burgdorferi, agent of Lyme disease. The characteristic greenish staining of the microbial antigen is well brought out.

The next two figures, *Figures 16* and *17*, contrast the differences in staining when the same preparation of human T-lymphocytes (HUT-78 strain) infected with HIV-1 (formerly HTLV-III) is tested by the IFA method and by the immunoperoxidase method. *Figure 16* shows the typical green fluorescence of viral material within the infected cell, and *Figure 17*, photographed by visible light, shows the typical brownish cast of material stained by horseradish peroxidase-labelled conjugate and the appropriate substrate (Diaminobenzidine). A simple and lucid explanation of the principles (and application) of these two techniques is given by Forghani (1985). The advantage of the immunoperoxidase procedure is ability to read test results directly by light microscopy and without recourse to special instruments.

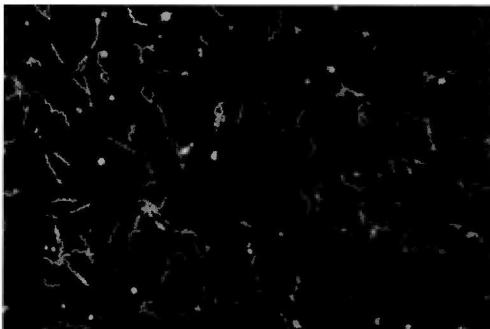


Figure 15. *Borrelia burgdorferi.* Indirect immunofluorescence (IFA) with FITC.

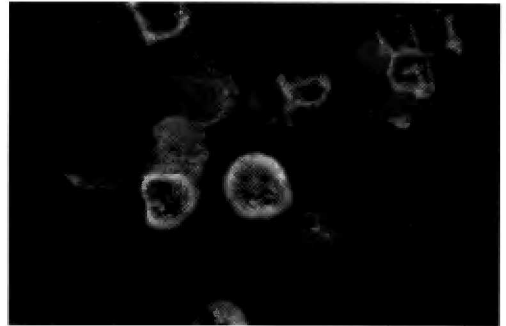


Figure 16. Human T-Lymphocytes (HUT-78) infected with Human Immunodeficiency Virus (HTLV-III, HIV-1). Indirect immunofluorescence (IFA) with FITC.

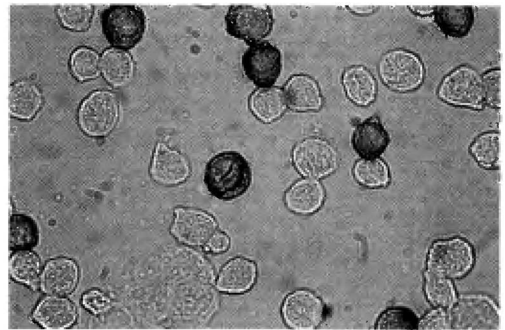


Figure 17. Human T-Lymphocytes (HUT-78) infected with Human Immunodeficiency Virus (HTLV-III, HIV-1). Immunoperoxidase method.

To summarize the status of the indirect immunofluorescent antibody (IFA) test, it is highly versatile method which can be used to quantitate antibodies in the various immunoglobulin fractions, and whose reading and interpretation is facilitated by the pattern of cellular distribution (nucleus, cytoplasm, membrane) of the specific antigen.

Complement-Fixation (CF) Test

The complement-fixation (CF) test, based on principles enunciated by Bordet (1895) and now nearly a century old, is still an important basic immunoserologic test in the field of medical microbiology. In the several areas of microbiology (viral, rickettsial, bacterial, fungal and parasitic serology) it has served as a reference standard against which other methods have been compared. Despite the recent development of procedures with much higher levels (in some cases) of sensitivity, or equal sensitivity but higher reactivity (in other cases), the test continues to plaÿ an important role in the serologic domain. Its partial displacement by other techniques is due not to problems inherent in the method, but rather to the unavailability of reagents of satisfactory quality.

The test is based on the fact that immune complexes formed during the interaction of antigen and antibody will bind complement, and thereby remove it from the reaction system. This *in vitro* test can thus be used to detect and to quantitate either antigen or antibody, as appropriate, and requires only that one or the other of these reactants be known.

The CF test is conducted in two stages. In the first (incubation overnight at 4-8° C), complement is irreversibly bound (fixed) to the antigen-antibody complexes which form if the patient's serum contains antibodies specific for the antigen used in the test. The magnitude of fixation reflects the relative concentration of antibody.

Complement, required for the test, is added to the antigen-antibody system in the form of fresh (or freshly re-constituted lyophilized) guinea pig serum. Since the concentration of complement in the reacting system must be accurately known (and so is carefully titrated for the test) and as all animal sera, including man, contain complement, the unwanted effect of unknown quantities of naturally occurring complement in the serum under examination is circumvented by heating the serum specimen at 56° C for 30 minutes (thermo-inactivation).

It was shown some 40 years ago that freeze-dried complement is stable at 15° C for at least 5 years (Flosdorf, 1949). If *lyophilized complement* is used it is *unnecessary* to perform daily titrations of the complement as still prescribed in some working instructions (cf, Palmer and Whaley, 1986). Theoretically, a single batch of lyophilized complement, tested carefully and found satisfactory, can be used over years in the same concentration (dilution), provided that other reactants in the system (antigen and erythrocytes) have not changed significantly.

The second stage of the CF test consists of the addition of an indicator system which permits reading and interpretation of the results. This stage is carried out at 37° C for 30 minutes.

To reaction mixtures which were incubated overnight in the first stage are added sheep erythrocytes which, shortly before, have been treated (sensitized) with antisheep red cell hemolytic serum (hemolysin). Lysis of the sensitized erythrocytes occurs only in the presence of free complement, which is unbound, or not «fixed» to the antigen-antibody complex. Thus, the indicator system provides visual evidence as to whether specific antigen-antibody union has occurred.

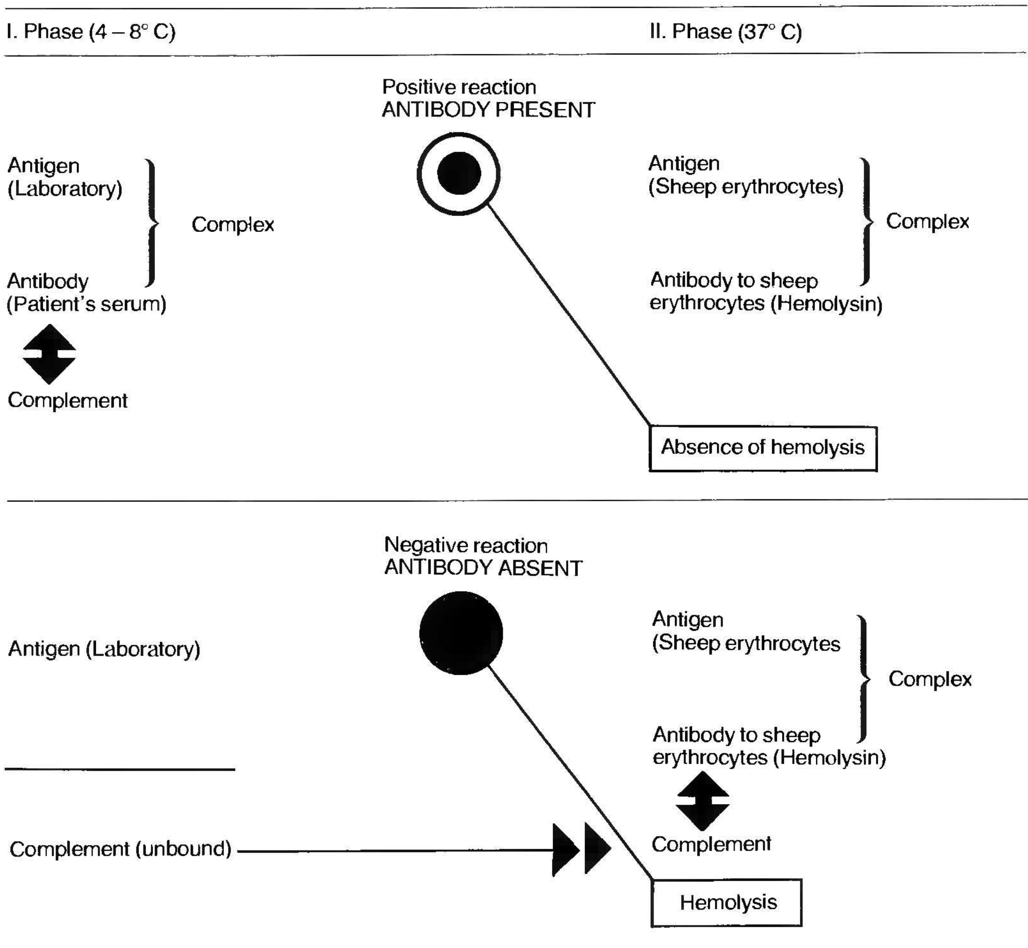
Stated differently, hemolysis of sheep erythrocytes reflects the action of free (unbound)

complement, i.e., antigen-antibody complexes were not formed, which means the absence of antibodies to the antigen used in the test. Inhibition of hemolysis, on the other hand, means the absence of free complement, which was bound by the antigen-antibody complexes formed in the first stage of the test. The result indicates the presence of antibodies, and if serial dilutions of the serum specimen were used in the test, the results can be quantitatively expressed.

Figure 18 is a schematic illustration of how the several components of the CF test react, and *Figures 19, 20* and *21* illustrate positive and negative reactions, both by the classic (now seldom done) test in small tubes, and the micro-test as done today in the wells of a 96-well polystyrene plate.

The CF test is characterized by a relatively low reactivity (sometimes equated with sensitivity) as compared to some of the more

Figure 18. Schema of the Complement-Fixation (CF) Test for Detection of Antibodies.



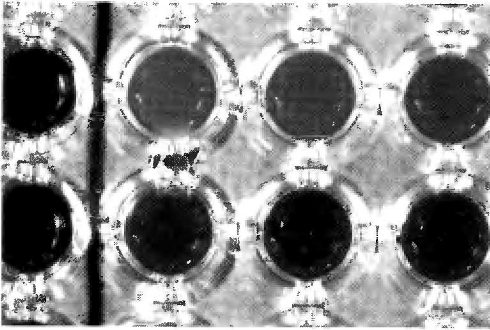


Figure 19. CF test in a 96-well test microplate using 0.025 ml amounts of reactants. 1st well: serum control; 2nd, 3rd wells: serial serum dilutions (1 : 8, 1 : 16, etc.).

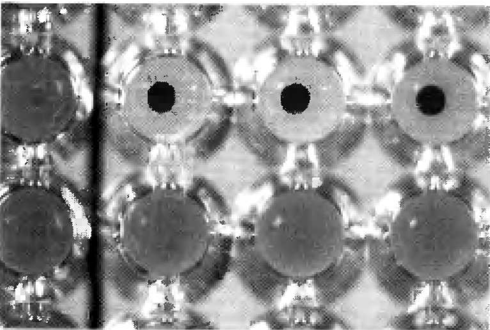


Figure 20. Same test as Figure 19 but plate centrifuged at 600 rpm for 5 minutes to stabilize the reaction, and permit exact reading even hours after stopping the incubation of the second (final) phase.

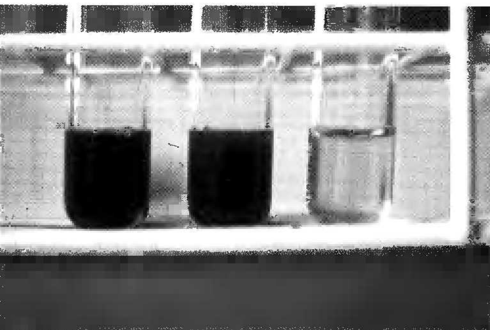


Figure 21. CF test in glass tubes with 0.2 ml of reactants, as done prior to the introduction of micromethods.

recently introduced diagnostic methods such as enzyme-immunoassays or radio-immunoassays. In examining sera for antibodies to viral agents such as cytomegalovirus (Booth, Hannington, Aziz and Stern, 1979); herpes simplex virus (Denoyel, Gaspar and Noyrigat, 1980); Epstein-Barr virus, *Toxoplasma gondii,* measles virus (Kleiman, Blackburn, Zimmerman and French, 1981; Kahane, Goldstein and Sarov, 1979) the sensitivity of the CF test in detection of antibodies correlated well with that of the widely-used enzyme immunoassays (and other tests as well). An evaluation of the comparative specificity and sensitivity of the CF test and an immuno-enzyme assay is given in *Table 5,* and shows that both tests are of comparable, and high sensitivity, as well as of equally high specificity. In another survey, a distribution plot was made of antibodies as detected by the CF test in comparison with those detected by indirect immunofluorescence; the results of the two methods are given in plot form in *Figure 22,* and the good concordance between methods is readily apparent.

It might be mentioned here that inasmuch as the CF test does not distinguish antibodies of the IgG and IgM classes, it provides a valuable means for the etiologic diagnosis of illnesses in the acute phase, at which time IgM antibodies make their appearance. *Table 6* lists the various classes and subclasses of serum immunoglobulins which fix complement.

As already noted, IgM class antibodies are characteristic of a recent infection, and, with some exceptions, are of relatively short persistence. However, IgG class viral antibodies detectable by the CF test persist for months or longer in the general run of many viral infections, but decline to very low or undetectable levels in bacterial infections. As shown in *Table 6,* the important, or dominant, subclasses of IgG are IgG1 and IgG3, which are

Table 5. Comparison of Complement-Fixation (CF) Test and Enzyme Immunoassay (EIA) for Cytomegalovirus (CMV).

EIA		Number	CF Results		Percent*	Percent**
Lot	Results	Samples	Positive	Negative	Sensitivity	Specificity
A	Positive	669	633	36	95.0 (633/669)	
	Negative	711	26	685		96.0 (685/711)
B	Positive	742	706	36	95.0 (706/742)	
	Negative	725	37	688		95.0 (688/725)
C	Positive	1429	1387	42	97.0 (1387/1429)	
	Negative	1438	117	1321		92.0 (1321/1438)
Total	Positive	2840	2726	114	96.0 (2726/2840)	
	Negative	2874	180	2694		94.0 (2694/2874)

* % Sensitivity = (No. CMV CF positive / No. CMV EIA positive) x 100
** % Specificity = (No. CMV CF negative / No. EIA negative) x 100

associated with infections with viruses such as herpes simplex, cytomegalovirus (Linde, Hammarström, Person et al., 1983), Epstein-Barr (Kaschka, Hilgers and Skvaril, 1982), varicella-zoster, poliovirus, rotavirus, influenza A, parainfluenza type 1, tick-borne encephalitis, rubella (Linde, 1985), rabies and HIV-1. In measles and mumps infections, the antibody response is dominated by the IgG1 subclass. IgG2 and IgG4 are subclasses with little or no antibody activity. One exception is hepatitis B antigen, to which antibodies are found in the IgG1, IgG3 and IgG4 subclasses (Morell, Roth-Wicky and Skvaril, 1983).

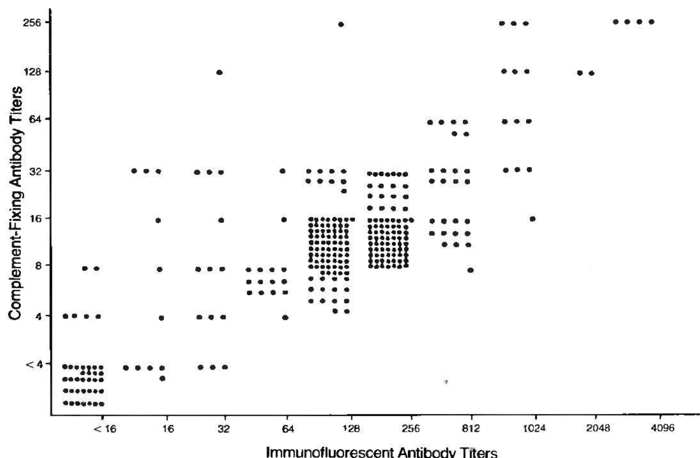


Figure 22. Comparative distribution of *Toxoplasma gondii* antibody titers by complement-fixation and indirect immunofluorescence test (296 cases).

Table 6. Immunoglobulin Classes and Subclasses.

IgG				IgM	IgA		IgD	IgE
IgG1	IgG2	IgG3	IgG4		IgA1	IgA2		
++	+	+++	–	+++	–	–	–	–

Interpretation of the results of CF tests has a decades-long background of experience. Serologic conversions or increases in antibody titers in paired or multiple sera are obviously significant, and need no further explanation. However, high CF antibody titers (1:64) may sometimes be of diagnostic importance, especially when the serum sample was taken late in the disease. Such high titers are of very short duration (4 to 8 weeks) in influenza, parainfluenza and mumps infections, but may on the other hand persist for many months in human herpes virus infections (cyto-megalovirus, Epstein-Barr virus, herpes simplex and herpes zoster virus). Although the height of the CF titer cannot be used as an absolute diagnostic criterion, the mere presence of such a titer correlates well with the detectability of IgM antibodies; the CF test rarely gives negative results in such cases *(Figure 23)*.

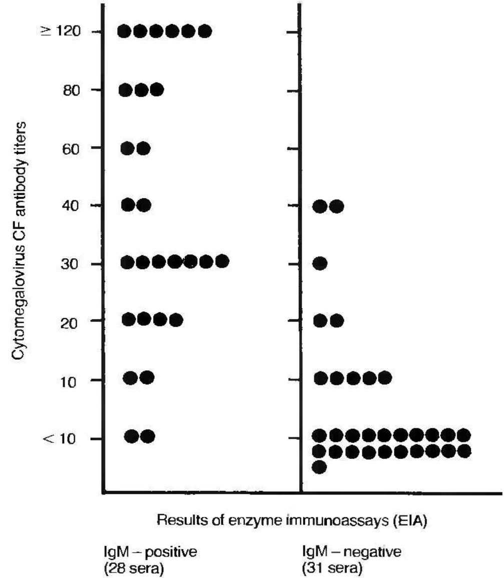


Figure 23. Cytomegalovirus complement-fixing antibody titers in IgM positive (left) and IgM negative (right) sera.

The results of CF tests in bacterial diseases are generally easier to interpret, since antibodies usually do not persist more than 3 to 9 months after infection, and only very briefly at high titers (≥ 1:64). A survey of antibody prevalences to a number of microbial agents in an apparently normal healthy population (blood donors, Switzerland) is summarized in *Table 7;* only titers of 1:64 were considered significant, for the reasons just stated, and it will be noted that such titers were associated only with viral infections.

In summary, the major advantages of the CF test are:

1. Freeze-dried antigens and antisera (Jung, 1985) as well as complement (Flosdorf, 1949) are stable over many years at 4-8° C.
2. The low cost per specimen as compared to other methods.
3. Markedly more potent antigens obtained through improved extraction methods are now available. An example how the potency of a native antigen may be increased by extraction with a buffer at high pH (Schmidt and Lennette, 1966; Krech and Jung, 1971) is given in *Table 8.*
4. For some diseases, the CF test is the only diagnostic test commercially available.
5. The large array of microbial antigens, now more than 45, available to the laboratory.

Table 7. Prevalence of High-titered CF Antibodies in a Healthy Population (Blood Donors, Switzerland) to a Variety of Microbial Antigens.

Antigens	Percent CF ≥1:64	Antigens	Percent CF ≥1:64	Antigens	Percent CF ≥1:64
Influenza A	0	Influenza B	0	Parainfluenza 1	0
Parainfluenza 2	0	Parainfluenza 3	0	Adenovirus	0
RS virus	1	Mumps	0	Picornaviruses	7
Cytomegalovirus	4	Epstein-Barr	1	Herpes simplex	7
Measles	9	Varicella-zoster	1	Arbo-B (european)	0
Rotavirus	1	Polyoma	0		
Brucella	0	Listeria	0	N. gonorrhoeae	0
Yersinia 03	0	Yersinia 09	0	Yersinia pseudotbc.	0
Camp. jejuni	0	Camp. fetus	0	Shigella dysenteriae	0
Shigella flexneri	0	Shigella sonnei	0	Leptospirae	0
Chlamydiae	0	M. pneumoniae	0	Coxiella burneti	0
Toxoplasma gondii	0	Entamoeba hist.	0	Echinococcus	0

(summarized from Jung, M., Serologic Diagnosis of Bacterial Infections, 1983)

Table 8. Comparison of Native and Glycine-Extracted Complement-Fixing Antigens for Cytomegalovirus.

Patients			Antigen titrations										
Age in months	Serum number	Antigen dilution	Native antigen Serum dilutions					Glycine-extract Serum dilutions					
			10	20	40	80	160	10	20	40	80	160	320
5	10471	1:1	2	2	0	0	0[1]	2	2	2	2	0	0
		1:2	2	2	0	0	0	2	2	2	2	0	0
		1:4	2	1	0	0	0	2	2	2	0	0	0
		1:8	0	0	0	0	0	2	2	2	0	0	0
		1:16	0	0	0	0	0	2	2	1	0	0	0
		1:32	0	0	0	0	0	2	2	0	0	0	0
6	10995	1:1	2	1	0	0	0	2	2	2	2	0	0
		1:2	2	2	0	0	0	2	2	2	2	0	0
		1:4	2	0	0	0	0	2	2	2	2	0	0
		1:8	0	0	0	0	0	2	2	2	2	0	0
		1:16	0	0	0	0	0	2	2	0	0	0	0
		1:32	0	0	0	0	0	2	2	0	0	0	0
8	9890	1:1	0	0	0	0	0	2	2	0	0	0	0
		1:2	0	0	0	0	0	2	2	0	0	0	0
		1:4	0	0	0	0	0	2	2	0	0	0	0
		1:8	0	0	0	0	0	2	2	0	0	0	0
		1:16	0	0	0	0	0	0	0	0	0	0	0
		1:32	0	0	0	0	0	0	0	0	0	0	0
3	9413	1:1	1	0	0	0	0	2	2	2	2	2	0
		1:2	0	0	0	0	0	2	2	2	2	2	0
		1:4	0	0	0	0	0	2	2	2	2	2	0
		1:8	0	0	0	0	0	2	2	2	2	2	0
		1:16	0	0	0	0	0	2	2	2	2	1	0
		1:32	0	0	0	0	0	2	0	0	0	0	0

Reproduced from Krech, U., Jung, M.: Z. Immun.-Forsch. *141*, 411-429, 1971 with permission of the publisher (Gustav Fischer Verlag, D-7000 Stuttgart).

(1) 2 = means 100 percent fixation
1 = means 50 percent fixation
0 = means no fixation of complement.

6. CF test is the only one which permits the use of a battery of antigens by simple incorporation into the test run. This is perhaps the most important advantage because additional antigens can be added to the run without disrupting the routine of the operation. The clear advantage of such an approach is well recognized (see, for example, Cremer, Devlin, Riggs et al., 1984). Increasing the spectrum of antigens correspondingly broadens the opportunities to identify the etiologic agent of the patient's illness, and no other method currently available possesses this versatility and at such low cost. This contrasts with some of the more recently introduced diagnostic methods, such as enzyme immunoassay, where testing against a series of microbial antigens is impractical (and expensive as well) because of the additional controls required by the multiplicity of antigens.

7. Antigenic cross-reactivities, where they occur, are well known and can be compensated for.

Direct Agglutination

Direct agglutination of bacteria can be used for the detection of antibody homologous to that organism, and hence for serologic diagnosis. The bacterial cells to be used as antigens may be living or killed in a variety of ways (formalin, phenol, boiling, etc.). The reaction is complex, involving a number of epitopes on the bacterial cell and determinants on the polyclonal antibodies. Agglutination is generally considered to be mainly to be function of the IgM immunoglobulin subclass (Dick, 1983).

The aggregates formed by the clumping of the bacteria in a positive reaction are readily visible to the naked eye, and antibody quantitation, based on the use of serial dilutions of the unknown serum specimen, is readily achieved.

In recent years agglutination tests have been adapted for use with 96-well polystyrene microplates permitting reduction in the amount of antigen needed for the test as well as automation of the procedure. Reading is best done by low-power magnification (6 to 10 ×) of a stereo-microscope. An agglutination test is illustrated in *Figure 24.*

Agglutination tests have been used mostly in bacterial serology for the detection of antibodies to Bordetella pertussis, Salmonellae, Brucellae, Yersiniae, Leptospirae, etc.). These tests are, in general, somewhat more serotype-specific than in the CF, and this must be considered if the test is used for diagnostic purposes.

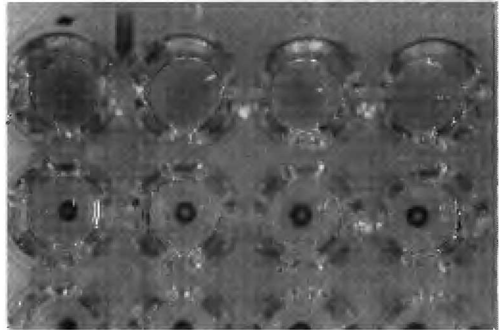


Figure 24. Serial dilutions of a patient's serum tested against two Salmonella-antigens. Upper row: antibodies present (agglutination positive – note the clumping ◎). Lower row: antibodies absent (agglutination negative; setting, not clumping, of the cells has occurred ⊙).

Indirect Agglutination

(Passive Agglutination and Passive Hemagglutination)

The passive agglutination (PA) technique has been known for quite some years, and although characterized by a high sensitivity, it has not found wide acceptance in the diagnostic laboratory. Kits for antibody detection are available for only a few agents, which might be interpreted as a reflection of the laboratories needs. On the other hand, the dearth of commercially-available kits may mirror the manufacturers lack of interest.

The principle of the test is quite simple. The antigen is attached to a carrier particle such as latex, bentonite or red blood cells, which can be done by any of several methods. When the «sensitized» carrier is added to a test serum, antibody, if present, will attach to the particle-bound antigen and produce grossly visible aggregates. Passive hemagglutination (PHA) is the designation for tests in which the carrier particle is a red blood cell.

Passive agglutination has perhaps found its widest use in the detection of Rheumatoid Factor (RF), an anti-human IgG antibody often encountered in the IgM fraction of normal human sera.

Figure 25. Homogenous suspension of IgG-coated latex particles mixed with the patient's serum which does not contain antibodies (RF) to IgG (negative reaction).

Figures 25 and *26* illustrate a latex agglutination test done on a plastic slide, and read under low (10×) microscope magnification (the clumping is also visible to the naked eye). Latex particles, coated with IgG immunoglobulins, were used to detect antibodies to IgG (RF or rheumatoid factor) which may, under certain circumstances, be present in the patient's serum.
Passive hemagglutination tests are available in the form of diagnostic kits for the detection of antibodies to *Treponema pallidum, Toxoplasma gondii, Entamoeba histolytica, Echinococcus granulosus* and, if coated with antibodies to Hepatitis B_s antigen, for the detection of Hepatitis B_s antigen. However, in recent years many of these tests have been replaced by indirect enzyme immunoassay for IgG antibodies to these agents.
Passive hemagglutination was also used in rubella for the determination of immunity status. It is not recommended for the diagnosis of rubella, or for determining recent rubella infection in the pregnant woman, because PHA antibodies to this virus appear later than do those detectable by the hemagglutination-inhibition test (HAI).

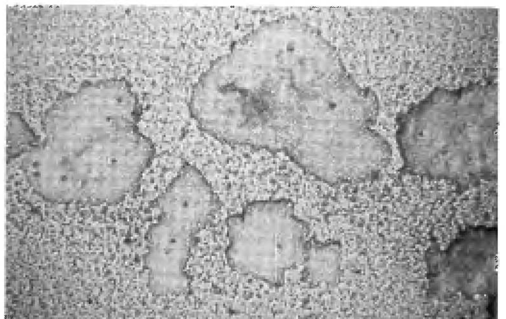


Figure 26. Agglutination of IgG-coated latex particles by antibodies (RF) to IgG present in the patient's serum (positive reaction).

Hemagglutination-Inhibition (HAI) Test

A number of viruses (within several taxa) possess the ability to agglutinate the erythrocytes of man and some animal species, and this phenomenon (hemagglutination) forms the basis for a sero-diagnostic test for this diverse group of viruses. Hemagglutinating viruses give rise to antibodies which *in vitro* can inhibit or negate the hemagglutinating activity of the virus, and so this test, known as the hemagglutination-inhibition test, can be used to quantitate this antibody.

The test is simple and inexpensive, and can be routinely applied to a large volume of specimens. A fixed, pre-determined dose of viral hemagglutinin (supernatant fluid from virus-infected cell cultures) is added to serial dilutions in a microplate of the serum specimen under test, the reactants are incubated, and then erythrocytes are added as an indicator system. If specific antigen-antibody union has occurred, there will be no agglutination, i.e., hemagglutinating capacity has been neutralized (inhibited) by the antibody, and the red cells will sediment in a confluent pattern. In a negative test, the cells will settle out at the bottom of the well as a discrete button.

One drawback to the HAI test is the need to remove non-specific (non-antibody) inhibitors of hemagglutination which occur in many human sera as a normal constituent, and may give false positive results unless inactivated or removed. This can be done in a number of ways by treatment with receptor destroying enzyme (derived from cultures of *Vibrio cholerae*), kaolin, potassium periodate (KIO_4), heparin-manganous chloride, the procedure(s) used varying with the virus. Some sera also contain natural hemagglutinins, which will agglutinate the red cells; if the serum control wells show red cell clumping (agglutination) in the absence of the virus, the specimen can be re-tested after absorption with red cells to remove the natural agglutinins.

Figure 27 illustrates the appearance of a HAI test.

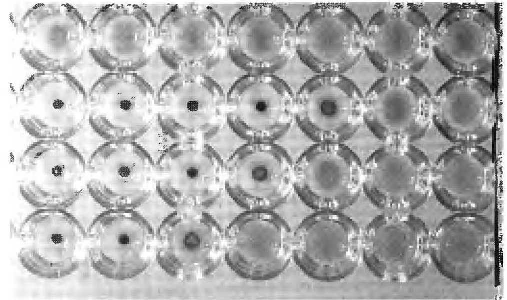


Figure 27. Hemagglutination-inhibition (HAI) test for antibodies to rubella virus. Erythrocyte sedimentation (⊙) means the presence of antibodies which have inhibited the hemagglutinating effect of the virus. Hemagglutination (◌) means the absence of antibodies, since the virus is able to exert its agglutinating properties.

The HAI test is used diagnostically to detect antibodies to influenza A and B viruses*, the parainfluenza viruses, mumps virus, measles virus, adenoviruses*, arboviruses, some picornaviruses and also to rubella virus. The HAI test for rubella virus is an excellent and readily reproducible test, both for the diagnosis of acute infection as well as for determining immunity status in the pregnant woman, or prior to vaccination. The test is also applicable to the determination of IgM class antibodies after fractionation of a serum or removal of IgG antibodies.

* Unlike the CF test, which measures group specific antigens common to influenza A virus types, the HAI test is type-specific and hence requires that antigenically appropriate virus strains be used. Similarly, the CF test is generally preferred for diagnosis of adenoviral infections because it measures group antigens, whereas the HAI test (and also neutralization test) have the limitation of being type specific.

Precipitation Test

When an antigen in solution is mixed with an antibody in serum, a visible precipitation, or flocculation, occurs. This test, known as the precipitin test, forms the basis for a number of tests devised for special purposes, and each with its own identifying designation. Among these, the Oudin test, the Ouchterlony test, the Mancini test and the immunoelectrophoresis technique come to mind. These were designed primarily to detect or separate antigens, although adaptation to detection of antibody has been effected, as in the case of the Ouchterlony and immunoelectrophoresis methods. In the Ouchterlony test, wells are cut out of the agar surface with a template die to give a pattern of a central well, into which the antigen is placed, and a series of peripheral wells into which the patients' test sera are placed – as the antibody diffuses towards the antigen, a precipitin line, or line of identity, will form.

In counter-immunoelectrophoresis (the opposite of immunoelectrophoresis), the antigen and the homologous antibody are pulled (or driven) toward each other by an electrical current, and a precipitin line forms in that area of the electrical field in which the antigen/antibody proportions are optimal.

Precipitin tests currently find little (Jung, Price, Kistler and Krech, 1973) or no application in the routine diagnostic laboratory for the detection and quantitation of antibodies. Immunodiffusion tests still have an important place, however, in identification of viral antigens.

Radioimmunoassay (RIA)

In diagnostic microbiology, radioimmunoassays have been developed primarily for virology, employing labelled antibody and using so-called non-competitive procedures.

The indirect RIA ist used for assay of antibodies. The viral antigen preparation is bound to a solid support such as the wells of a microtiter plate or to glass beads or a shell vial; the antigen preparation may be virus-infected cells or a lysate of such cells. To the support-bound antigen is added the test serum, followed, after an incubation period to permit of antigen-antibody union, by an anti-human IgG serum labelled with a radio-isotope, generally ^{125}I. After further incubation, the test is read by the number of counts per minute emitted by the label as compared with a control preparation without specific antigen.

RIA has virtually disappeared from the routine diagnostic laboratory because of a) the cost of the radio-label, b) its short shelf life, c) the hazard associated with the use of radio-active material, and d) the difficulties of disposing of such material. This test has been replaced in major part by immuno-enzymatic methods.

Neutralization Test (NT)

The neutralization (NT) test is one of the oldest and best known tests in diagnostic virolo gy, dating from the early work of Sternberg (1898) on vaccinia virus. Almost from its inception, it has been recognized as a «gold standard» against which other serologic methods have been compared and evaluated.

Sternberg noted that when vaccinia virus is mixed with its corresponding homologous antibody, it may be rendered non-infective for a susceptible host; the infective capacity of the virus is blocked, or «neutralized». This property thus provides a means for detecting (and measuring) an antibody response and, conversely, using a specific immune serum, for identifying a virus. The test can be done in any susceptible host system – animal, embryonated hens egg, or cell culture – although cell cultures are usually employed today because of the broad variety of susceptible cell lines and strains available. In a cell culture, free virus produces pathologic cellular changes, the so-called «cytopathic effect» and, in general, also death of the infected cell. If infectivity of the virus is neutralized by antibody, the cytopathogenic properties of the virus are blocked and cellular destruction does not occur.

In principle, the test is usually conducted by mixing a pre-determined dose of virus with serial (usually two fold) dilutions of the patient's serum, and inoculating the mixtures into cell cultures or animals, usually mice. Because of variations in susceptibility between individual cell cultures or individual animals, each mixture is inoculated into several cultures or animals to permit statistical evaluation of the data and computation of a 50% endpoint (see Hawkes, 1979; Leland and French, 1988).

The neutralization test is relatively simple in execution, but is seldom used in diagnostic laboratories for antibody assays because of several reasons: (1) it is labor intensive, (2) the material costs are high, especially the cost of cell cultures when supplied commercially, (3) several days may be required before the final reading can be done, and (4) the impracticality and prohibitive manufacturing cost of ready-to-use kits.
Figures 28 and *29* illustrate typical appearance of a neutralization test performed in tissue cultured cells.

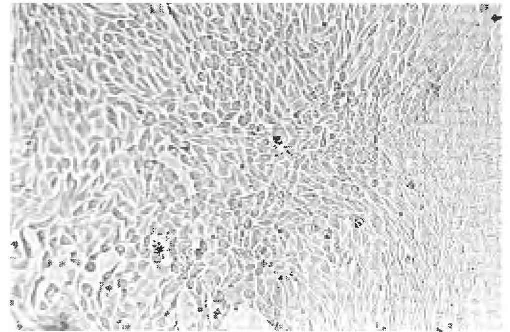


Figure 28. Neutralization test for antibodies to Coxsackie B-1 virus. Intact cells indicate neutralization of the virus. Monkey kidney cell culture. Magnification × 100.

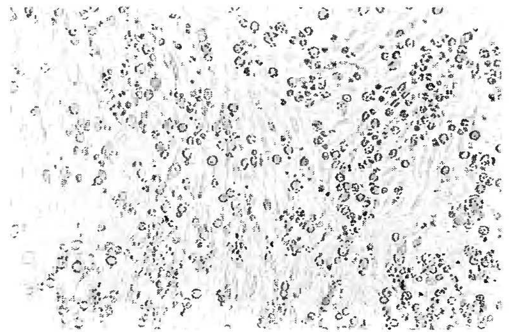


Figure 29. Typical cytopathic effect produced by Coxsackie B-1 virus which has not been neutralized (patient's serum does not contain specific antibodies). Monkey kidney cell culture. Magnification × 100.

Neutralization tests are used on occasion to detect antibodies to the polio- and Coxsackie viruses, types B 1-6; for epidemiologic surveys; and for determining the immunity status of an individual or the success of vaccination, mostly in cases of polio, rabies and measles vaccination.

Western Blot Method

The Western blot method as used in serology is an adaptation of the technique first described by Towbin, Staehelin and Gordon (1979) for electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. For the purposes of this test, the proteins from a sucrose-density gradient are electrophoretically separated on a polyacrylamide gel and then transferred to a nitrocellulose sheet, which is cut into strips for storage.

In the test itself, a strip is incubated with the patient's serum, then washed and treated with an anti-human IgG conjugated with horseradish peroxidase. After washing, the substrate and color reagent (di-aminobenzidine) is added. Antibodies reacting with the different viral antigens on the strip appear as distinct, separated bands with a brownish color.

The high molecular weight proteins band near the top of the strip, the lower molecular weights stratify toward the bottom. The color intensity of the bands is a function of both the relative concentration of the viral protein on the strip and the concentration of antibodies in the specimen reactive to that protein.

The Western blot is a highly sensitive and very specific test for antibodies to HIV-1, and since its sensitivity surpasses that of the ELISA test used for screening, it is employed as a confirmatory test on all specimens which prove positive by the ELISA technique.

Patients exhibit various response patterns, both qualitative and quantitative, by the Western Blot test. A number of protein antigens have been identified as associated with HIV, viz., p15, p17, p24, p31, gp41, p51, p55, p66, gp120, and gp160. Of these, the most frequently encountered in individuals with HIV infection are p24 and gp41. The position of these bands, and their arrangement according to molecular weight, is shown in *Figure 30.*

At present there is no general consensus on the interpretation of antibody reactivities detected by Western blot. However, the presence of antibodies to p24 or gp41, alone or in combination with other bands, may be regarded as a positive test (Morb. Mort. Weekly Report, 1985, 34, 1-5). Staining of p24 together with gp41 is considered unequivocally positive. (N.B.: the criteria for positivity vary among countries: refer to the guidelines of the local national health agency).

According to the Third Consensus Conference on HIV testing (Hausler, W.J., 1988) the major bands of diagnostic significance are gp160 or gp120, gp41, p24. A reactive blot must contain two of the three major bands of diagnostic significance.

Interpretation of the results is relatively simple when reactions with a number of antigens are present, and when the bands are strong.

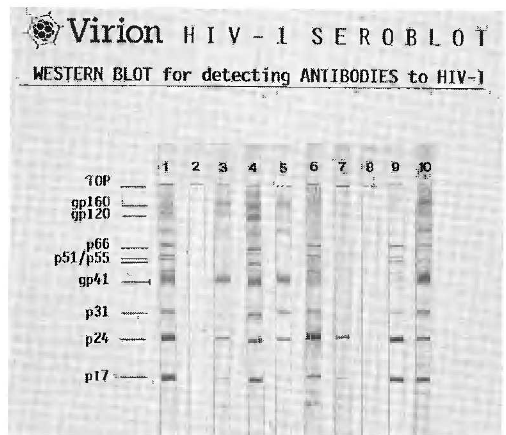


Figure 30. Western Blot for detecting antibodies to HIV-1.

Clinical Considerations

Quite often when the patient is first seen by a physician, the presenting signs and symtoms are those of a syndrome rather than markers characteristic of a specific disease. The aid of the laboratory is thus generally invoked to establish a definitive diagnosis, and to achieve this, certain important criteria must be met on the part of both the clinician and the laboratorian.

A cardinal need of the laboratorian is to maintain a good liaison and a good working relationship with general practitioners and specialists, veterinarians, epidemiologists, public health officers, and with colleagues at other diagnostic and reference laboratories. The practice of medicine today is a team effort involving participation of specialists from various medical disciplines, and all must work as part of an inter-dependent effort. Ideally, the working relationship between the attending physician and the laboratory should be a close one, so that the efforts of both are complementary and aimed at the common objective, the identification and management of the patient's illness.

Perhaps the greater responsibility in this dual partnership falls on the clinician, who should be aware that the laboratorian needs good, relevant clinical information to decide on a strategic plan of approach to the diagnostic problem as much as does the physician for a clinical diagnosis and for patient management. It also falls to him to see that the appropriate clinical specimens are properly collected, at the proper time, and are transported to the laboratory promptly and under the right conditions. This latter responsibility is outlined with clarity and in some detail by Madeley (1977) and, more recently, by Madeley, Lennette and Halonen (1988), whom the reader might consult.

Basic personal information required or clinically desired, includes name, address, sex, age/date of birth, identification number, occupation, pregnancy (gravida, parity) and family history, if important relevant background information is elicited. Clinical and epidemiologic background information includes date of onset and signs and symtoms of the illness, immunodeficiency state or immunosuppressive treatment, travel (when, where), prophylactic inoculation (vaccine, immunoglobulin), etc. Specimens for laboratory examination should indicate, in addition to the usual patient identifying data, the date of collection.

To assist the clinician-microbiologist team in the compilation and application of such information, we have divided the common infectious disease presentations into eleven categories, each division represented by a table. Each table has five headings for easy access to the desired information, viz., a) mode of transmission, b) incubation period, c) type of onset, d) disease symptoms/syndromes and e) complications. Only the salient characteristics are tabulated, and all descriptive material is omitted, so that each table constitutes a precis of the disease of interest. The numbers and titles of the several tabulations follow:

Table 9 Respiratory Diseases
Table 10 Infectious-Mononucleosis-Like Diseases
Table 11 Venereal Diseases
Table 12 Reactive Arthritis
Table 13 Pregnancy, Congenital Infections
Table 14 Central Nervous System Infections
Table 15 Exanthematic Diseases
Table 16 Infections in The Immunocompromised Individual

Table 17 Infectious Diarrheas
Table 18 Liver Diseases
Table 19 Ocular Infections

These tabulations are presented as a part of this Section.

A complementary tabulation, using the same disease categories, is presented according to the causal agents generally to be considered in selecting laboratory tests for diagnosis. Because of differences in geographic distribution or localization of more than just the commonest infectious diseases, the tabulations perforce cannot include every known infectious disease, and so are incomplete in this respect. Despite this, the various permutations listed should prove helpful to the medical microbiologist with respect to the different etiological agents which can produce the same clinical picture or syndrome and in the selection of test methods for the detection of these agents. The clinician also may find these tabulations of interest and help with respect to differential clinical diagnosis, and in requesting specific serologic tests.

Table 9. Respiratory Diseases

						Respiratory syncytial virus		Parainfluenza 1–3 viruses		Adenoviruses				Respiratory symptoms only		
		M. pneumoniae	Chlamydiae (psittaci, trachomatis)	Legionella pneumophila	Influenza A + B	Young children	Adults	Primary	Reinfection	Acute respiratory disease (ARD)	Pharyngo-conjunctival fever	Coxiella burnetii (Q-Fever)	Bordetella pertussis	(1) Cytomegalo-virus	(2) Measles virus	(3) Varicella virus (adult)
Mode of transmission	Respiratory route	+	+	+	+	+	+	+	+	+	+	+	+			
	Bite of a bird		+													
	Conjunctival inoculation					+	+									
Incubation period (days)		21	7–15	2–10	4–6	3–6		3–7		5–6		14–39	7–10			
Onset	Sudden		+	+	+	+	+	+	+	+	+	+				
	Insidious	+	+										+			
Disease symptoms / Syndromes	Fever	+	+	++	+	+	+	+	+	+	+	++	+/−			
	Chills	+	++	+	+	+	+					+				
	Rhinitis	+			+	+	+	+	+	+	+		+			
	Pharyngitis	+			+	+		+	+	++	++					
	Laryngitis (Croup)					+	+	++							+	
	Tracheitis, bronchitis	+				+	+	+	+/−	+			++			
	Bronchiolitis					++		+		+					+	
	Pneumonia	+	++	+		+	+	+		+		++		+		
	Conjunctivitis					+/−	+				++		+			
	Otitis media					+										
	Myalgias; arthralgias	+	+	+	+							+/−				
	Arthritis	+														
	Lymphadenopathy				+						+					
	Hepatomegaly		+									+				
	Splenomegaly		++									+				
	Rash		+									−				
	Nausea, vomiting		+	+/−												
	Cough with vomiting												++			
	Diarrhea		+	++												
	Meningitis						+									
	Confusion, delirium, coma		+	+												
Complications	Otitis media	+					+/−						++			
	Sinusitis	+														
	Epistaxis												++			
	Pneumonia	+			+		+						++	+	+	+
	Atelectasis												+			
	Pneumothorax												+/−			
	Bronchiectasis												+/−			
	Myo-, endocarditis	+	+		+/−							+			+/−	
	Rash	+														
	Arthritis		+													
	(Meningo) encephalitis	+			+/−		+									
	Mono-, polyneuritis	+														
	Convulsions												+/−			
	Ulceration of the frenulum linguae												++			
	Hernias, rectal prolapse												+			

(1) See also Tables 10, 12, 13, 16, 18

(2) See also Tables 14, 15

(3) See also Tables 12, 13, 14, 15, 16

Table 10. Infectious-Mononucleosis-Like Diseases

		(1) Cytomegalo-virus			(2) T. gondii	(3)
		Postnatal infection (Children)	Infection in normal adults	Epstein-Barr virus	Toxoplasmosis (in im-munocompetent patient)	Human immunodefici-ency virus-1 (HIV-1)
Mode of transmission	Perinatal / postnatal	+				
	Genital route		+			+
	Blood, urine, saliva, organs	+	+	+	+	+
	Parenteral route		+			+
	Fecal-oral route				+	
Incubation period (days)			20–60	30–60		>6
Onset	Sudden		+	++	+	+
	Insidious		++	+	+	
Disease symptoms / Syndromes	Fever		+	+		++
	Chills		+	+		+
	Pharyngitis	+	+/–		+	+
	Bronchitis	+				
	Myalgias, arthralgias		+		+	+
	Lyphadenopathy		+	+	+	++
	Hepatitis		+	+	+/–	
	Splenomegaly		+	+	+	+
	Rash			+	+	+
	Atypical mononucleosis	++	++	+	+	
	Heterophile antibodies	–	–	+		
	(Chorio)retinitis				+	
	Thrombocytopenia		+			
Complications	Pneumonia	+	+	+/–	+/–	
	Myocarditis		+	+/–	+/–	
	Arthritis		+			
	Rash		+			
	(Meningo) encephalitis		+	+		
	Guillain-Barré Syndrome		+	+		

(1) See also Tables 9, 12, 13, 16, 18
(2) See also Tables 13, 14, 16, 19
(3) See also Tables 11, 13, 14, 16, 17

Table 11. Venereal Diseases

		T. pallidum (Syphilis)			Chlamydia (trachomatis)					N. gonorrhoeae (Gonorrhoea)						Herpes simplex virus					(1)	(2)	(3)
																		Recurrent infection					
		Primary syphilis	Secondary syphilis	Tertiary (late) syphilis	Lyphogranuloma venereum	Nongonococcal urethritis	Epididymitis	Proctitis, colitis	Genital Chlam (Women)	Genital (Men)	Genital (Women)	In pregnant women	Pharyngeal gonorrhoea	Anorectal gonorrhoea	Disseminated gono-coccal infection	Primary infection	Primary genital infection	Herpes labialis	Genital lesions	Ocular lesions	Cytomegalovirus	Hepatitis B	Human immunodefi-ciency virus-1 (HIV-1)
Mode of transmission	Genital route	+			+	+	+	+	+	+	+	+									+	+	+
	Laboratory accident	+			+	+	+																
	Parenteral route	+/−																					+
	Fomites, devices, currettage				+	+	+		+														
Incubation period (days)		3–90	14–60	years	3–21					2–7	2–7					2–12	2–7						
Onset	Sudden		+		+	+			+	+	+					+	+	+	+	+			
	Insidious	+	+		+	+					++				+								
Disease symptoms / Syndromes	Fever		+		+										+	+	+						
	Chills		+		+										++	+	+						
	Gingivostomatitis															++							
	Pharyngitis		+										+			++							
	Pneumonia														+/−								
	Lymphadenopathy	+	+		++			+/−					+			+	+	+/−					+
	Conjunctivitis																			+			
	Atrhritis				+										++								
	Skin lesions	+	++		++										++								+
	Mucous membrane lesions	+																					
	Skin-, mucosal vesicles		–													++	++	++	++				
	Neurosyphilis, gumma			+																			
	Abortion, perinatal mortality											+											
	Urethritis				+	+	+		+	+	+/−	+/−											
	Proctitis							+			+			+									
	Cervicitis								++		++												
Complications	Fistulas				+																		
	Strictures				+																		
	Peritonitis								+		+												
	Perihepatitis								+		+												
	Epididymitis						+			+													
	Inguin. lymphadenopathy									+													
	Endometritis										+												
	Salpingitis										+												
	Bartholinitis										+												
	Ectopic pregnancy								+		+												
	Infertility								+		+												
	Meningitis														+/−								
	Keratitis																			++			

(1) See also Tables 9, **10**, 13, 16, 18 (2) See also Table 18 (3) See also Tables 13, 14, 16, 17

Table 12. Reactive Arthritis

		Neisseria gonorrhoeae	Chlamydiae	Campylobacter jejuni, C. fetus	Yersinia enterocolitica	Brucella	Salmonellae	Shigellae	Picornaviruses (ECHO, Coxsackie)	Borrelia burgdorferi (Lime disease)	Rubella virus	Mycoplasma pneumoniae	Adenoviruses	Cytomegalovirus	Varicella virus	Hepatitis B virus	T. pallidum (Secondary syphilis)
Mode of transmission	Respiratory route		+						+		+	+	+	+	+		
	Bite of a bird		+														
	Laboratory accident		+														+
	Fomites, devices, currettage		+										+	+			
	Fecal-oral route			+	+	+	+	+	+								
	Genital route	+	+											+		+	+
	Arthropod-borne									+							
	Parenteral route													+		+	+
Incubation period of primary disease (days)		2–7	3–21	2–6		7–21	1/4–2	1–7	2–14	4–18	14–21	21	5–6	20–60	10–21	30–180	3–90
Disease symptoms / Syndromes	Fever	+	+	+	+	+	+	+	+	++	+/−	+	+	++	+	+	+/−
	Arthritis	++	+	+	+	+	+	+	+/−	+/−	++	+	+/−	+	+/−	+	+
	Lymphadenopathy	+/−	++	+/−	+/−	++	+/−				++		+	+			+
	Rash	+				+	+/−		+	+	++		+		++	+	++
	Pneumonia		+			+/−				+/−		++	+	+/−			
	Hepatomegaly					+	+			+				+/−		+	
	Splenomegaly					++	+			+	+/−			+		+	
	Enteritis, colitis			+	+		+	++	+								
	Meningitis	+/−				++			+	++					+/−		
	Encephalitis					++			+		+/−				+		

Table 13. Pregnancy, Congenital Infections

							Listeria monocytogenes				(1)	(2)	(3)	(4)			(5)	
								Child										
			Rubella virus	Cytomegalovirus	Treponema pallidum	Toxoplasma gondii	Mother	Granulomatosis infantiseptica	Sepsis	Herpes simplex viruses 1 and 2	Hepatitis B virus	Neisseria gonorrhoeae	Coxsackie B viruses	Mumps / Parotitis virus	Chlamydiae (C. trachomatis)	Varicella virus	Human immunodeficiency virus-1 (HIV-1)	Measles virus
Mode of transmission	to the mother	Respiratory route	+							+			+	+		+		+
		Laboratory accident			+	+												
		Fomites, devices		+						+				+	+			
		Fecal-oral route				+	+						+					
		Genital route		+	+					+	+	+			+		+	
		Parenteral route		+	+	+					+						+	
	to the child	Intrauterine infection	+	+	+	+		+		+		+		+		+	+	+
		Perinatal (genital tract)		+					+	+		+	+					
		postnatal: Maternal milk, -excretions		+								+						
		postnatal: Transfusion		+														
		postnatal: Devices								+								
Disease symptoms / Syndromes in child		Lymphadenopathy			+	++												
		Rash			++	+		+	+				+					
		Pneumonia			+	+												
		Osteochondritis			++													
		Abortion, prematurity	+	+	+			+		+						+		+
		Stillbirth	+		+			+						+				
		Congenital heart disease	++															
		Deafness	++		+											+		
		Cataract	++													+		
		Retinopathy	+	+		++				++						+		
		Hepatosplenomegaly	+	++	+	++				+			+/−					
		Meningoencephalitis	+	+		+		+	++				+					
		Mental retardation	+	+		+				+						+		
		Low birth weight	+	+										+				
		Thrombocytopenia	+	++	+	+				+			+					
		Abscesses and granulomas in various organs						++										
		Conjunctivitis								+		+			+			
		Skin vesicles								++								
		Arthritis										+						
		Proctitis										+						
		Skin scarring														++		
		Fever							+				+/−					
		Disseminated infection											+					

(1) See also Tables 12, **18**
(2) See also Tables **11,** 12, 15, 19
(3) See also Tables 12, **14,** 15, 17
(4) See also Table **14**
(5) See also Tables 10, **11,** 16

Table 14. Central Nervous System Infections

		Mumps / Parotitis virus	Adenoviruses	Picorna viruses (ECHO, Coxsackie)	Tick-borne encephalitis (RSSE)	Varicella virus		Borrelia burgdorferi (Lime disease)	Rickettsiae	Polioviruses 1–3	Neurological symptoms only						
						Varicella	Herpes zoster				(1) Epstein-Barr virus	(2) Herpes simplex virus 1	(3) Leptospirae	(4) Toxoplasma gondii	(5) Measles virus	(6) Human immunodeficiency virus (HIV-1)	(7) Treponema pallidum
Mode of transmission	Respiratory route	+	+	+		+						+			+		
	Fomites	+										+					
	Fecal-oral route			+						+			+	+			
	Arthropod-borne				+			+	+								
	Reactivation of latent virus						+					+					
	Blood, urine, saliva, organs										+					+	
	Genital route											+				++	+
	Exposure to the animals												+/–				
	Laboratory accident													+			+
	Parenteral route													+		++	+
Incubation period (days)		14–28	5–6	2–14	7–20	10–21		4–18	7–10	5–35	30–60	2–12	7–12		10–14		3–90
Onset	Sudden	+	+	+	+	+	+	+	+	+	++	+	+		+	+	
	Insidious			+			+				+					+	
Disease symptoms / Syndromes	Fever	++	+	+	++	+	+/–	++	++	+	+	+/–	++				
	Chills								+				++				
	Headache	+			++			++	++	+			++				
	Meningitis		+		++			++		+			++			+	
	Encephalitis		+		+/–					+/–						+	
	Parotitis	++															
	Lymphadenopathy	+/–															
	Pharyngitis		+	+						+							
	Pneumonia		+					+									
	Conjunctivitis			+									++				
	Rash			+		++	+	++	++								
	Myalgia					+		+	+	++			++				
	Arthralgia					+		+									
	Lesions on the mucosa					+											
	Myocarditis							+									
	Radicular pain						++										
	Hepatosplenomegaly							++	+								
	Conjunctival suffusions								++				++				
	Paresis, paralysis									++							+
	Sensory loss									–							
Complications	Sialadenitis	+															
	Epididymo-orchitis	++															
	Oophoritis	+															
	Pancreatitis	+															
	Meningitis	++		+		+/–	+				+	+/–		+/–			
	Encephalitis	+/–		+/–		+	++				+	+		+/–	+/–		
	Myocarditis	+		+		+				+							
	Pneumonia					+	+		+/–	+							
	Arthritis	+				+		+									
	Rash				+/–		+										
	Nystagmus				++												
	Tremor				++												
	Paresis, paralysis				+/–			+									
	Confusion, lethargy, coma				+/–			+	+							+	
	Guillain-Barre Syndrome						+				+						
	Subacute sclerosing panencephalitis (SSPE)														+		
	Respiratory failure									++							
	Hepatitis					+											
	«Zoster ophthalmicus»						++										
	Recurrences						+	++									
	Generalisation						+										

(1) See also Tables **10**, 15, 18
(2) See also Tables **10**, 13, 16, 19
(3) See also Table 18
(4) See also Tables **10**, **13**, 16, 18
(5) See also Tables 9, **15**
(6) See also Tables **10**, **11**, 13, 16, 17
(7) See also Tables **11**, 12, **13**, 15

Table 15. Exanthematic Diseases

		Rubella virus	Measles virus: Measles	Measles virus: Modified measles (After γ-globuline)	Measles virus: Atypical measles (After vaccination)	Picorna viruses (ECHO, Coxsackie)	Epstein-Barr Virus	Varicella virus: Varicella	Varicella virus: Herpes zoster	Cat-scratch-disease	Skin and mucous membranes symptoms only: (1) Herpes simplex 1 and 2	Skin and mucous membranes symptoms only: (2) T. pallidum	Skin and mucous membranes symptoms only: (3) Rickettsiae	Skin and mucous membranes symptoms only: (4) Gonorrhoea (disseminated)
Mode of transmission	Respiratory route	+	+	+		+		+			+			
	Fomites										+			
	Fecal-oral route					+								
	Arthropod-borne												+	
	Reactivation of latent virus								+		+			
	Blood, urine, saliva, organs						+							
	Genital route										+	+		+
	Laboratory accident											+		
	Parenteral route				+							+		
	Cat contact / scratch									+				
Incubation period (days)		14–21	10–14	>14		2–14	30–60	10–21		3–10	2–12	3–90	7–10	2–7
Onset	Sudden	+				+	++	+	+				+	
	Insidious		+			+	+		+			+		
Disease symptoms / Syndromes	Fever	+/–	+		++	+	+	+		+/–	+/–			
	Chills					+	+	+						
	Conjunctivitis		++	+/–		+				+/–				
	Enanthem	+/–										–		
	Koplik-Spots		+	+–/–										
	Rhinitis		++											
	Pharyngitis					+	+							
	Bronchitis		++											
	Pneumonia				+									
	Myalgia, Arthralgia							+						
	Myocarditis					+								
	Lymphadenopathy	++					+			++				
	Rash	++	++	–/+–	+	++	+	++	++	+/–		+/++	++	++
	Skin, mucosal vesicles							++			++			
	Hepatitis						+							
	Menigitis					++								
	Splenomegaly	+/–					+			+/–				
Complications	Guillain-Barré Syndrome					+	+	+	+					
	Paralyses					+								
	Subacute scerosing panencephalitis (SSPE)		+											
	Meningitis							+/–	+					
	Encephalitis	+/–	+/–				+	+	++					
	Myocarditis						+/–	+						
	Hepatitis							+						
	Pneumonia		++				+	+	+	+				
	Arthritis	++						+						
	Lymph node suppuration									+/–				
	«Zoster ophthalmicus»								+					
	Generalized infection								+					

(1) See also Tables 10, **11**, 13, 14, 16, 19
(2) See also Tables **11**, 12, 13, 14
(3) See also Tables **14**, 15
(4) See also Tables **11**, 12, 13, 19

Table 16. Infections in the Immunocompromised Individual

		Cytomegalovirus	Respiratory syncytial virus	Herpes simplex viruses 1 and 2	Polyomaviruses	Varicella / Zoster virus	Listeria monocytogenes (1)	Toxoplasma gondii (2)	Candida
Mode of infection	Organ transplantation	+				+	+	+	
	Transfusion	+						+	
	Immunosuppressive drugs	+	+	+	+	+	+		
	Respiratory route		+	+					
	Fomites			+					
	Genital route	+		+					
	Fecal-oral route						+	+	
	Parenteral route	+							
	Reactivation of latent infection	+		+		+		+	
Disease symtoms / Syndromes	Fever	++	+				+		
	Atypical mononucleosis	++							
	Lymphadenopathy	+		+				++	
	Tracheobronchitis			+					
	Pneumonia	++	+	+		+		+	
	Retinitis	+						+	
	Esophagitis			+					
	Myocarditis	+						+	
	Rash	+					+		
	Arthralgias	+					+		
	Myalgias	+							
	Skin and mucosa vesicles			+					
	Ulcerative gastritis and colitis	+							
	Hepatitis	+					+		
	Splenomegaly	+							
	Herpes zoster					+			
	Meningitis	+		+/−		+/−	++	+	
	Encephalitis	+		+		++		++	
	Progressive multifocal leukoencephalopathy (PML)				+				
	Generalised lesions					+	+		+

(1) See also Table 13

(2) See also Tables **10,** 13, 14, 19

Table 17. Infectious Diarrheas

		Salmonellosis													(1)
			Enteric fever												
				Other salmonelloses											
		Enterocolitis	Typhoid fever	Gastro-enteritis	Enteric or para-typhoid fever	Bacteremia	Shigellosis	Campylobacter jejuni	Yersinia enterocolitica	Rotavirus	Picornaviruses (ECHO, Coxackie)	Adenoviruses	Giardia lamblia	Entamoeba histolytica	Human immunodeficiency virus (HIV-1)
Mode of trans-mission	Respiratory route										+	+			
	Fecal-oral route	+	+	+	+	+	+	+	+	+	+	?	+	+	
	Genital route														+
	Parenteral route														+
Incubation period (days)		1/4–2	7–21	1/2–2			1–7	2–6			2–14		7–21		?
Onset	Sudden	+		+	+		+	+	+	+	+		+	+	+
	Insidious		+		+						+			+	+
Disease symptoms / Syndromes	Fever		++	+	++	++	++	+	+	+	+	+	+/–	+/–	++
	Chills		–	+	+		+				+				
	Headache		++		+		+								+
	Pharyngitis								+	+/–	+				+
	Conjunctivitis										+				
	Diarrhea	+		++	+	+/–	++	++	++	++	+	+	++	+	++
	Constipation		+		+										
	Vomiting	+		+		+/–	+			++					
	Tenesmus			+	+		++								
	Lymphadenopathy		+/–												++
	«Rose spots»		+		+/++	–									
	Exanthema										+				+
	Hepatomegaly		+		+										
	Splenomegaly		+		+										+
	Dry cough		++												
	Leukopenia		+			–									
	Relative bradycardia		+												
	Mesenteric adenitis							+	+						
	Ileitis								+						
	Myo-, pericarditis										+				
	Meningitis										++				
	Ecephalitis										+/–				
	Myalgia							+							
Complications	Pneumonia		+			+									
	Lung abscess					+									
	Parotitis		+												
	Arthritis		+			++	+	+	+						
	Thrombophlebitis		+												
	Intestinal hemorrhage		++												
	Intestinal perforation		++												
	Splenic abscess		+						+						
	Hepatic abscess		+						+					+	
	Osteomyelitis		+/–			++			+						
	Endo-, myocarditis		+			+		+/–							
	Conjunctivitis						+								
	Meningitis		+/–			+		+/–	+						
	Paralyses										+				
	Guillain-Barré syndrome										+				
	Pyelonephritis					+									
	Chronic diarrhoea												+	+	
	Relapse		+												
	Chronic carrier state		+/–												

(1) See also Tables **10**, 11, **13**, 14, 16

Table 18. Liver Diseases

		Hepatitis viruses				Brucella						(1)	(2)	
		Hepatitis A	Hepatitis B	Cytomegalovirus	Epstein-Barr virus	Acute and subacute brucellosis	Relapsing brucellosis	Chronic brucellosis	Localised disease	Leptospirae	Coxiella burnetii (Q-Fever)	T. pallidum (Syphilis I and II)	Entamoeba histolytica	Echinococcus
Mode of transmission	Respiratory route										+			
	Fecal-oral route	+				+				+			+	+
	Fomites			+										
	Genital route		+	+								+		
	Parenteral route		+	+								+		
	Blood, urine, saliva, organs			+	+									
	Exposure to the animals									+				+
Incubation period (days)		14–45	30–180	20–60	30–60	7–21				7–12	14–39			
Onset	Sudden	++	+	+	++	+	+			+	+			
	Insidious	+	++	++	+	+		+						
Disease symptoms / Syndromes	Fever	+	+	+	++	+	++	+/–		++	++		+/–	
	Chills	+	+	+	+	+				++	+			
	Headache	+	+							++	++			
	Myalgias	+	+	+						++	+			
	Arthralgias	+	+	+							+/–			
	Pharyngitis	+	+		+									
	Jaundice	++	+		+/–					+/–	+	+/–		
	Hepatomegaly	++	+	+/–		+	+	+		+				+
	Splenomegaly	+	+	+/–	+	++	+	++		+	+			
	Hepatitis	+	+	+	+				++	+	+	+		
	Arthritis	+/–	+			+	+	+	+/–					
	Lymphadenopathy	+/–	+/–	+	+	++	+			+				
	Atypical mononucleosis			++	+									
	Heterophile antibodies			–	+									
	Rash	+/–		+	+	+			+	+	–	+/–		
	Pneumonia								+	+	++			
	Endocarditis								++		+			
	Meningitis								++	++				
	Encephalitis								++					
	Neuritis								+					
	Keratitis, retinopathy								+					
	Osteomyelitis								++					
	Orchitis, prostatitis								+					
Complications	Liver necrosis	+	+											
	Chronic active hepatitis		+											
	Hepatic abscess												+	
	Pneumonia			+	+/–									
	Arthritis			+										
	Meningitis			+	+									
	Encephalitis			+	+	+	+							
	Guillain-Barré syndrome			+	+									
	Myocarditis			+	+					+				
	Thrombocytopenia									+				
	Renal failure									+				
	Neuropsychiatric symptoms							+						
	Delirium									+				

(1) See also Tables 11, 12, 13, 14, 15
(2) See also Table 17

Table 19. Ocular Infections

		Adeno-viruses (1)		Chlamydia trachomatis (2)			Herpes simplex virus 1 (3)		Varicella/Zoster virus			Toxoplasma gondii (4)			
				Inclusion conjunctivitis					Varicella						
		Pharyngoconjunctival fever	Epidemic keratoconjunctivitis	Neonates	Adults	Trachoma	Congenital	Acquired	Congenital	Acquired	Herpes zoster	Congenital	Acquired	Toxocara canis	N.gonorrhoeae
Mode of transmission	Respiratory route	+								+					
	Fecal-oral route (cysts, eggs)												+	+	
	Genital route				+										+
	Fomites, fingers, towels		+		+	+									+
	Parenteral route												+		
	Laboratory accident												+		
	Reactivation of latent virus										+				
	to the child: Intrauterine infection						+		+			+			+
	to the child: Perinatal (genital tract)			+			+								+
	to the child: Maternal milk, -excreta														+
	to the child: Devices						+								
Incubation period (days)			4–24					2–12		10–21					
Onset	Sudden	+			+					+	+				+
	Insidious		+			+					+			+	
Disease symptoms / Syndromes	Conjunctivitis	++		+	+		+			+					+
	Keratoconjunctivitis		++			+		+							
	Chorioretinitis						++		+			++	+		
	Strabismus											+		+	
	Cataract								+						
	Glaucoma										+				
	Endophthalmitis													+	
	Blindness								+			+		+	
	Retinopathy													+	
	Zoster ophthalmicus										+				
	Pharyngitis	++													
Complications	Blepharitis							++							++
	Keratitis							+							
	Blindness					+					+				
	Reinfections					+									
	Relapses of chorioretinitis											+	+		

(1) See also Table 9
(2) See also Tables 9, 11
(3) See also Table 11
(4) See also Tables 10,13

Choice of Diagnostic Tests

Table 20.

LABORATORY METHODS FOR THE SEROLOGICAL DIAGNOSIS OF COMMON INFECTIOUS DISEASES AND ANTIBODY SCREENING FOR EPIDEMIOLOGICAL PURPOSES

Clinical syndroms and causative agents * Occasional involvement	Laboratory methods(1)				
	CF(2)	IFA(3)	EIA(4)	Other methods(5)	
RESPIRATORY DISEASES					
Mycoplasma pneumoniae	+ +(6)	0	0	+	Latex agglutination
Chlamydiae (psittaci, trachomatis)	+ +	+	+	0	
Legionella pneumophila	+	+ +	0	0	
Influenza A and B viruses	+ +	0	0	+	HAI
Respiratory syncytial virus	+ +	0	+	0	
Parainfluenza 1–3 viruses	+ +	0	0	+	HAI
Adenoviruses	+ +	0	0	0	
Coxiella burneti	+ +	+	0	0	
* Cytomegalovirus	+ + S	+ S	+ S	S	PHA, Agglutination
* Measles virus	+ +	0	+ S	+	HAI
* Bordetella pertussis	+ +	+ +	+	+	Agglutination
* Varicella (adults)	+ +	+	+ S	0	
INFECTIOUS-MONONUCLEOSIS-LIKE DISEASES, LYMPHADENOPATHY, AIDS (SUSPECTED)					
Cytomegalovirus	+ + S	+ S	+ S	S	PHA, Agglutination
Epstein-Barr virus	+	+ +	+	+ +	Paul-Bunnell
Toxoplasma gondii	+ + S	+ + S	+ S	+	Agglutination, Dye test
HIV-1	0	+ + S	S	+ +	Western blot et al.
REACTIVE ARTHRITIS					
Neisseria gonorrhoeae	+	0	0	+	Agglutination et al.
Campylobacter jejuni/fetus	+ +	0	0	0	
Yersinia enterocolitica/pseudotuberculosis	+ +	0	0	+ +	Agglutination
Brucella	+ +	0	+ S	+ +	Agglutination
Salmonellae	0	0	0	+ +	Agglutination
Shigellae	+	0	0	+	Agglutination
Picornaviruses (Echo, Coxsackie)	+ +	0	0	0	
Borrelia burgdorferi/Lyme disease	0	+ +	+	0	
Rubella virus	+	0	+ S	+ + S	HAI, PHA
Strepto/Staphylococcus	0	0	0	+	Agglutination
* Mycoplasma pneumoniae	+ +	0	0	0	
* Adenoviruses	+ +	0	0	0	
* Cytomegalovirus	+ + S	+ S	+ S	S	PHA, Agglutination
* Varicella	+ +	+	+ S	0	
* Hepatitis B	0	0	+ +	+ +	PHA, RIA
* Treponema pallidum	+	+ + S	0	+ + S	PHA

Table 21.

VENEREAL DISEASES	CF	IFA	EIA	Other methods
Treponema pallidum	+	+ + S	0	+ + S PHA
Chlamydia (trachomatis)	+ +	+	+	0
Neisseria gonorrhoeae	+	0	0	+ Agglutination et al.
Herpes simplex	+ + S	+ S	+ S	0
HIV-1	0	+ + S	S	+ + Western blot et al.
* Hepatitis B	0	0	+ +	+ + PHA, RIA
* Cytomegalovirus	+ + S	+ S	+ S	S PHA, Agglutination
PREGNANCY, CONGENITAL INFECTIONS				
Rubella virus	+	0	+ S	+ + S HAI, PHA
Cytomegalovirus	+ + S	+ S	+ S	S PHA, Agglutination
Treponema pallidum	+	+ + S	0	+ + S PHA
Toxoplasma gondii	+ + S	+ + S	+ S	+ Agglutination, Dye test
Listeria monocytogenes	+ +	0	0	+ Agglutination
Herpes simplex types 1 and 2	+ + S	+ S	+ S	0
* Hepatitis B	0	0	+ +	+ + PHA, RIA
* Neisseria gonorrhoeae	+	0	0	+ Agglutination et al.
* Coxsackie B viruses	+ +	0	0	+ Neutralization
* Mumps / Parotitis virus	+ +	0	+ S	0
* Chlamydiae (trachomatis)	+ +	+	+	0
* Varicella	+ +	+	+ S	0
* HIV-1	0	+ + S	S	+ + Western blot et al.
CENTRAL NERVOUS SYSTEM INFECTIONS				
Mumps / Parotitis virus	+ +	0	+ S	0
Adenoviruses	+ +	0	0	0
Picornaviruses (Echo, Coxsackie)	+ +	0	0	0
Tick-borne encephalitis (RSSE)	+ +	0	+	+ HAI
Varicella/Zoster	+ +	+	+ S	0
Epstein-Barr virus	+	+ +	+	+ + Paul-Bunnell
* Borrelia burgdorferi / Lyme disease	0	+ +	+	0
* Leptospirae	+ +	0	0	+ Agglutination, Lysis
* Rickettsiae	+ +	0	0	+ Agglutination
* Toxoplasma gondii	+ + S	+ + S	+ S	+ Agglutination, Dye test
* Measles virus	+ +	0	+ S	+ HAI
* HIV-1	0	+ + S	S	+ + Western blot, etc.
* Polioviruses 1–3	+ +	0	0	+ Neutralization
* Treponema pallidum	+	+ + S	0	+ + S PHA
EXANTHEMATIC DISEASES				
Rubella virus	+	0	+ S	+ + S HAI, PHA
Measles virus	+ +	0	+ S	+ HAI
Picornaviruses (Echo, Coxsackie)	+ +	0	0	0
Adenoviruses	+ +	0	0	0
Epstein-Barr virus	+	+ +	+	+ + Paul-Bunnell
* Varicella/Zoster	+ +	+	+ S	0
* Herpes simplex types 1 and 2	+ + S	+ S	+ S	0
* Treponema pallidum	+	+ + S	0	+ + S PHA
* Rickettsiae	+	0	0	+ Agglutination
* Cat-Scratch-Disease (Chlamydia-related)	+	+	+	0

Table 22.

INFECTIONS DURING IMMUNOSUPPRESSIVE THERAPY	CF	IFA	EIA	Other methods	
Cytomegalovirus	+ + S	+ S	+ S	S	PHA, Agglutination
Respiratory syncytial virus	+ +	0	+	0	
Herpes simplex viruses types 1 and 2	+ + S	+ S	+ S	0	
Polyoma viruses	+ +	0	0	+	HAI
Varicella / Zoster virus	+ +	+	+ S	0	
* Listeria monocytogenes	+	0	0	+	Agglutination
* Toxoplasma gondii	+ + S	+ + S	+ S	+	Agglutination, Dye test
* Candida	0	0	0	+	PHA
INFECTIOUS DIARRHOEAS					
Salmonellae	0	0	0	+ +	Agglutination
Shigellae	+	0	0	+	Agglutination
Campylobacter jejuni, fetus	+ +	0	0	0	
Yersinia enterocolitica, pseudotuberculosis	+ +	0	0	+ +	Agglutination
Rotavirus	+ +	0	+	0	
Picornaviruses (Echo, Coxsackie)	+ +	0	0	0	
Adenoviruses	+ +	0	0	0	
Brucella	+ +	0	+ S	+ + S	Agglutination
Giardia lamblia	+	0	0	0	
Entamoeba histolytica	+ +	+	0	+ +	PHA
LIVER DISEASES					
Hepatitis A and B	0	0	+ +	+ +	PHA, RIA
Cytomegalovirus	+ + S	+ S	+ S	S	PHA, Agglutination
Epstein-Barr virus	+	+ +	+	+ +	Paul-Bunnell
Brucella	+ +	0	+	+ + S	Agglutination
Leptospirae	+ +	0	0	+	Agglutination, Lysis
* Entamoeba histolytica	+ +	0	0	+ +	PHA
* Echinococcus	+ +	+	0	+ +	PHA
OCULAR INFECTIONS					
Adenoviruses	+ +	0	0	0	
Chlamydiae (trachomatis)	+ +	+	+	0	
Herpes simplex virus type 1	+ + S	+ S	+ S	0	
Zoster virus (Varicella)	+ +	+	+ S	0	
* Toxoplasma gondii	+ + S	+ + S	+ S	+	Agglutination, Dye test
* Toxocara canis	0	+	0	0	

(1) + + accepted as a routine diagnostic method for acute disease and, in general, available from commercial sources

S accepted as a routine screening method for presence/absence of antibodies and available, in general, from commercial sources

\+ accepted as a diagnostic method, but seldom performed because of any of several reasons:
- (a) too laborious
- (b) not suitable for laboratories dealing with only a few serum specimens daily
- (c) too expensive
- (d) some methods require special (expensive) equipment
- (e) limited availability of the reagents commercially
- (f) incomplete standardization and unsatisfactory comparability of the products from different manufacturers
- (g) combined use of reagents from different manufacturers, and even from different kits of the same manufacturer, is not possible

0 attemps were sometimes made on experimental scale, but the method is not yet available for diagnostic purposes

(2) Complement-fixation method

(3) Indirect fluorescent antibody method

(4) Enzyme immunoassay

(5) HAI Haemagglutination inhibition test
PHA Passive haemagglutination test
Agglutination: agglutination of sensitized particulate material coated with antigens (bacterial, parasitic, viral)
RIA Radio Immunoassay

References

Alexander, J.G.: Diagnosis of enterovirus (picornavirus) infections by the complement-fixation test using a group picornavirus commercial antigen. J. Infection *4*. 265-267, 1982.

Allen; J.C. and Kunkel, H.G.: Hidden rheumatoid factors with specificity for native gamma-globulin. Arthritis Rheum. *9*, 758-768, 1966.

Anderson, L.J.: Paramyxoviridae: Respiratory syncytial virus. Chapter in Laboratory Diagnosis of Infectious Diseases. Principles and Practice. II. Viral, Rickettsial and Chlamydial Diseases. E.H. Lennette, P. Halonen and F.A. Murphy (Editors). Pages 540-570. Springer Verlag, New York, 1988.

Ankerst, J., Christensen, P., Kjellén, L. and Kronvall, G.: A routine diagnostic test for IgA and IgM antibodies to rubella virus: Absorption of IgG with staphylococcus aureus. J. Inf. Dis. *130*, 268-273, 1974.

Benjamin, D.R.: Immunoenzymatic methods. Chapter in Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections. E.H. Lennette and N.J. Schmidt (Editors), pages 153-170. 5th Ed., Amer. Pbl. Hlth. Assn., Washington, D.C., 1979.

Bjorek, L. and Kronvall, G.: Purification and some properties of streptococcal protein G, a novel IgG-binding reagent. J. Immunol. *133*, 969-974, 1984.

Booth, J.C., Hannington, G. Aziz, T.A.G. and Stern, H.: Comparison of enzyme-linked immunosorbent assay (ELISA) technique and complement-fixation test for estimation of cytomegalovirus IgG antibody. J. Clin. Pathol. *32*, 122-127, 1979.

Bordet, J.: See Miles, A.: History. Chapter in Topley and Wilson's Principles of Bacteriology, Virology and Immunity. I. General Microbiology and Immunity. W. Graham and H.M. Dick (Editors). 7th Ed., pages 1-15. Edward Arnold, London, 1984.

Bordet, J.: Ann. Inst. Pasteur, *12*, 688, 1898. Cited from Dick, H.M.: Antigen-antibody reactions in vitro. Chapter in Topley and Wilson's Principles of Bacteriology, Virology and Immunity. I. General Microbiology and Immunity. G. Wilson and H.M. Dick (Editors). 7th Ed., pages 319-329. Edward Arnold, London, 1984.

Brown, G.C., Baublis, J.V. and O'Leary, T.P.: Development and duration of mumps fluorescent antibodies in various immunoglobulin fractions of human serum. J. Immunol. *104*, 86-94, 1970.

Bürgin-Wolff, A., Hernandez, R. and Just, M.: Separation of rubella IgM, IgA and IgG antibodies by gel filtration on agarose. Lancet *2*, 1278-1280, 1971.

Chantler, S., Devries, E., Allen, P.R. and Hurn, B.A.L.: A rapid immunofluorescent procedure for the detection of specific IgG and IgM antibody in sera using Staphylococcus aureus and Latex-IgG as absorbents. J. Immunol. Methods *13*, 367-380, 1976.

Cohen, I.R., Norins, L.C. and Julian A.J.: Competition between, and effectiveness of, IgG and IgM antibodies in indirect fluorescent antibody and other tests. J. Immunol. *98*, 143-149, 1967.

Coons, A.H., Creech, H.J. and Jones, R.N.: Immunological properties of an antibody containing a fluorescent group. Proc. Soc. Exp. Biol. Med. *47*, 200-202, 1941.

Cradock-Watson, J.E., Bourne, M.S. and Vandervelde, E.M.: IgG, IgA and IgM responses in acute rubella determined by the immunofluorescent technique. J. Hyg. (Cambridge) *70*, 473-485, 1972.

Cremer, N.E., Devlin, V.L., Riggs, J.L. and Hagens, S.J.: Anomalous antibody responses in viral infection: Specific stimulation or polyclonal activation? J. Clin. Microbiol. *20*, 468-472, 1984.

Cremer, N.E.: Antibodies in serodiagnosis of viral infections. Chapter in Laboratory Diagnosis of Viral Infections. E.H. Lennette (Editor), pages 73-85. Marcel Dekker, Inc., New York, 1985.

Csango, P.A., Sarov R., Schiotz, H. and Sarov, I.: Comparison between cell culture and serology for detecting chlamydia trachomatis in women seeking abortion. J. Clin. Pathol. *41*, 89-92, 1988.

Denoyel, G.A., Gaspar, A. and Nouyrigat, C.: Enzyme immunoassay for measurement of antibodies to herpes simplex virus infection: Comparison with complement-fixation, immunofluorescent-antibody, and neutralization techniques. J. Clin. Microbiol. *11*, 114-119, 1980.

Deutsch, H.F. and Morton, J.I.: Dissociation of human serum macroglobulins. Science *125*, 600-600, 1957.

Dick, H.M.: Antigen-antibody reactions in vitro. Chapter in Topley and Wilson's Principles of Bacteriology, Virology and Immunity. I. General Microbiology and Immunity. G. Wilson and H.M. Dick

(Editors). 7th Ed., pages 319-329. Edward Arnold, London, 1984.

Dick, H.M. and Kirkwood, E.: Immunity to infections – immunoglobulins. Chapter in Topley and Wilson's Principles of Bacteriology, Virology and Immunity. I. General Microbiology and Immunity. G. Wilson and H.M. Dick (Editors). 7th Ed., pages 374-383. Edward Arnold, London, 1984.

Duermeyer, W., Wielaard, F. and van der Veen, J.: A new principle for the detection of specific IgM antibodies applied in an ELISA for hepatitis A. J. Med. Virol. *4,* 25-32, 1979.

Engvall, E. and Perlmann, P.: Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of IgG. Immunochemistry *8,* 871-874, 1971.

Engvall, E. and Perlmann, P.: Enzyme-linked immunosorbent assay. ELISA. III. Quantitation of specific antibodies by enzyme-linked anti-immunoglobulin in antigen coated tubes. J. Immunol. *109,* 129-135, 1972.

Fahnestock, S.R.: GammaBind™ G: Genetically engineered, recombinant protein G, a new IgG binding reagent with advantages over protein A. DuPont Biotech Update *4,* 10-11, 1989.

Flosdorf, E.W.: Freeze-Drying (Drying by Sublimation). Page 84. Reinhold Publishing Corporation, New York, 1949.

Forghani, B., Schmidt, N.J. and Lennette, E.H.: Demonstration of rubella IgM antibody by indirect fluorescent antibody staining, sucrose density gradient centrifugation and mercaptoethanol reduction. Intervirology *1,* 48-59, 1973.

Forghani, B.: Enzyme immunoassay systems. Chapter in Laboratory diagnosis of viral infections. E.H. Lennette (Editor), pages 105-118. Marcel Dekker Inc., New York, 1985.

Fraser, K.B., Shirodaria, P.V. and Stanford, C.F.: Fluorescent staining and human IgM. Brit. Med J. *3,* 307-307, 1971.

Galen, R.S.: Use of predictive value theory in clinical immunology. Chapter in Manual of Clinical Immunology. Rose, N.R., Friedman, N. and Fahey, J.L. (Editors), pages 966-970. Amer. Pbl. Hlth. Assn., 3rd Ed., Washington, D.C., 1986.

Gispen, R., Nagel, J., Brand-Saathof, B. and De-Graaf, S.: Immunofluorescence test for IgM rubella antibodies in whole serum after absorption with anti-gamma Fc. Clin. Exp. Immunol. *22,* 431-437, 1975.

Goding, J.W.: The chromic chloride method of coupling antigens to erythrocytes: Definition of some important parameters. J. Immunol. Methods *10,* 61-66, 1976.

Harboe, M. and Folling, I.: Recognition of two distinct groups of human IgM and IgA based on different binding to Staphylococci. Scand. J. Immunol. *3,* 471-482, 1974.

Hausler, W.J.: Third Consensus Conference on HIV testing. Infection, Control and Hospital Epidemiology. *9,* 345-349, 1988.

Hawkes, R.A.: General principles underlying laboratory diagnosis of viral infections. Chapter in Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections. E.H. Lennette and N.J. Schmidt (Editors), pages 3-48. 5th Ed., Amer. Pbl. Hlth. Assn., Washington, D.C., 1979.

Joassin, L. and Reginster, M.: Elimination of nonspecific cytomegalovirus immunoglobulin M activities in the enzyme-linked immunosorbent assay by using anti-human immunoglobulin G. J. Clin. Microbiol. *23,* 576-581, 1986.

Jung, M., Price, P.C., Kistler, G.S. and Krech, U.: Immunoprecipitation studies with antigens of human cytomegalovirus. Z. Immun.-Forsch. *145,* 191-198, 1973.

Jung, M.: Serologic diagnosis of bacterial infections. Virion Edition, Cham, Switzerland, 1983.

Jung, M.: Long term stability of diagnostic reagents after freeze-drying. 6th International Chartridge Freeze-Drying Symposium, Chartridge Lodge, March 25-27, 1985. Edwards High Vacuum, Manor Royal, Crawley, Sussex, England.

Kagan, I.G.: Serodiagnosis of parasitic diseases. Chapter in Manual of Clinical Immunology. Rose, N.R., Friedman, H. and Fahey, J.L. (Editors), pages 467-487. 3rd Ed., Amer. Soc. Microbiol., Washington, D.C., 1986.

Kahane, S., Goldstein, V. and Sarov, I.: Detection of IgG antibodies specific for measles virus by enzyme-linked immunosorbent assay (ELISA). Intervirology *12,* 39-46, 1979.

Kaschka, W.P., Hilgers, R. and Skvaril, F.: Humoral immune response in Epstein-Barr virus infections. I. Elevated serum concentration of the IgG1 subclass in infectious mononucleosis and nasopharyngeal carcinoma. Clin. Exp. Immunol. *49,* 149-156, 1982.

Kenny, G.E.: Mycoplasmas. Chapter in Manual of Clinical Microbiology. E.H. Lennette, A. Balows, W.J. Hausler, Jr. and H.J. Shadomy (Editors). Pages 407-411. 4th Ed., Amer. Soc. Microbiol., Washington, D.C., 1985.

Kleiman, M.B., Blackburn, C.K.L., Zimmerman, S.E. and French, M.L.V.: Comparison of enzyme-linked immunosorbent assay for acute measles with hemagglutination inhibition, complement-fixation and fluorescent-antibody methods. J. Clin. Microbiol. *14,* 147-152, 1981.

Krech, U., Jung, M. et al.: Study of complement-fixing, fluorescent and neutralizing antibodies in human cytomegalovirus infections. Z. Immun.-Forsch. *141,* 411-429, 1971.

Krech, U., Jung, M. et al.: Identification of human cytomegalovirus isolates by means of indirect immunofluorescence employing an improved system for fluorescent microscopy. Z. Immun.-Forsch. *143,* 354-362, 1972.

Kurtz, J.B. and Malic, A.: Rubella-specific IgM detected by an antibody capture assay/ELISA technique. J. Clin. Pathol. *34,* 1392-1395, 1981.

Leinikki, P.O., Shekarchi, I., Dorsett, P. and Sever, J.L.: Determination of virus-specific IgM antibodies by using ELISA: elimination of false positive results with Protein A-sepharose absorption and subsequent IgM antibody assay. J. Lab. Clin. Med. *92,* 849-857, 1978.

Leland, D.S. and French, M.L.V.: Virus isolation and identification. Chapter in Laboratory Diagnosis of Infectious Diseases. Principle and Practice. II. Viral, Rickettsial and Chlamydial Diseases. A. Balows, W.J. Hausler and E.H. Lennette (Editors), pages 39-59. Springer Verlag, New York, 1988.

Levy, E. and Sarov, I.: Determination of IgA antibodies to human cytomegalovirus by enzyme-linked immunosorbent assay (ELISA). J. Med. Virol. *6,* 249-257, 1980.

Lind, I., Harboe, M. and Følling, I.: Protein A reactivity of two distinct groups of human monoclonal IgM. Scand. J. Immunol. *4,* 843-848. 1975.

Linde, G.A., Hammarström, L., Persson, M.A.A., Smith, C.I.E., Sundquist, V.A. and Wahren, B.: Virus-specific antibody activity of different subclasses of immunoglobulins G and A in cytomegalovirus infections. Infection & Immunity *42,* 237-244, 1983.

Linde, G.A.: Subclass distribution of rubella virus-specific immunoglobulin G. J. Clin. Microbiol. *21,* 117-121, 1985.

Madeley, C.R.: Guide to the collection and transport of virological specimens, 40 p., World Health Organization, Geneva, 1977.

Madeley, C.R., Lennette, D.A. and Halonen, P.: Specimen collection and transport. Chapter in Laboratory Diagnosis of infectious Diseases. Principle and Practice. II. Viral, Rickettsial and Chlamydial Diseases. A. Balows, W.J. Hausler and E.H. Lennette (Editors), pages 1-11. Springer Verlag, New York, 1988.

Mancini, G., Carbonara, A.O. and Heremans, J.F.: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry *2,* 235-254, 1965.

Morell, A., Roth-Wicky, B. and Skvaril, F.: Immunoglobulin G subclass restriction of antibodies against hepatitis B surface antigen. Infection & Immunity *39,* 565-568, 1983.

Morris, G.K. and Patton, C.M.: Campylobacter. Chapter in Manual of Clinical Immunology. E.H. Lennette, A. Balows, W.J. Hausler, Jr. and H.J. Shadomy (Editors), pages 302-308. 4th Ed., Amer. Soc. Microbiol.. Washington, D.C., 1985.

Naot, Y., Barnett, E.V. and Remington, J.S.: Method for avoiding false-positive results occurring in immunoglobulin M enzyme-linked immunosorbent assays due to presence of both rheumatoid factor and antinuclear antibodies. J. Clin. Microbiol. *14,* 73-78, 1981.

Nicholls. A.C.: Enzyme immunoassay in microbiology. In: Future directions for non-isotopic immunoassays. R.S. First Incorporated, Munich, 1982.

Osborne, N.G., Hecht, Y., Gorsline, J., Forbes, B.A., Morgenstern, F. and Winkelman, J.: A comparison of culture, direct fluorescent antibody test, and a quantitative indirect immunoperoxidase assay for detection of chlamydia trachomatis in pregnant women. Obstet. Gynecol. *71,* 412-415, 1988.

Palmer, D.F. and Whaley, S.D.: Complement-fixation test. Chapter in Manual of Clinical Immunology. Rose, N.R., Friedman, H. and Fahey, J.L. (Editors). Amer. Soc. Microbiol., Washington, D.C. Third Edition, 1986.

Riggs, J.L., Seiwald, R.I., Burckhalter, J.H., Downs, C.M. and Metcalf, T.G.: Isothiocyanate compounds as fluorescein labeling agents for immune serum. Amer. J. Pathol. *34,* 1081-1097, 1958.

Ritchie, R.F.: Introduction. Chapter in Manual of Clinical Immunology. N.R. Rose, H. Friedman and J.L. Fahey (Editors), pages 1-3. 3rd Ed., Amer. Soc. Microbiol., Washington, D.C., 1986.

Salonen, E.-M., Vaheri, A., Suni, J. and Wager, O.: Rheumatoid factor in acute viral infections: Interference with determination of IgM, IgG, and IgA antibodies in an enzyme immunoassay. J. Inf. Dis. *142,* 250-255, 1980.

Salvedt, E. and Harboe, M.: Binding of IgA to Protein A containing Staphylococci: Relationship to subclasses. Scand. J. Immunol. *5,* 1103-1108, 1976.

Sarov, I., Andersen, P. and Andersen, H.K.: Enzyme linked immunosorbent assay (ELISA) for determination of IgG antibodies to human cytomegalovirus. Acta Path. Microbiol. Scand. *88,* 1-9, 1980.

Schmidt, N.J. and Lennette, E.H.: Rubella complement-fixing antigen derived from the fluid and cellular phases of infected BHK-21 cells: extraction of cell-associated antigen with alkaline buffers. J. Immunol. *100,* 815-821, 1966.

Shirodaria, P.V., Fraser, K.B. and Stanford, F.: Secondary fluorescent staining of viral antigens by rheumatoid factor and fluorescein-conjugated anti-IgM. Ann. Rheum. Dis. *32,* 53-57, 1973.

Singer, J.M. and Plotz, C.M.: I. The latex fixation test. Application to the serologic diagnosis of rheumatoid arthritis. Amer. J. Med. *21,* 888-892, 1956.

Skaug, K. and Gaarder, P.I.: An indirect immunofluorescent antibody test for determination of rubella virus specific IgM antibodies. Elimination of secondary IgM rheumatoid factor staining after absorption of serum IgG with staphylococcal Protein A. Acta Path. Microbiol. Scand. Sect. C. *86,* 33-35, 1978.

Swack, N.S.: Mycoplasmoses. Chapter in Laboratory Diagnosis of Infectious Diseases. Principles and Practice, I. Bacterial. Mycotic and Parasitic Diseases. A. Balows, W.J. Hausler, M. Ohashi and A. Turano (Editors), pages 396-402. Springer Verlag, New York, 1988.

Tenover, F.: DNA probes – Where are they taking us? Eur. J. Microbiol. Infect. Dis. *7,* 457-459, 1988.

Tilton, R.C.: Microbiology in a changing health care world. Diagnostic Medicine 1985, February, pps. 1-5, Published by Medical Economics Company, Inc., Oradell. N.J. 07649.

Towbin, H., Staehelin, T. and Gordon, J.: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA *76,* 4350-4354, 1979.

Tuokko, H.: Comparison of nonspecific reactivity in indirect and reverse immunoassays for measles and mumps immunoglobulin M antibodies. J. Clin. Microbiol. *20,* 972-976, 1984.

Vesikari, T. and Vaheri, A.: Rubella: a method for rapid diagnosis of a recent infection by demonstration of the IgM antibodies. Brit. Med. J. *1,* 221-223, 1968.

Wang, A.C.: The structure of immunoglobulins. Chapter in Basis and Clinical Immunology. H.H. Fudenberg, D.P. Stites, J.L. Caldwell and J.V. Wells (Editors). Pages 15-31. Lange Medical Publications, Los Altos, California, 1974.