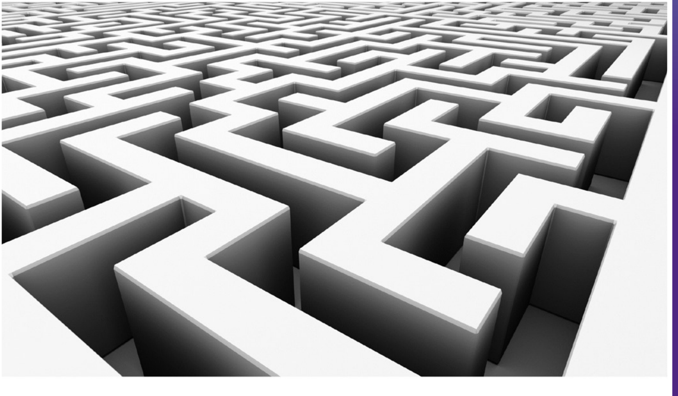
Healthcare Sterilisation: Challenging Practices Volume 2



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Preface

Beyond the steadfast and traditional techniques of steam, dry heat and irradiation, sterilisation remains a challenge in the 21st century. Some chemical sterilisation methods such as ethylene oxide, are carcinogenic, explosive, hazardous and toxic. Non-traditional methods (e.g., hydrogen peroxide and plasma and ozone) and alternative and newer methods (chlorine dioxide, peracetic acid, hydrogen peroxide and oxides of nitrogen) have attempted to replace these dangers and hazards. They frequently have their limitations such as less penetration, adverse effects on some materials (e.g., corrosion, damage, oxidising and residuals), and some are less reliable than the steadfast and traditional methods of steam, dry heat and irradiation. Sterilisation is not a singular problem or discipline but an interfacial area of investigation of materials, biocompatibility, biocontainment, chemistry, engineering, microbiology, material, drug and patient safety, product design, statistics and validation. It requires a mulitidiciplinary effort and synergism.

In parallel to these challenges, design of package, products and processing may be created that are optimal for the final sterilisation outcome(s). Optimal design, material and process development, and innovation can help to improve the current challenges of sterilisation.

To achieve sterilisation without adversely affecting package and product quality and sterility, requires validation, statistics and improved scientific approaches to optimally accomplish sterility.

This volume makes you question and think what you are doing in selection, design, statistics, in following standards and performing validations for sterilisation and sterile claims.

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1 Traditional Chemical Sterilisation Methods

There are no miraculous chemical sterilants, only magical ways of using them …

Chemical sterilisation is not the oldest, most common or traditional form of sterilisation however, its magical inactivating powers have been significantly recognised in the twentieth century and continue as we go into the twenty-first century.

Traditional chemical sterilisation typically consists in two forms: gaseous and liquid. They have many characteristics and qualities in common. Both gaseous and liquid sterilisation improves with heat to inactivate microbes.

Ethylene oxide (EO; gas) and glutaraldehyde (liquid) kills all microbes, if they are penetrable or contactable. Both require slightly higher temperatures than ambient to inactivate all microbes including resistant spores within reasonable exposures, but lower temperatures are possible with increased exposure time and concentrations. As the sterilising temperatures from moist heat are reduced, an increasing number of polymers and materials become more compatible and tolerant to these traditional chemical methods. Both EO and glutaraldehyde have different toxic residues or wastes to the ones produced by use of radiation and oxidising agents), but they use more hazardous levels of chemicals.

Although there are numerous chemical antimicrobial agents, only a few have classically been available to use for sterilisation (e.g., inactivation of spores) such as EO, propylene oxide, β-propiolactone (BPL), aldehydes (e.g., formaldehyde), dialdehydes (e.g., glutaraldehyde, glyoxal), and oxidising agents such as potassium permanganate, halogens (e.g., chlorine, hypochlorites, iodine), ozone (O_3), peracetic acid (PAA), and hydrogen peroxide (H_2O_2) (high percentage). Beyond traditional approaches PAA, H_2O_2, chlorine dioxide, and O_3 have been redefined, and oxides of nitrogen (the oxides of nitrogen are referred to as reactive nitrogen species and include nitrogen oxide and nitrogen dioxide). Also, considerable information on the compatibility and manipulation of polymers and materials for use with these chemical sterilants (e.g., EO and glutaraldehyde) exists.

All chemical sterilants are not safe, unless used safely, there are no 'inherently' environmentally friendly chemical sterilants. What matters is operator and patient

safety when it comes to chemical sterilisation. Inhalation levels indicate the toxicity of working with several chemical sterilants (see **Table 1.1**).

Table 1.1 Different safe levels for different chemical sterilants

Chemical	Safe levels
EO	TWA: 1 ppm
Propylene oxide	OSHA PEL: 20 ppm
Glutaraldehyde	ACGIH TLV: 0.05 ppm
Glyoxal	OSHA –not listed
H_2O_2	OSHA PEL: 1 ppm (TWA)
O_3	OSHA PEL: 0.1 ppm
PAA	EPA AEGL 1: 0.17 ppm
Chlorine	ACGIH: 0.5 ppm TWA
Chlorine dioxide	ACGIH: 0.1 ppm TWA
Methyl bromide	ACGIH: 5 ppm TWA
BPL	ACGIH TLV: 0.5 ppm TWA: 1.5 mg/m^3

PEL: Permissible exposure limit(s)
TLV: Threshold limit value(s)
TWA: Time weighted average
Adapted with data taken from the American Conference of Industrial Hygienists (ACGIH); Environmental Protection Agency (EPA); Acute Exposure Guideline Level(s) (AEGL); and Occupational Safety and Health Administration (OSHA).

Paradoxically, while chemical sterilants are capable of inactivating all microbes that cause disease, they are also capable of being toxic and causing death of people. For example, contact with liquids (glutaraldehyde) may cause irritation to the eyes or skin and EO and BPL are carcinogens. Oxidising agents such as PAA, H_2O_2 and O_3 can cause irritation to the eyes, and the respiratory system. Chemical sterilants by their nature are hazardous, otherwise they would not function well as sterilants of highly resistant microbes such as spores.

In this chapter there will be a discussion of the future of EO, and of the similarities and differences of other potential classical-traditional sterilisation methods, in particular:

EO, propylene oxide, chlorine dioxide, BPL, glyoxal, glutaraldehyde, PAA, H_2O_2 (no plasma) and so on. But discussion of alternative sterilants (e.g., H_2O_2 and plasma, O_3 and so on) will be addressed more thoroughly in **Chapter 5**.

1.1 Ethylene Oxide Sterilisation

The wizardry of ethylene oxide material compatibility and penetration is like an ethereal and gentle breeze of a magical sterilising wand.

Originally EO was used for the decontamination of spices, but EO sterilisation has probably been the predominant method of gaseous chemical sterilisation used in healthcare for more than 25 years, and has remained second to steam sterilisation in the hospitals, but now there is tremendous pressure to reduce its use, and it is not available in as many hospitals as it once was. However, like old soldiers, it will never 'totally' die, but just fade away, and be eventually replaced by a quasi-alternative, traditional sterilant, for certain cases and situations unfilled by current alternative or novel methods. Some overall characteristics of EO sterilisation are:

- Chemical:
  - Gaseous
  - Toxic, flammable, explosive sterilant
  - Toxic residues
- Complex sterilisation process:
  - Numerous parameters
  - Requires complex equipment and facilities
  - Limited penetration, but better than moist heat or oxidising sterilants
  - Requires air removal, conditioning, temperature control, exposure and degassing monitoring
  - Requires breathable packaging
  - Requires environmental control and monitoring
- Advantages:
  - Wide material compatibility

- Inactivates a wide range of microbes
- Widely available

EO became one of the most common traditional sterilants because it can sterilise a wide range of heat and moisture sensitive materials as well as heat tolerant polymers and non-polymers. It is compatible with nearly all polymeric and other materials (see American Association of Medical Instrumentation (AAMI) Technical Information Report (TIR) 17 [1]). Some materials with hydrophilic coatings may be sensitive, but can be overcome. Some polystyrenes may craze and distort with moderate sterilising temperatures, but this can be overcome. Residuals in processed materials may result, which may be toxic and require aeration and a degassing process. Ethylene oxide sterilisation is still commonly used in manufacturing and in healthcare facilities (e.g., contract, hospitals).

1.1.1 Green sterilisation (Benefits **versus** *Risks)*

It is typically not considered a green sterilisation process, because of potential carcinogenicity, safety, toxicity, and hazards, but its principle benefits of low temperature, permeability and compatibility overcome its risks. It has had moderate costs for equipment and consumables, however, other costs include: aeration, gas mixtures, reclamation or conversions, monitoring, and use of these can increase total costs. It can be synergised with humidity (moisture), temperature, and some chemicals, and it has a high level of material compatibility.

It still has an advantage over many newer green processes (e.g., chlorine dioxide, H_2O_2, low temperature peroxide plasma processes, and O_3) through its penetration abilities and greater material compatibility capacity (e.g., papers, some metals, some polymers and adhesives). Many polymers can be multiple sterilised. However, EO may not be able to multiple sterilise some polyacrylics and polystyrene (PS) at relatively high temperatures (e.g., 60 °C) without some effects (e.g., warping, and sticking), and may not sterilise bioabsorbables, at ordinary sterilising parameters.

Due to its high hazard, toxicity, carcinogenicity and by-products it is not regarded as a green process as such. However, it has improved equipment (gas recovery systems, scrubbers, and aeration chambers), enhanced processing (reduced EO gas) and better handling (gas mixtures). EO sterilisation has created a safer and more efficacious process. It becomes a more green process by using less sterilant, reducing EO concentration, recovery and re-using EO gas, and aerating to remove toxic residues and emissions from the environment.

EO is a 'versatile' organic compound with the formula C_2H_4O. It is a ring compound (**Figure 1.1**), which means that it is composed of two alkyl groups attached to an oxygen (O_2) atom in a cyclic shape (triangular), without any reactive radicals unless the ring is opened.

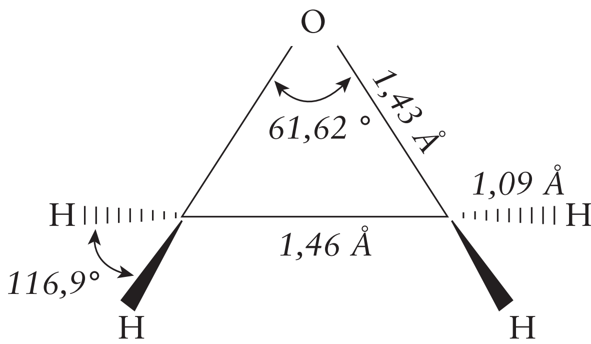
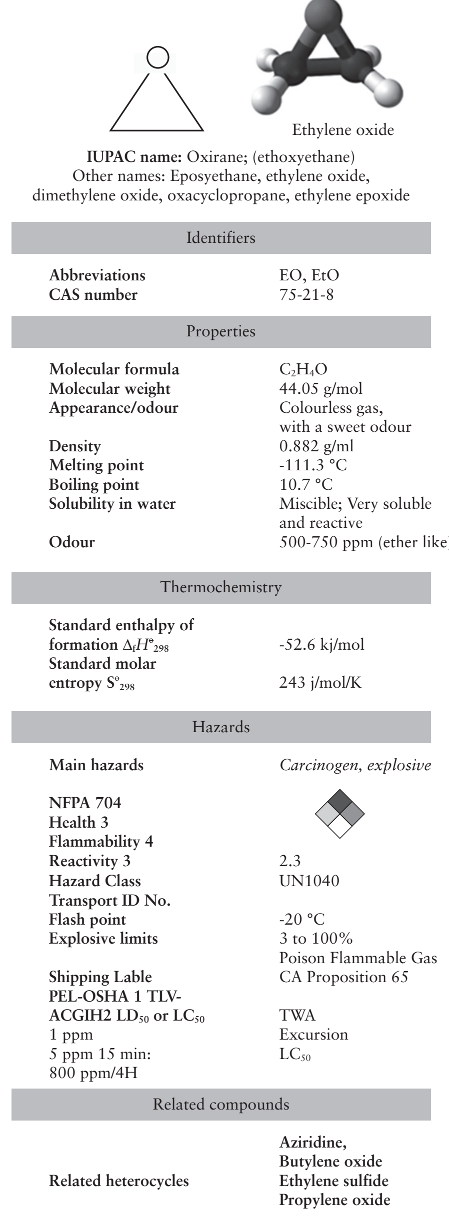


Figure 1.1 Structure of ethylene oxide (C_2H_2O)

Ethers are typically flammable and not chemically reactive, but EO is stable until its ring is opened. Most of the gaseous EO sterilant used is never reacted, but remains throughout the sterilisation process in a stable form. Virtually only the SH, COOH, NH_2, and OH side radicals or other compounds of organic matter or water is typically reacted [2]. Other strong oxidising and irradiating agents, react with many other chemicals and substances, and they are not soft and mild toward materials as EO is. EO is a traditional method that is able to sterilise many polymers, but not liquids. It may craze some polymers, and it can leave toxic residues and by products, but in another view it is very gentle on most materials as compared to other methods. It has been an exceptional sterilisation method. However, EO is a very hazardous substance, reactive and toxic (see **Figure 1.2**). It is highly explosive and can form toxic by-products, however, EO is a very versatile molecule and has unique properties.



IUPAC name: Oxirane; (ethoxyethane)
Other names: Eposyethane, ethylene oxide, dimethylene oxide, oxacyclopropane, ethylene epoxide

Identifiers	
Abbreviations	EO, EtO
CAS number	75-21-8
Properties	
Molecular formula	C_2H_4O
Molecular weight	44.05 g/mol
Appearance/odour	Colourless gas, with a sweet odour
Density	0.882 g/ml
Melting point	-111.3 °C
Boiling point	10.7 °C
Solubility in water	Miscible; Very soluble and reactive
Odour	500-750 ppm (ether like)
Thermochemistry	
Standard enthalpy of formation $\Delta_f H^o_{298}$	-52.6 kj/mol
Standard molar entropy S^o_{298}	243 j/mol/K
Hazards	
Main hazards	*Carcinogen, explosive*
NFPA 704	
Health 3	
Flammability 4	
Reactivity 3	2.3
Hazard Class	UN1040
Transport ID No.	
Flash point	-20 °C
Explosive limits	3 to 100%
	Poison Flammable Gas
Shipping Lable	CA Proposition 65
PEL-OSHA 1 TLV-	
ACGIH2 LD_{50} or LC_{50}	TWA
1 ppm	Excursion
5 ppm 15 min:	LC_{50}
800 ppm/4H	
Related compounds	
Related heterocycles	**Aziridine, Butylene oxide Ethylene sulfide Propylene oxide**

Figure 1.2 Ethylene oxide - physical and chemical properties. CAS: Chemical Abstract Service; IUPAC: International Union of Pure and Applied Chemistry; LC_{50}: lethal concentration – the concentration of a chemical in air that kills 50% of a group of test animals in a given time (usually 4 h); LD_{50}: lethal dose – the amount of a material, given all at once, which causes the death of 50% of a group of test animals; and NFPA: The National Fire Protection Association. Reproduced with permission from the National Fire Protection Association. ©National Fire Protection Association

For more physical properties of EO see reference [3]. EO is a colourless gas at 25 °C and is a mobile liquid at 0 °C, however, its boiling point would increase to 57.5 °C at 2 atmospheres. The viscosity of liquid EO at 0 °C is about 5.5 times lower than that of water, however, it is fully miscible with water. The gas has a characteristic sweet odour of ether, noticeable when its concentration in air exceeds 500-700 ppm, but acceptable levels in air are only 0.5-1 ppm (TWA) or 5 ppm (short-term exposure limits (STEL)). EO is not only readily soluble in water but also in ethanol, diethyl ether and many organic solvents, however, it is 1.5 times heavier than air and tends to settle along the floor, where it can become a hazard because of its explosiveness, flammability, and toxicity. Some of the characteristics of EO are:

- It is dangerous and hazardous.
- It is a gentle cyclic ether $(CH_2)_2O$, to most materials, polymers.
- It has a molecular weight of 44.05, the same as carbon dioxide (CO_2).
- Its primary action is alkylation (replacement of a hydrogen atom in an organic compound with an alkyl group like that in deoxyribonucleic acid (DNA)).
- It boils at 10.8 °C at normal pressure.
- It is a gas at normal temperature.
- It freezes at -11 °C, with water.
- It is highly explosive in its natural state – this can be reduced by mixing with Freon, nitrogen or CO_2.
- It is extremely toxic and potentially carcinogenic – it produces erythema and oedema,
- It is soluble in water and other solvents.
- It is highly permeable through cardboard, leather, many papers, plastics and rubbers.
- It is gentle on most metals, polymers and rubbers, unlike steam, heat, radiation, and oxidising agents.
- It is bactericidal on most microbes and biological organisms, including spores, but not necessarily prions.

EO is a technique that has some penetration capabilities, but requires a long time for the overall process (e.g., preconditioning, sterilising, and aeration). EO is an effective and soft (gentle) sterilant for most medical materials, polymers and devices and is re-usable.

It is used in 'both' hospitals and industrial manufacturing applications for manufacture of disposables. EO will sterilise most polymeric materials [1]. Common limitations of EO sterilisation relate to inadequate humidification, diffusion barriers, gas concentration, process time, and interactions. Diffusion barriers present a limitation to the efficacy of EO sterilisation if the EO gas, temperature and humidity necessary for the sterilisation process cannot penetrate into all locations within the device (e.g., into a stopcock or very long, thin lumen or large dense product load). Long overall process times can be an economic limitation to the application of EO due to long preconditioning periods, extended exposure times, post-sterilisation aeration times and post-processing biological indicator testing. Parametric release has traditionally been difficult to achieve uniformly with this method, but it is improving but faster release times can be achieved with the use of 'rapid (release) biological indicator incubation times (e.g., 4 or 72 h).

1.1.2 Ethylene Oxide Residuals

Hazardous material and toxic residuals are important considerations since EO is regarded as an explosive chemical, and a potential human carcinogen and a reproductive toxicant. It requires gas mixtures or special handling, robust scrubbers on gas emissions and worker exposure is a significant consideration. Post-sterilisation evaluation for toxic residuals (EO and ethylene chlorohydrin) must be performed before release or during the validation of the sterilisation process with each device.

EO sterilisation process modalities under certain conditions may retain residual sterilant. These residuals must be removed to levels required in other standards or in demonstration of biocompatibility. The International Organization for Standardization (ISO) 10993-7 [4] standard presents the allowable limits for EO and ethylene chlorohydrin residuals in medical devices. The ISO standard is accepted by most of the world, but some countries have added their own constraints on top of the international requirements (e.g., AAMI TIR 19 [5]).

In EO sterilisation, EO and ethylene chlorohydrin are the primary residuals of concern. However, other toxic residual chemicals may form, such as ethylene glycol (ETG), ethylene diglycol, dioxane and so on. Ethylene chlorohydrin is formed when the EO reacts with chloride ions, or active chlorine-containing chemicals in the load. Thus, if a device contains no chlorine atoms, and was not cleaned with a chlorine-containing compound prior to sterilisation, it is likely that there will be no ethylene chlorohydrin formed. If a device was treated with a chlorine-containing cleaning agent to lower the bioburden prior to sterilisation, there may be very large quantities of chlorohydrin formed. In general, ethylene chlorohydrin is not removed by aeration.

Materials vary considerably in their ability to absorb, retain, and release EO. Polyvinyl chloride (PVC) with plasticisers, and polyurethane (PU) may absorb a lot of EO, and have a great affinity for EO, but will desorb fairly well, however, materials such as actylonitrile-butadiene-styrene (ABS) and styrene acrylonitrile may absorb EO, but desorb slowly. Collagen is a poor material because it has high absorbency and slow desorption. Polytetrafluoroethylene ((PTFE) Teflon) is a good material, because it absorbs EO at low levels and has a slow desorption. Kynar is an excellent material because it is not affected by EO residuals Silcone is an excellent material as it absorbs EO but desorbs it quickly.

When conversion of EO to ethylene chlorohydrin (ECH) is possible, two similar devices made of different materials are likely to have very different residue profiles. The concentration of ECH varies greatly in materials that contain a source of free chloride ions. Some acceptable EO and ECH residual levels in devices are shown in **Table 1.2**.

Table 1.2 Acceptable EO residual levels (2008) taken from ISO 10993-7:2008 [4]

Type of device	Length of EO treatment	EO residual level	ECH residual level
Standard devices	<24 h	4 mg	9 mg
Standard devices	24 h-30 days	60 mg/30 days and 2 mg/24 h	60 mg/30 days and 9 mg/24 h
Standard device - permanent implant	>30 days	2.5 g/lifetime 60 mg/30 days and 4 mg in the first 24 h (average of 0.1 mg/day)	10 g/lifetime 60 mg/30 days and 9 mg in the first 24 h (average of 0.4 mg/day)
For intraocular lenses	Device exposure contact period	0.5 μg/lens/day or 1.25 μg/lens (maximum)	4 × EO limit(s)
For blood oxygenators	Device exposure contact period	60 mg/device	45 mg/device
For cardiopulmonary by-pass procedures	Device exposure contact period	20 mg (maximum allowable limit)	9 mg (maximum allowable limit)
Drapes that are intended to contact intact skin	Patient exposure contact period	10 μg/cm^2 (maximum tolerable contact limit)	5 μg/cm^2 (maximum tolerable contact limit)
Blood cell separator	Device exposure contact period	10 mg/device	22 mg/device
Extra corporeal blood purification devices, e.g., haemodialysers	Assuming 13 devices used per month. *Note:* Allowable lifetime EO may be exceeded	Should not exceed 4.6 mg/device	Should not exceed 4.6 mg/device

1.1.3 Advantages of Ethylene Oxide Sterilisation

Despite the creation of toxic residuals, EO has many extrinsic advantages as a sterilising process, at and beyond its classical usage. EO is gentle to materials and items compared to other sterilising methods. It is an effective sterilant for most medical devices, some pharmaceuticals, and many hospital products. It has become more costly with the necessity for safety and monitoring equipment. Its chemical and physical properties are well understood, and skilled users can rapidly develop and validate effective sterilisation cycles. Standard EO sterilisation cycles are executed at relatively low temperatures, moderate humidities, which make the process ideal for most polymeric materials, which would be destroyed by higher temperatures and moisture. In addition, there are no free radicals which can destroy many materials. In addition, most electronic devices can be successfully processed in EO, where they would be destroyed by other processes.

Use of EO has evolved from a classical sterilant to a continually evolving traditional method by reducing EO concentrations, sterilising temperatures, controlling relative humidities and balancing pressures. Classically it was used to sterilise spices and some biologics, but it is now capable of sterilising some enzymes and active electronics including electrical equipment without adverse effects.

Some of the principle advantages of EO are:

- Can be used at low temperatures.
- Good penetration and diffusability.
- EO is commonly used and easily available.
- Highly compatible with many heat and moisture sensitive plastics, polymers, materials and even some enzymes.
- Moderate costs for equipment and consumables - Other costs: aeration, reclamation or conversions and monitoring.
- Can be synergised with humidity (moisture), temperature and some chemicals.
- Highly bactericidal, and its classical EO gas concentrations can be significantly reduced.
- Residuals and toxic residues can be eliminated or reduced to safe levels.
- EO gas can be reclaimed and reused, or modified into a reusable by-product.
- EO gas that is released from sterilisers can be destroyed and rendered safe.

- EO sterilised product can be parametrically released.
- EO controllers, equipment and systems can be made fully automated, controllable and versatile.
- Modern EO cycles are easy to run, control, monitor and document.
- Particularly suitable for medical devices containing electronic components.
- The EO steriliser and aeration chamber can be continuous.

Multiple products that can be sterilised by EO are:

- Combination device and drug products.
- Implants.
- Multiple reuseable hospital items.
- Procedural and surgical trays.
- Single use-disposable devices.
- Some pharmaceuticals.
- Mummies (preserved bodies).
- Decontamination of enclosures (e.g., gnotobiotics).
- Unusual items in the Library of Congress.
- Nerve gas antidotes.
- Decontamination of powders

1.1.4 Some interdependent variables of using Ethylene Oxide

There are many interdependent variables within EO sterilisation cycles, which consists of multiple processing steps and parameters:

- Preconditioning - temperature, time and % relative humidity (RH).
- Initial vacuum - rate, depth and time.
- Pre-humidification -%RH, temperature and time.
- Gas injection rate, pressure change and time.

- Exposure - EO concentration and gas make up.
- Exposure – temperature and temperature range allowed.
- Exposure - pressure changes, sterilant or diluent gas make up.
- Dwell/Exposure - time, out of specification conditions.
- Post-sterilisation aeration - vacuum and pressure changes, number of dilutions and rates.
- Aeration.

Some different EO sterilisation process sources and methods are:

- 100% EO cycle with/without nitrogen.
- Standard EO gas mixtures.
- Balance pressure cycle.
- Air displacement cycle.
- EO/CO_2 gas mixtures.
- Low temperature processes.
- Dynamic preconditioning.
- Un-evacuated enclosures.
- Combination Processing of EO steriliser and aeration chamber.

Some miscellaneous considerations of successful EO sterilisation are:

- Aeration needed for residual removal, however, some by-products can be formed (e.g., ethylene chlorohydrin and ETG).
- Wetting organic matter can reduce its sterilisation effectiveness in the vapour state.
- Some materials are impermeable to EO (e.g., glass, (polyamide (PA) except with moisture), metals).
- Some materials (polymers) can be crazed or stick (e.g., styrene).
- Some thermotolerant spores can be more resistant than the biological indicator organisms.Current commercial biological indicators may not be an adequate monitor in low %RH conditions, since they are standardised at moderate %RH conditions and not against low %RH conditions.

- EO sterilisation can be synergised.
- EO can sterilise a multiplicity of polymers without significant damage.
- It is a gentle process.
- It can be used to sterilise re-useables.

1.1.5 Material Considerations with Ethylene Oxide Sterilisation

EO sterilisation uses multiple conditions - heat, moisture, pressure changes and exposure to EO or non-flammable diluents that could affect materials or products.

Consideration should be given to the potential physical, chemical, biological effects of these conditions and formation of residuals or by-products. During sterilisation products, or materials are subjected to environmental stresses of changes of vacuum pressure, elevated temperature and changes in humidity. Changes in pressure could affect the strength of package seals. Most polymer materials are compatible with use of EO, but liquids typically are not. Some materials vary with change of process parameters.

EO can sterilise glass, most polymers, metals, many heat stable polymers, celluloses, many drugs, biologics, pharmaceuticals, some medical devices and re-usable, but not infectious wastes that other methods such as steam can. It is moderately expensive, and has toxic residues or sources. It is not truly environmentally friendly, scientific, and compatible with many polymers, devices, and materials.

EO sterilisation in the form of minimum concentrated gas under pressure, is by far one of the more reliable mediums, known for decimal reduction value/death value (D-value) calculations (the destruction of all microbes logarithmically). It is the preferred method for packaged devices and items that are heat sensitive but not vulnerable to moisture. It is not widely used to decontaminate hazardous and contaminated wastes. EO is relatively expensive, not environmentally friendly, and previously widely available. The major concern with EO sterilisation is not the damage, degradation or destruction of materials by heat or moisture, but the significant safety and hazardous concerns. However, EO can sterilise many sensitive material materials such as: acetal, fabrics, glass, metals, PA, polycarbonate (PC), polypropylene (PP), polysulfones, PTFE, fibres, or celluloses (papers) that can be damaged or be rendered impractical by other methods. It can re-sterilise many (re-usable) materials that other methods cannot. If prudently applied and controlled, EO does not corrode metals, and can sterilise many other polymers (high-density polyethylene, PVC, many rubbers and heat sensitive materials, if the temperature is reduced (e.g., <45 °C) and the

exposure time is increased. If there is no 'clinical' or risk concern with thermophilic 'spores' as there is with irradiation or as with validation of other major sterilisation methods (e.g., H_2O_2 and O_3), then EO sterilisation could be applied at even lower temperatures (e.g., ambient to 30 °C), and under other conditions to sterilise even more heat sensitive materials, (e.g., enzymes). There has been a continuation of EO as a method for industrial sterilisation and to sterilise radiation damaged materials, tight fitting devices, cellulosics, medical devices, as well as enzymes and electronics that O_3 and H_2O_2 cannot typically sterilise easily.

1.1.6 Principles of Ethylene Oxide Sterilisation

The killing power of EO is due principally to the alkylation or chemical alteration of proteins in viable forms of life. EO like heat can inactivate all microbes, including the most resistant bacterial spores, and most moulds, except for *Pyronema domesticum* that may arise from desiccated or dry cotton that cannot be sterilised unless with pretreated or pre-sterilised with steam instead of humidity at elevated temperatures. Prions within brain tissue, or on contaminated instruments are recommended to be removed with steam sterilisation, not EO.

EO can sterilise at typically low temperatures and pressures. A minimum concentration of EO under pressure can typically run at low temperatures (e.g., <60 °C). Lower temperatures have been used (e.g., ambient to 35 °C) to prevent degradation or damage of heat sensitive material, polymers, certain enzymes and certain adhesives. EO typically does not destroy resistant prions or pyrogens. Higher temperatures (e.g., >65 °C) could be used for faster sterilisation with steam. The killing power of EO is due principally to the alkylation of proteins in viable forms of life - this can include chemically altered DNA and breakdown of vital enzymes. As the temperature and EO gas pressure increases, the time to sterilise decreases and conversely as the temperature and minimum gas EO pressure decrease, the time taken to sterilise increases.

Sterilisation with EO typically requires humidity. Less than saturation (e.g., >85%), humidity typically decreases the time to sterilise but EO may lose the ability to sterilise at below 15% RH of desiccated microbes. Theoretically, approximately 33% RH is an optimal humidity for EO microbiocidal activity, however, there are other considerations such as whether materials are desiccated, or moisture absorbing.

Salt encrusted materials, require higher humidities to dissolve the salts for inactivation or sterilisation [6].

The 'sterilising power' of EO is largely due to its alkylation capacity. As EO is delivered, it diffuses, and as its concentration increases, it causes an increase in

pressure, and then the volume of EO is absorbed, which sets up a drop in positive pressure that draws in more EO until it reaches a set point and equilibrium. With EO diffusion, lethal amounts of the chemical are delivered to microbes, until a gas, humidity and temperature equilibrium is reached.

Sterilisation with EO occurs when the available surfaces are treated with humidity through pre-conditioning or pre-humidification and penetration of gas is achieved through and into porous and permeable materials. An acidic pH can decrease the time and temperature required to sterilise with EO. Fats, oils, grease, low or insoluble salt crystals, biofilms or organic matter, slow or prevent penetration of a minimum concentration of EO and increase time or even prevent sterilisation.

1.1.7 Factors Affecting Lethality and Demonstrating Effectiveness

The minimum EO concentration, temperature, humidity, exposure time, gas concentration and gas exposure time are the primary factors affecting effectiveness of sterilisation. But cleanliness and quantity of bioburden will also have an influence.

For effective sterilisation, reusable items and equipment must be thoroughly cleaned. Simply wiping may not be sufficient. Cleaning may include disassembly, brushing, flushing, and rinsing. Cleaning may vary with product. Products can be divided into various categories:

- Solid items or devices – there are no hidden surfaces or lumens or blind spots. An example may be a wound retractor, a colposcope, spatula or rigid endoscopes such as laparoscopes and arthroscopes.
- Hinged items or devices - scissors and instruments with insert closures such as a box lock are examples. It must be possible to wash or rinse out hidden areas.
- Sliding shaft items or devices - these devices should be dissembled if possible, otherwise they must be washed or rinsed out.
- Tubular items or devices - examples are tubular instruments such as suction devices, trocars or other instruments with lumens such as shavers for arthroscopy. These may be flushed and brushed.
- Microsurgical items or devices - cleaning methods must be tailored to these extremely delicate instruments, such as hand cleaning.
- Special items or devices - these are items that cannot be assigned to other groups such as orthopaedic instruments, such as socket boring and pneumatic motor systems.

- Flexible items or devices - examples may be biopsy forceps, flexible instruments (endoscopes) used in minimally invasive surgery.

Cleaning can be problematic with items that have textured surfaces, hinges, springs, dead end lumens, inaccessible cracks and crevices, braided cables, and pliable materials such as silicone and rubber increase the difficulty of cleaning. Automated cleaning (e.g., washer-disinfector, ultrasonic cleaners) may have an advantage over manual cleaning that is not consistent, but at times automated cleaning may not work.

There are a variety of tests to determine if a product, device, or item is clean:

- Visual examination.
- Adenosine triphosphate test that produces light in the presence of microbes.
- Microscopy.
- Washing and rinsing with soil, protein solubilising agent and filtering.
- Follow manufacturer's instructions.

Cleaning before sterilisation will also depend upon the nature of the product, device or item's use. Critical devices or items are surgical instruments, implantable items such as hips, screws, and plates in orthopaedics, pacemakers, stents and so on. Semi-critical devices or items that are minimally invasive and may contact mucous tissues such as flexible endoscopes and so on. Non-critical devices that may contact intake skin, tissue, but are not invasive, such as stethoscopes, electrodes, bed rails, patient furniture, floors, all of which can contribute to cross contamination and so on.

Cleaning is particularly important when using liquid glutaraldehyde as it may not penetrate as well as EO, but EO may not penetrate organic encrustations, salt occlusions and so on. Both EO and glutaraldehyde as well as other chemical sterilants are enhanced by use of heat or increase in temperature, however, chemical sterilants are used for temperature sensitive items.

Temperature: the higher the temperature of the product or item being sterilised, the higher the lethality of the cycle. The temperature difference required to cause a 1-fold change in D-value (Z-value) for EO to change lethality one log or 90%, is about ~38 °C. So if the temperature is increased from ambient (e.g., 25 °C to 63 °C, the lethality could be increased ~10 times. Its temperature coefficient (Q_{10}) value is about 1.5 to 1.8, the lethality of the process would increase in 10 °C, by 1.5 to 1.8 times, not quite double.

Q_{10} value = 1.5 to 1.8

Z-value = ~38 °C

Heat is transferred to the sterilisation load during its pre-environmental treatment or preconditioning, during humidity dwell *via* a controlled steam process, and during gas injection. The sterilisation vessel temperature setting will also affect the heat transfer to the sterilisation load. A typical temperature range is 38-57 °C.

Without a minimum amount of moisture (humidity), EO sterilisation may not be effective. Contact with %RH is critical. Optimal %RH may be ~33%, but in reality 40-50% may be more practical, and a upper %RH of 80% may be useful.

Moisture has three important roles: lethality, heating and permeation and in particular it facilitates the lethality of EO to microbes. Higher humidities are required for more drier and desiccated materials. Moisture (sub-atmospheric pressure steam) also provides heating for higher temperature processes. Moisture also helps to get EO through materials such as PA, which are very polar. Paradoxically EO also helps to make moisture transfer through non-polar materials easier. A minimum concentration of EO is effective at an above ambient temperature.

Moisture and heat must penetrate to all parts of a product, or device to be effective. Typically heat flows into cooler areas. Air can be a barrier to moisture/humidity diffusion, as it is with moist heat sterilisation. Consequently device design, construction, conductivity and packaging are important considerations toward influencing the ability to kill microbes (see **Table 1.3**). There are a number of factors that will impair the ability of EO to inactivate microbes.

1.1.8 Microbial Effectiveness of Sterilisation

The microbial effectiveness or SAL of the EO sterilisation process should be confirmed, not just accepted. Consequently biological indicators (BI) of dormant spores, with thick coats are among the most resistant forms of organisms to EO sterilisation. Typically viruses are easier to sterilise than vegetative (no spores) microbes, but this may be in part due to the temperature and humidity of an EO process. Most viruses are very heat sensitive. Vegetative microbes (e.g., *Staphylococcus, Streptococcus, Micrococcus*) are easily inactivated unless desiccated, and typically microbial spores are among the more resistant (some are killed at slightly above ambient temperatures, others may require higher temperatures). Prions are even more resistant and may not be inactivated by EO, but chemically by a combination of formaldehyde and

formic acid. What constitutes complete destruction or sterilisation varies, but it has been defined as the demonstration of the inability of microbes to readily grow and reproduce on their own when placed in suitable growth medium.

Table 1.3 Some factors influencing the capacity of EO sterilisation to kill microbes

Factors relating to lethality of EO	Factors relating to the organisms to be killed
Intensity (concentration)	Types of microbes, e.g., spores, *Mycobacterium*
Exposure time	Number of microbes to be killed
Temperature	Level of SAL (probability)
Presence of air (desiccation)	Conditions that impair inactivation
%RH	Reduces sterilising effectiveness – salt, dirt
Distribution of heat, gas, humidity	Previous history of microbes to be killed
Related to product families (similar product)	Related to packaging families (similar packaging)
Length of lumens	Removal of air
Air pockets	Permeation of humidity and gas
Mated surfaces	Vacuum and air purge integrity
Absorbable material	Absorbable and insulation within boxes
Load mass	Packaging area of breathability
SAL: Sterility assurance level(s)	

Bioburden is a pre-sterilisation population of viable micro-organisms on an item or product of a sterile barrier system that need to be inactivated for sterilisation. Bioburdens are characterised as colony forming units (CFU), as a viable growth of microbes on culture plates or as tubes arising (growing and reproducing) from single or multiple cells.

When microbes (bioburden) cannot reproduce or grow under suitable post-sterilisation 'recovery' conditions they are designated as inactivated or killed. However, the presence of some substances (bacteriostatic, fungistatic, or sporostatic) can inhibit the outgrowth of viable organisms. To verify that such substances are not present requires a bacteriostatic or fungistatic test of materials or devices before performing a bioburden or sterility test. Unlike most microbes prions are not identified until after autopsy. They reproduce without intrinsic DNA or ribonucleic acid (RNA).

It is virtually impossible to test every sterilised product without sacrificing the entire product from a lot or load, but testing only a few samples of a final sterilised product can be misleading and a result of passing an unsterile product. However, by evaluating survival of microbes under fractional conditions and with a small sample size, it can enhance the opportunities of prediction of sterile product under full cycle or process conditions. Understanding that less than 100% of products will result in a probability (e.g., 10^{-1}) that some non-sterility can still persist is important. There is a relationship between small sample size and the probability of passing an unsterile sample in a batch, from the results of a conventional sterility test.

Microbes typically die or fail to reproduce logarithmically. The EO sterilisation method has one of the more perfect inactivation-logarithmic curves. There is no activation shoulder as there is with steam, and no tailing unless there is desiccation, and heterogeneous bioburden population, or clumping.

The dynamics of microbial inactivation by EO reveals, in general, that microbes are destroyed in a logarithmic or first order rate. An old explanation of this phenomenon is that the logarithmic order of death is due to an expression of a monomolecular reaction of protein penetration or damage, e.g., of one DNA gene essential to reproduction. *Note:* EO sterilises by alkylation of proteins or DNA, particularly the amine group of adenine or guanine. EO was found to be an irreversible agent. It is bactericidal in nature, not bacteriostatic. It provides one of the best examples of the logarithmic nature of death.

It should be realised that the microbial death observed is really a failure of the microbe to reproduce when placed under favourable environmental conditions and in an optimal recovery medium. What are deemed to be favorable conditions can vary. For example, *Bacillus atrophaeus* can be cultured to grow well at mesophilic temperatures (e.g., 30-35 °C) but not at thermophilic temperatures (e.g., 55-60 °C), for example *Geobacillus stearothermophilus*.

Statistics of EO sterilisation are based on the assumption that all micro-organisms die or are inactivated in a logarithmic or first order reaction rate. This assumption is reasonably true under laboratory or pure environmental conditions. As Mark Twain said, ‘there are three ways to lie: lies, damn lies and statistics'. Some exceptions exist, for example, EO sterilisation characteristically does not kill in a logarithmic way with desiccated microbes, clumped microbes, organic encrustation, barriers or salt occluded microbes. But since microbes in homogeneity with EO die in a logarithmic manner, it is possible to confirm sterilisation without testing all or various sample sizes after full sterilisation by taking samples after fractional cycles and evaluating results and outcomes, and calculating the D-value.

Death value or D_{10} value: is the time, timed-condition, or dose (EO concentration) required to achieve inactivation of 90% of a population (or one logarithm) of the test micro-organism population under stated conditions.

D_{EO} is a D-value obtained at parameters for EO sterilisation - see ISO 11461 [7].

$$\text{D value} = \frac{\text{Exposure time}}{\text{Log N}_o - \log \text{N}_b} \quad (1.1)$$

Where N_o (A) is the initial microbial or spore population prior to exposure and N_b (B) is the surviving microbial or spore population after time of exposure. Time or dose typically is an incremental or sub-exposure to the sterilising agent that allows the possibility of survivors. **Equation 1.1** for the D-value is the Stumbo equation.

The D-value is the backbone of sterilisation statistics for EO sterilisation and provides a characterisation of the resistance of a particular microbial population to a sterilisation method. Sometimes it becomes difficult to determine a D-value because the microbial population is heterogeneous, the population and resistance are extremely low, and the indigenous population does not follow a perfect logarithmic order of death. In many cases it is easier to perform D-value measurement for a particular process on bacteria spore populations used in Biological indicator(s) (BI) because they can be prepared as an homogeneous population, with a high resistance and demonstrate an ideal D-value curve to the sterilising agent.

To statistically perform a D-value measurement, one really needs at least four fractional D-value test run results. The new ISO 11135-1 [8] may allow two, but for the purpose of statistically evaluation, a singular D-value has merit, particularly by applying the Stumbo or Halvorsen–Zieglar equations and approach.

The more complex D-value is the Stumbo equation modified with Halvorsen-Zieglar (most probable number) equation, where:

$$D_v = \frac{\text{Exposure time}}{\text{Log N}_o - \log(2.3 \log^{n/s})} \quad (1.2)$$

Where:

- D_v is the time taken to inactivate one log or 90% of the population,

- N_o is the initial spore population,
- n is number of spore carriers or BI, and
- s is the number of sterile BI or fractional negatives.

An overkill cycle is the time to inactivate 12 time D-value or 10^{12} resistant spores,or 10^6 spores of a BI with an additional probability of 1 in a million or 10^{-6} chance of survival.

The Z-value is the temperature increase to reduce the killing time by 1/10th. The Z-value may be derived from the following equation:

$$Z = \frac{T_x - T_o}{\text{Log } D_o - \log D_x} \tag{1.3}$$

Where:

- T is the temperature at time o and time x, and
- D is D-values at time o and time x.

Demonstration of microbial effectiveness is based upon statistics, knowledge of product bioburden (ISO 11737-1 [9]) and microbial resistance. Micro-organisms with a high resistance to heat are referred to as bacterial spores or spore enzymes in BI.

A BI is typically a solution or carrier consisting of a known concentration of 'resistant' spores (e.g., *B. atrophaeus*) that are highly resistant to EO sterilisation, which can be used to predict lethality to the pre-sterilisation bioburden by use of the BI system, because the spores are intended to be more resistant than the bioburden on the product. BI results typically take 3-7 days at 30-35 °C for results, but more rapid indicators may be available (e.g., 48 h, 4 h, or possibly less). BI are used to check the cycle parameters.

The types of spores used as BI or monitors of the EO sterilisation cycle may vary, but for EO sterilisation *B. atrophaeus* is deemed to be the most resistant spore to inactivate. *Note:* Chemical indicators can also be used to assess parameters such as: time, EO concentration, %RH and temperature (EO labels and cards) as well as adequate moisture (RH sensor tests).

Today, many processes are released without using any BI, but rely primarily on physical and accurate chemical measurements.

Standard BI release: Typically product that is EO sterilised is released by the BI, which is to demonstrate that the BI after sterilisation will not grow in an optimal medium at 30-35 °C for 7 days, as well as documentation and acceptance of cycle parameters.

Parametric release: This uses EO analysis for concentration measurement, however, during validation, either BI and/or bioburden inactivation can be used to substantiate the parametric release.

Rapid BI release: As compared to classical seven day BI incubation testing, the use of a rapid release BI that allows after 4 h incubation is fast and excellent for quickly sterilised product availability. Rapid BI release allows for an easier validation than that required for a parametric release or process control release.

A process challenge device (PCD): This can be used in place of a BI or inoculated (spore) on or within product(s) or items that are designed to constitute a defined (greater) resistance to a sterilisation process than any bioburden hidden on a product. The PCD or BI is used to assess the performance of the process with 'fractional (short) cycles' to show lethality or 'half cycles' to demonstrate total inactivation with a 10^{-6} probability of assurance under full or routine cycles.

The purpose (or reason) of periodically performing bioburden measurements is to ascertain that the pre-sterilisation bioburden count or bioburden estimate on products (sometimes referred to as bioburden load) produced in a controlled environment are of sufficiently low enough population and resistance that the 'appropriateness of use' of BI or PCD validated continue to indicate that successful sterilisation by EO exposure is appropriate.

In general there are three ways to test for sterility: product sampling and product sterility testing, BI or PCD, and or a combination approach (product and BI). Unless one tests every sample from a load there still remains the possibility that there will be an unsterile unit, unless the test is performed under fractional exposure time. Product sterility can be performed according to ISO 11737-2 [10] or by appropriate pharmacopeia methods.

Product sterility can be performed directly with the product immersed in sterility media or indirectly/directly by passing wash solutions through a filtration membrane.

BI testing for sterility is an indirect approach. The application and use of BI with resistant spores can be used *in lieu* of product testing. To be effective, the BI must be demonstrated to be more resistant than the product bioburden, during qualification and validation. BI can be a form or monitor of sterility evaluation. BI generally consists of spores or spore enzymes of highly resistant microbes, which are placed on or in the product load prior to sterilisation. These indicators generally have a high microbial

population (e.g., 10^6) in excess of what is naturally occurring on the product. The combination of high microbial population and high resistance to a specific sterilisation process make these indicators a fairly reliable tool for determination of process efficacy or product sterility.

A combination of bioburden and product sterility after fractional cycles is another way of verifying sufficient SAL. This is performed typically during validations. Product (bioburden) and sterility testing must be typically carried out according to ISO 11737-1 [9] and ISO 111737-2 [10] or according to appropriate pharmacopeia methods. Since EO sterilisation methods destroy or eliminate microbes logarithmically, it is possible to measure the kill time logarithmically (D-value), from the measured bioburden and product sterility survivors from a fractional cycle and extrapolate the inactivation or sterility to a probability, e.g., SAL.

EO sterilisation effectiveness can be validated and tested to determine its SAL, which is denoted typically as a 10^{-6} probability of finding survivors. Requirements and guidance for this (EO sterilisation) testing and validation are within ISO 11135-1 [8].

Validation of a sterilisation process and equipment is a process of obtaining, demonstrating and documenting evidence that the equipment (and process), as installed and operated in accordance with operational procedures, consistently performs in accordance with pre-determined criteria and thereby yields product meeting its specification, with protocol (plan) and documentation.

What constitutes complete destruction or removal of viable microbes varies. It is virtually impossible to test every sterilised product without sacrificing the entire product from a lot or load. Since most sterilisation methods destroy or eliminate microbes logarithmically, it possible to measure the kill time logarithmically (D-value), and extrapolate inactivation or sterilisation as a probability. Sterilisation by heat is predictable because it can destroy microbes logarithmically and thus be evaluated statistically, through validation. There are three methods or approaches of microbiological validation.

The overkill method consists typically of three half cycles in which none of the (10^6) spores from BI or PCD survive. Other methods include a comparison between the BI or PCD and bioburden on the product with typically a demonstration of 10^{-6} probability of survivor of microbes on the product. The bioburden testing is performed to show that the BI is of potential greater resistance than the natural bioburden. *Note:* Validation is a step-by-step documentation approach to demonstrate sterility.

In a total bioburden approach, the bioburden testing will be determined to show its inactivation and resistance to the actual process and demonstrate a 10^{-6} probability of

survivors. Non-invasive medical devices may require only 10^{-3} probability of assurance.

There are a variety of reasons why an EO sterilisation process may fail as well as why it is successful.

1.1.9 Process Variables and Types of Ethylene Oxide Sterilisation

EO Sterilisation = Gas (*conc*) + *Temperature* + *%RH* + *Time*

EO sterilisation may not be faster at high concentrations and have more parameters (steps) to deal with than other non-heat methods (e.g., H_2O_2, with plasma; O_3) of sterilisation, and, it may have more parameters than sterilisation by moist heat, because of the need to aerate.

1.1.9.1 Process Variables

EO concentration, %RH, temperature and pressure and exposure time are the primary process variables for EO sterilisation.

1.1.9.1.1 Ethylene Oxide Concentration

EO gas concentration is measured in milligrams per litre (mg/l). The typical effective range concentration is between 400-1,200 mg/l. The higher the EO concentration without any changes to %RH or temperature, the shorter the time taken to sterilise. At high EO concentrations the inactivation reaction becomes zero order, but below a threshold concentration(s), the inactivation reaction is first order under ideal conditions, but not necessarily with product mass, where EO is absorbed. EO concentration will influence the amount of EO residuals produced. Consequently a reduced EO concentration may be required to achieve acceptable EO residual levels. Gas concentration and gas time may be similar above 440 mg/l between 42-55 °C, and EO concentrations of 300 mg/l may be maintained above 55 or 60 °C [6] where under ideal conditions there is no product mass or barriers. However, standards and manufacturer's instructions should be carefully followed, unless demonstrated with repeatable results to the contrary and validated differently. Typically inactivation times are directly proportional to changes in EO concentration. However, as temperature increases, the minimum EO concentration activity will begin to plateau, but this will vary with product mass and barriers, where higher EO concentrations may be required. However, the minimum active EO concentration may not plateau with 100% active saturated steam, as the temperature increases.

1.1.9.1.2 Temperature

EO vapourises above ~11 °C. Consequently it theoretically can sterilise at temperatures as low as ~12 °C, which may be optimal for sterilising biological materials (e.g., bone marrow) or tissues. But its typical effective sterilising temperature is as low as 21 °C. Optimal operating temperatures for EO sterilisers typically range between 20-60 °C. Some pharmaceuticals with pre-sterilised syringes in packaging have been sterilised as temperatures as low as 20-25 °C. Temperature also affects the pressure of the gas. As the temperature rises, the pressure also rises. So a gas mixture with an inert gas [e.g., chloroflurocarbon (CFC) or CO_2] may not be able to deliver as high a concentration as 100% EO. A preheated steriliser will allow the EO to remain in a gaseous state. Careful attention must be paid to the chamber temperature and pressure.

The Q_{10} value of EO may be 1.5 or 1.8. So every 10 °C rise may halve the sterilising time. Its Z-value is approximately 38 °C. So a temperature change from an ambient temperature (22 °C) to 60 °C may decrease sterilising time by 10%. Increasing the temperature for EO may be limited because of the formation of its by-product (ETG), with EO and moisture (humidity) of the process, which can be toxic at high concentrations.

1.1.9.1.3 Relative Humidity

The standard conditions described for use of %RH are typical of the McDonald pre-humidification EO process. It is humidifying under vacuum with a 'static dwell' time prior to the injection of gas. This process injects steam after air has been removed under vacuum followed by a 'static dwell' period prior to admission of the gas. Pre-humidification is regarded as one of the most important steps in the sterilisation process, because it is important to strategically place moisture under vacuum (with removal of air) prior to admission of EO gas, for the following reasons:

- The number of water molecules even in a highly humidified environment is overwhelmed by the greater number of EO gas molecules.
- The diffusivity of EO surpasses that of water vapour.
- Water readily reacts with EO and CO_2 through hydrogen bonding which would create aggregates that would impede diffusivity of water vapour.
- Molecular interference such as air pockets and expanded heat sealed plastic bags prevent effective permeation of the water vapour.

In practice steam is introduced into a steriliser after air has been removed to a minimum

of 35% RH and commonly at a high level of RH usually 40 to 80% RH, and allowed to dwell or 'soak' for a period of time to allow the moisture to permeate the load. The rule of thumb is always to provide moisture in excess of a minimum of 35% RH. However, the higher the humidity level, the greater the driving force, but while 100% humidity or saturated steam may be the highest driving force, it may not be desired because it could wet the materials. It is important to inject steam at a slow rate, otherwise dry product may overheat due to excessive hydration or over moisture of certain materials (e.g., dry cardboard boxes), causing an exothermic response.

To achieve more uniform and optimal pre-humidification, for static dwell process(es), a deep vacuum should be considered, so that as much air removal as possible can be achieved with the equipment and system.

To prevent thermal lag between the chamber and the load, a slower steam rate should be considered depending upon the density, level of moisture, and size of the load.

Moisture in EO sterilisation is typically measured as %RH – this is the ratio of the amount of water actually present in the air to the greatest amount (saturation) in air. While EO sterilisation may be considered a dry process, typically some moisture must be present, but not at a saturation level. The optimal %RH is between 30-60%, but %RH values are kept between 40-80% because of variations and absorption of moisture in product loads, but higher %RH levels are needed for salt encrusted spores [6].

Moisture plays an important role in sterilisation. It may help in the alkylation process of EO to inactivate microbes, but it may also help to moisturise, desiccated or dry microbes to allow EO in, by possibly softening the surfaces of the spores. Organic and salt encrustation of spores, can require higher humidities for inactivation or sterilisation.

Moisture will help EO transfer/pass through polar materials such as PA and cellophane, while EO will help to drive moisture through non-polar materials such as polyethylene and polypropylene. In industrial sterilisation, it is frequently recommended to pre-condition product loads to overcome product dryness. So in addition to a 30-60 min period in vessel pre-humidification, or products may be pre-conditioned prior to sterilisation for 8-24 h under ambient pressure conditions.

Moisture in the form of pre-conditioning or pre-humidification may help to heat the product load and bring it to an equilibrium. Some pre-conditioning has also been shown to reduce bioburden on the product prior to sterilisation. Moisture under very deep vacuums with little air, may also help dilute residual air to keep EO within the chamber outside of its potential explosive range.

RH plays a very important role in all sterilisation processes. RH plays a critical role in EO sterilisation processes for several different reasons: these reasons are discussed in order to determine and establish further %RH parameter limits such as exist in, in-house dynamic environmental conditioning.

RH is necessary for effective EO sterilisation, no matter what cycle is used. Scientifically it has been demonstrated that the microbiocidal action of EO was 10 times faster at 28% RH than at 97% RH, and four times faster at 28% RH than at 65% RH, during gas exposure. Currently %RH is measured indirectly (as steam pressure) before exposure.

Paradoxically it has been shown that sterilising efficiency increased with increased RH, essentially when loads or product were extremely dry, and higher temperatures of sterilisation were required.

Microbiocidal activity is minimal when spores are extremely dry or desiccated and high RH are required to reduce the resistance of desiccated or very dry spores. This desiccated state may not be readily overcome by a high RH level unless the spores are exposed to 100% RH or intentionally wetted. Concern over wetting has been expressed, because EO gas may be absorbed into moisture, particularly on absorbable or porous material like corrugated cardboard, which can lead to a significant decrease in EO concentration. For example it has been suggested that a zone of high moisture could have a diluting effect on EO reducing its availability to the micro-organism especially when the EO environment is minimal.

When bacteria are occluded in organic matter, crystals, or even sweat, they can become extremely resistant, which consequently has lead to the use of high humidities. The humidity necessary to kill the *B. atrophaeus* spores in a salt preparation was found by the Statens Serum Institut of Copenhagen, to be at least 76% RH at 20 °C, a value which was recognised officially by the Nordic Pharmacopoeia. It was used as the constant humidity condition to dissolve sodium chloride in sweat or at 60 °C, it would be minimally 75% RH. Several years ago, Sweden had asked that their spores be inactivated with the salt preparation. Only a dynamic (environmental) conditioning cycle, (i.e., a cycle where steam was pulsed into the steriliser prior to admission of gas), was able to show any inactivation of that indicator. However, they accepted cycles without full inactivation of their BI, when there was extremely low bioburden on the product.

Most sterilisation processes inject steam and measure RH during pre-conditioning (before and outside the vessel), and other measurements are taken during pre-humidification or during dynamic conditioning, before admission of EO. This is regarded as one of the most important steps in the sterilisation process, because it is

important to strategically place moisture under vacuum and prior to admission of EO gas, for the following reasons:

- The number of water molecules even in a highly humidified environment is overwhelmed by the greater number of EO molecules.
- The diffusivity of EO surpasses that of water vapour.
- Water readily reacts with EO and carbon dioxide through hydrogen bonding which would create aggregates that would impede diffusivity of water vapour.
- Molecular interference such as air pockets and expanded heat sealed plastic bags prevent effective permeation of water vapour.

1.1.9.1.4 Exposure

Of the four sterilising variables essential to EO sterilisation, exposure time may be the easiest to measure and control. A change in any of the variables %RH, temperature, pressure, or EO concentration may influence exposure. Typical EO exposure times are 1-12 h. These times are dependent upon pre-environmental conditions, the load mass, barriers to gas, product design and construction, humidity, and temperature. The ability to sterilise depends upon the conditions that exist surrounding the microbes during exposure.

With longer exposure times, it becomes imperative to either make up gas that is absorbed in the product load, or to verify that a minimum gas pressure or concentration that was validated is achieved at the end of exposure. Exposure is the period of the cycle in which the microbes and product are in contact with minimum concentration of EO at a set temperature for a certain period of time, necessary to inactivate all microbes, typically for a probability of survivor of 10^{-6} or better. Typical relationships of time - gas concentration and temperature result in a certain pressure.

Pressure during exposure will vary directly according to the concentration of EO and the temperature applied. If the EO pressure and temperature do not correlate per the manufacturer's instructions or procedures, then other variations exist and sterilisation may not be achieved. Typically as gas pressure increases above atmospheric pressure, the %RH will be observed to increase. Therefore, %RH is observed and monitored for quality prior to EO gas injection.

Bioburden affects lethality. With EO, low bioburden and bioburden control are factors affecting lethality of processing. Unlike EO sterilisation, steam can have a non-logarithmic inactivation death curve where heat activation (an initial shoulder)

occurs in the decline curve because very dormant spores are virtually resurrected by the initial excess of heat energy from the steam. However, clumping or heterogeneous populations can lead to tailing of an inactivation curve of virtually any sterilisation methods, including EO, particularly with large microbial populations. Consequently, EO is best performed with pre-sterilisation low spore bioburden levels below 1,000 CFU/device.

With the chemically altered proteins within microbes, EO sterilisation becomes more effective at very low temperatures for non-spore microbes. With temperatures of less than pasteurisation temperatures (e.g., 62-72 °C) that are required for non-spores (vegetative cells), EO sterilisation is typically effective at above ambient conditions for inactivating both vegetative and bacterial spores, however, in the presence of enzymes and other heat and chemically sensitive biological matter, EO can inactivate spores at lower (sterilising) temperatures (e.g., 11-22 °C), and longer exposure times.

Product or material must be brought up to the sterilising temperature. EO is many times more effective in materials and products that have been heated up than with dry heat. Time to heat-up can be very lengthy, unless performed under optimal pre-conditioning, humidification and gas injection.

Proper loading for EO penetration and distribution is necessary for less dense loads and heavy packaging. Traditional temperatures for EO are 30-60 °C. These temperatures kill only microbes but do not depyrogenate. The temperature for just killing microbes must typically be in excess of 22-25 °C. Medical sterilisation of 'very' heat sensitive enzymes or polymers (e.g., ABS, acrylic, styrene and so on) may require lower temperatures (e.g., 30-40 °C) than some typical traditional EO methods at temperatures of 50-60 °C.

Exposure or contact with EO can vary with types of polymers and materials (e.g., textiles, fabrics and so on). Where EO cannot easily penetrate, it will not be very effective, unless it heats up the product with EO. If the sterilant is glutaraldehyde, less inactivation will occur and longer exposure time is required, without any heating up of the product.

Temperature, heat and EO must penetrate all parts of a device to be effective. Typically EO flows or diffuses into many areas (long lumens, some mated or adjacent surfaces), which many other chemicals will not, because of EO's ring structure and chemistry. It goes into dense areas, mated surfaces, and where air is a barrier to permeation. Consequently device designs, construction, conductivity, packaging, and loading are critically important to achieving adequate lethality and ultimate sterility. Besides sterilisation cycle parameters, other steps in the process must be considered.

1.1.10 Typical Process Sequences for Ethylene Oxide Sterilisation

Sterilisation is still considered an art not a science, but is more a solution of microbiological effectiveness and engineering efficacy.

1.1.10.1 Staging

Staging is the placing and positioning of the product during loading so that EO or humidity *via* steam injection can be easily dispersed, disseminated and permeable to all surfaces that need to be sterilised. Some miscellaneous precautions:

- Prior to sterilisation, all instruments and other items to be sterilised should be decontaminated, cleaned and not desiccated).
- All jointed or mated surface instruments should be in the opened or unlocked position, while instruments composed of more than one part or sliding parts should be disassembled.
- In larger sterilisation chambers, EO may be constantly flowing or mixed with either a fan or a blower, because without it, stratification may occur.
- Instruments should not be held tightly together by rubber bands, clamps or any other means that will prevent EO contact with all surfaces.
- Packs should be arranged in the chamber to allow free circulation and penetration of EO to all surfaces.
- When using an EO steriliser, it is best to package devices in appropriate packaging materials.

Besides air and moisture permeable packaging (e.g., paper, spun polyolefin), EO can penetrate non-polar packaging (polyethylene) and drive moisture through the film, it will not penetrate polar materials (PA) except with the help of moisture from humidity. EO works best with materials that have similar qualities to itself, for example, EO is non-polar and likes non-polar materials (polyethylene). Moisture (water) is polar and likes polar materials (e.g., PA). However, moisture, humidity will diffuse easier through porous materials such as paper and spun polyolefin than PA. For large production loads, loading arrangements must be specified and validated for effective %RH, temperature and gas distribution and/or penetration.

1.1.10.2 Air Removal

Air must be removed or displaced so that moisture and EO can effectively contact all surfaces and penetrate areas to be sterilised. This may be performed by various methods.

The air displacement method is where air which is lighter than EO mixtures is exhausted through the top of chamber (the reverse of gravity displacement by steam). Air displacement is typically used to sterilise packages entrained with air. The typical rate of exhaust is slow to remove air around the product package to prevent them from bursting. The air displacement method is simple and requires typically less equipment than deep evacuation pumps. A dynamic displacement method (see **Figure 1.3**) will include a steam purge and steam pulsing to improve sterilisation efficacy through dense or troublesome product loads. This dynamic phase is the same as with a saturated steam cycle. In both situations, it can help not only moisturise, but also heat a product load.

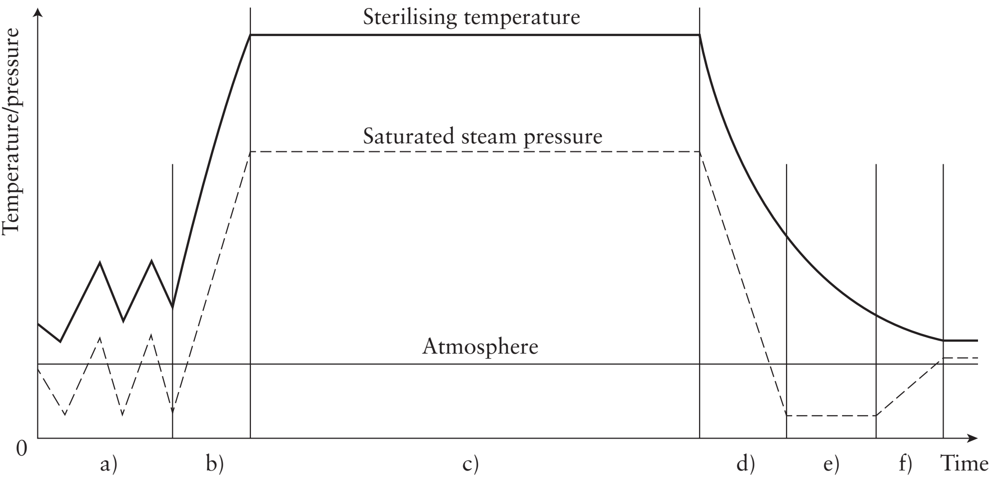


Figure 1.3 Phases of a dynamic (environmental) conditioning process for an EO or saturated steam process

This process is intended to sterilise products where air is difficult to remove. An example of a chamber temperature and pressure profile is given in figure. The EO sterilisation process like a saturated steam process is performed with a forced-air-removal system consisting of six major phases:

a) *Air removal/conditioning*: After the chamber is loaded with carriers or pallets of product, the door is closed and secured. Air is dynamically removed from the chamber and load by a deep vacuum and a dynamic series of vacuum and steam pulses.

b) *Charge (EO injection) or come-up time (saturated steam)*: EO gas is injected like saturated steam in to the chamber until the sterilisation temperature and pressure are attained.

c) *Exposure*: The sterilising temperature and pressure are maintained in the chamber by ethylene gas or ethylene gas mixture like a saturated steam process, for a specified exposure time.

d) *Exhaust (vacuum)*: EO is exhausted from the chamber by a vacuum and may be drawn to a pre-determined level.

e) *Aeration:* For products that are required to be aerated or (dried after steam) the temperature in the jacket and the vacuum in the chamber are maintained for a pre-determined period.

f) *Vacuum relief*: Air is admitted to the chamber through a microbiologically retentive filter until atmospheric pressure is reached.

High evacuation EO processing removes the air before the EO is injected. Air removal can be checked by a monitoring leak test (where vacuum is held) or by a Bowie Dick test, which monitors the diffusion of moisture. Pre-vacuum cycles are typically faster than balance pressure methods, but the product must be able to withstand vacuums and pressure/vacuum rates or peel pouches may burst, or covers from pipe ends may blow off. The control rates typically need to be controlled. Package bursting usually occurs during the post-vacuum phase of the cycles, but a post-cycle hold is typically used to de-gas the load. Hold times may vary between 20 and 45 min or longer.

Dynamic steam pulsing removes air from the steriliser and entrained air in the packed load. This type of method usually includes a steam purge followed by EO injection and gas exposure, then post-evacuation for improved aeration (see **Figure 1.3**). Slow post-evacuation will generally not keep the product from bursting as much as use of an EO overpressure cycle, but it can maintain the product configuration. However, over-pressurised process cycles have been developed to prevent bursting.

EO processes are typically processes (**Table 1.4**) that have some air in order to prevent the sealed packaging from bursting. In such cases recirculation fans are used to mix moisture, EO and air, because otherwise entrained air is a barrier to moisture/EO diffusion and penetration. EO - air mixtures are less effective than deep vacuum EO

processes with no air. However, they (package with air) can be efficient for keeping the product moist and allowing EO to diffuse into the retained air (e.g., mixing), and keep the package from bursting at the end of the cycle. This cycle will require de-gassing to remove residuals.

Table 1.4 Typical (industrial) EO cycle parameters*

Phases	**Parameter Conditions**
Pre-conditioning parameters	
Time	8-12 h
Temperature	Ambient to 44 °C
RH	30-80%
In-vessel parameters	
Initial vacuum	
Vacuum	From ambient (atmospheric) pressure to 0.09 MPa (vacuum), for example
Pre-humidification and/or pre-conditioning	
RH	30-80%
Dwell time	15-120 min
Exposure time	
Gas concentration	400-1,500 mg/l
Temperature	25-65 °C
Dwell time	30 min to 16 h
Vacuum/air wash	
Vacuum/pressure	0.09 MPa (vacuum) to ambient (atmospheric) pressure
Number of washes	1-3, typical
Post-aeration parameters	
Temperature	Time
60 °C	8 h
50 °C	12 h
25 °C	4 days

*Please note: the figures and parameters given in this table are just examples. Significant variations may occur due to changes in load sizes, gas absorption EO residuals, and improvements in sterilisation.

1.1.10.3 Typical Profile, Phases or Steps in an Ethylene Oxide Sterilisation Process

Pre-conditioning (optional with industrial sterilisation, is not typically applied in hospital sterilisation cycles) is a period of time to condition a product load under controlled temperature and %RH conditions.

Pre-evacuation is used to remove air prior to admission of steam for humidification.

The humidity and heat-up is the steam period for conditioning with the %RH and heat.

Subsequent gas injection of EO involves gassing up to the desired exposure, pressure and temperature. During the gassing phases microbial inactivation may begin occurring before achieving the set point in large industrial sterilisers. A longer conditioning time typically reduces exposure time, if optimal lethality is integrated. Humidification and heat-up time enhances the conditioning and heating of material for alkylation of EO of microbes on or within material.

Exposure is the period of the cycle in which microbes and product are in contact with a minimum concentration of EO, at a set temperature for a certain period of time, which is necessary to inactivate all microbes, typically with a probability of survivors of 10^{-6} or better. Typical relationships of time, gas concentration and temperature are expressed in terms of temperature and time (**Table 1.3**). The minimum concentrated EO pressure (without carrier) under normal atmospheric pressure and temperature is approximately 0.1 MPa or less than 0.1 MPa. Pressure will vary directly according to the concentration of EO and the temperature applied. High temperatures will have a minimum concentration of EO (e.g., 400 mg/l at greater than 44 °C and higher, above which a higher EO concentration is not required unless for reasons of absorption and so on). If EO pressure and temperature do not correlate to the manufacturer's instructions or to the minimum concentrated EO mg/l, then other variations exist and sterilisation may not be achieved.

The post-evacuation period is the period or step after exposure to bring pressure, temperature, EO concentration and moisture down to reduced conditions. This may be a period where packaging or containers (with entrained air) may burst or distort with the change in internal pressure *versus* external pressure and may require positive pressure overlay in the chamber to prevent it.

During the degassing period, microbial inactivation may be continuing to occur beyond simple exposure to EO. A longer degassing period may reduce exposure time, through integration of lethality during this period, and can help reduce the overall (total) process exposure appropriately.

Aeration is the period following EO exposure and the degassing period where residuals are allowed to degas. Aeration may also remove residual moisture and help polymer hydration. Post-heated aeration can provide additional inactivation by not allowing damaged microbes to repair themselves through nucleic acid annealing that might otherwise be observed as a slow growth, sterility incubation phenomena. Aeration with circulation and some heat can help eliminate water marks, and restore material distortion.

Post-sterilisation heat aeration may complete the inactivation of a few microbes that managed to survive after the heat-up, exposure, and degassing phases of the cycle for full inactivation. After a typical EO cycle, the product may be post-sterilisation heated and aerated, to achieve an addition reduction of residual microbes spores by 1-2 logs or more of population before putting them on test. If the product were not heated and held, but refrigerated, further reduction of residual microbes or spores will not occur.

1.1.11 Some Types of Ethylene Oxide Sterilisation Cycles

Standard EO cycles vary, but two are predominant:

- 100% EO cycle with/without nitrogen, which is typically used in industry and the standard EO/Freon (CFC) cycle. The cycle generally begins with an initial vacuum to remove air, and then steam is injected for pre-humidity, because steam diffuses slower than EO. After pre-humidification, EO gas is injected (either 100% EO with or without nitrogen or an EO gas mix with CFC). Then items are exposed for a period of time, and the EO gas is removed by vacuum and air purging, and then a final air injection to atmospheric pressure.

- Air displacement - is method to displace air in the chamber by a flow of EO with a diluent that is heavier than air to displace the air through the vent at the top of the chamber.

- Pre-vacuum – McDonald cycle - a vacuum is pulled into the chamber before allowing steam in (first) before injecting EO, because EO diffuses faster than humidity.

- Dynamic environmental conditioning – this type of cycle is used in place of the McDonald process - a pre-vacuum with steam pressure pulsing and vacuuming is applied - where a series of vacuums and steam pressurisations are performed before injecting EO gas to a pressure setting for exposure, to drive out any residual or hidden air pockets in the load, and thoroughly heat the load.

- Match flow is a very rapid method of injecting steam under vacuum at the same rate air is being exhausted prior to injection of a minimum concentration EO sterilisation process.

1.1.11.1 Other Ethylene Oxide Sterilisation Methods or Cycles

Steam EO would be a process with temperatures in excess of 65-70 °C where steam is injected (typically under vacuum) to bring the load up to 65 °C or higher and then injecting EO gas. Under these conditions saturated steam is used to effectively pre-heat the product before EO gas is injected. Since elevated temperatures are provided, there is activation and some inactivation of microbial spores, making the subsequent killing by EO easier. However, ETG may be an additional EO residual that is more tolerable than pure EO or ECH, as residuals.

Balance pressured EO–air mixture – EO is mixed with air to keep some sealed packages with entrained air from bursting. This often requires circulation fans. Air cooling is often used during the post-exposure portion of the cycle. It is less effective than humidity, but useful.

Package injection or EO and diffusion - is a method to keep products uniform and packages/containers from distorting.

A truly magical EO sterilisation process is a specialised, specific low EO concentration (<300 mg/l) and low residual (<2 ppm) process that has proved very effective and efficacious (see **Table 1.5**). It begins with a deep vacuum pre-humidification (without any pre-conditioning), involves a slow heating injection of saturated steam to a set point under deep vacuum to >85% RH for maximum heating >60 °C for high temperature of the load and low EO concentration (<300 mg/l) from a dilute EO/CO_2 (heated) gas mixture. The gas mixture is injected to nearly double pressurisation (e.g., ~0.2 MPa) of the chamber. *Note:* EO boils at ~57 °C at 0.2 MPa. The use of the EO/CO_2 gas mixture is thought to drive the EO gas through very difficult to permeate areas until pressure equilibrium of the external and internal gases is achieved. The high pressure CO_2 gas may create a slightly acidic environment to help open the epoxide ring. This process includes a continuous EO cycle including heated aeration as part of the total cycle. It employs a non-flammable, low 6-8% EO/CO_2 gas mixture. In this process heated aeration continues to inactivate microbes and spores. BI or PCD are not removed until after the heated aeration. Consequently the EO/CO_2 gas is removed and product is (drawn) moved directly into heated aeration for an additional period of approximately 6-12 h. High levels of spore inactivation have been achieved with <300 mg/l EO. This process (300 mg/l) provided equivalent results to a standard 1,200 mg/l cycle with the same difficult to sterilise device. Also

extremely low EO residuals of less than 2 ppm were achieved. Anything toxic taken in a small dose (concentration) is actually stimulatory. This excellent EO residual level is the result of a combination of very low EO sterilising concentration and heated aeration (>45 °C). Because of the 'statistical' uniqueness of this process a '12D' BI/PCD is applied and employed 'routinely' on every load, to verify overkill continuously, and not just during validation or requalification.

Table 1.5 A Specialised EO cycle

Phases	**Parameter conditions**
Preconditioning	Not applied
Initial vacuum	
Vacuum	Ambient (atmospheric) pressure (0.1 to lower than 0.09 MPa vacuum*)
Pre-humidification	
%RH	>85% to less than 100% RH (no wetting) steam injected slowly (over 20-30 min) to a set point.
Procedure time	20-30 min
Exposure	
Gas concentration	<300 mg/l* 6% EO/CO_2 gas pressurised to ~0.2 MPa
Temperature	60 °C
Dwell time	4 h
Vacuum/air wash	
Vacuum/pressure	To 0.09 MPa (vacuum) and then up to ambient (atmospheric) pressure
Number of washes	Repeat 1-3 times - final pressure to atmospheric pressure
Post-sterilisation aeration in an aeration chamber contingent to or within the steriliser (may be eliminated with lower EO concentrations (e.g., 50-100 mg/l*)	
Temperature	Time
60 °C	8 h
Cycle end	-
12D BI/PCD removed	Rapid BI 4 h
*The parameters and phases can vary depending upon the load and the load mass configuration, moisture, gas absorption and so on.	

Each type of sterilisation, discussed previously has its 'individual' advantages and disadvantages. The selection of the particular process type is dependent upon a

variety of factors such as the material/product/packaging capabilities, and the end use characteristic of the product and package.

1.1.12 Advantages and Disadvantages of Ethylene Oxide Sterilisation

EO sterilisation, although widely used, is not as ideal or a perfect sterilisation method as it once was, by any means. It has inherent advantages and disadvantages, frequently requiring a benefit risk analysis.

1.1.12.1 Advantages

The inherent advantages of EO sterilisation are numerous and varied:

- It is a mild and soft treatment compared to irradiation, steam, dry heat and strong oxidising agents.
- It is 'now' a reasonable sterilisation method.
- It is more compatible with more polymers than other methods.
- EO sterilisation has been generally been reserved for heat, oxidising, moisture and radiation sensitive materials.
- EO is capable of destroying most viable forms of life. It is a recommended method for destroying more resistant spores and hard to target viruses by irradiation.
- EO sterilisation is frequently part of aseptic processing (e.g., in-line parts).
- It has been used as a method of choice for most hospitals and healthcare facilities in the USA.
- Typically EO is the most dependable sterilant for surgical procedural operational trays.
- EO is frequently and routinely used for decontamination of reusable (hospital) electrical supplies and equipment.
- It can sterilise locations or sites such as mated surfaces, long and restrictive lumens or spaces by 'diffusing between near mated surfaces or spaces (e.g., syringes in glass containers and overlapping instruments).
- EO sterilisation can sterilise-in-place (SIP) some devices within sealed packages.

1.1.12.2 Disadvantages

High-pressure EO sterilisation is a very effective method of sterilisation but it can be difficult to do correctly, maintain, and control. Some inherent disadvantages of EO sterilisation are:

- EO sterilisation cannot sterilise (inactivate) the desiccated cotton mould, *Pyronema domesticum*, easily (without pre-conditioning with steam).
- To use and operate EO sterilisers and to perform sterilisation properly requires special 'training' of personnel who will be using the technique. Well trained personnel and safe practices during operation are required to avoid any leakage of this flammable, explosive, carcinogenic and very hazardous gas.
- EO must reach an acceptable pressure for an effective sterilant concentration to occur, and boilers must be maintained, so that they do not produce toxic additives.
- EO and its by products can be carcinogenic and toxic.
- Concentrated or pure EO may be less effective than a low concentration with diluted gas mixes.
- EO may be deleterious to EO adsorptive materials.
- It may be incompatible to certain polymers under certain conditions, such as ABS, acrylics, copper, low-density polyethylene, some standard styrene and some urethanes.
- It may eventually (through numerous re-sterilisations) damage some materials.
- The loading and packing configuration may be critical to performance.
- Under most circumstances it is not as penetrable as gamma irradiation.
- It cannot sterilise electronic devices, which are damaged or interfered with by moisture from pre-conditioning or pre-humidification.
- The source of 'aged' EO may be contaminated with high non-volatile residues.
- The quality of EO must be good, without any polymerisation or tendency to polymerise.
- Air, salts, organic matter, matched or matted surfaces, long tubing, or enclosed spaces can be a barrier to humidity and/or EO diffusion.
- It cannot sterilise oils, wet material, as dry heat or steam can.
- EO cannot inactivate (depyrogenate) endotoxins, as dry heat can.

- EO cannot inactivate prions as steam can.
- Absorbable or hygroscopic materials such as polyglycolic acid cannot be used with EO. EO sterilisable materials must be stable to both moisture and heat.
- EO cannot sterilise liquids in the same way as steam and filtration can.

1.1.13 Recommended/Non-recommended uses of Ethylene Oxide Sterilisation

The recommended uses of EO sterilisation vary:

- EO sterilisation is typically the recommended method of choice, when items are heat sensitive or moisture damaged or if they contain sensitive electronics.
- It is the gold standard of gaseous sterilants because of its penetration capabilities.
- It must be compatible with the environment and local health and safety, and state, and national regulations.
- The number of possible materials, polymers, and some metals capable of tolerating its EO and moisture vary (**Table 1.6**) with reduced temperatures and longer exposure times and so on.
- In hospitals and laboratories where re-usable materials are frequently and routinely sterilised, EO sterilisation is predominantly used and readily available.
- It is not a recognised method for inactivating *Pyronema domesticum* on cotton, unless steam is used during pre-conditioning.
- It can sterilise glass, acetals, most polymers, natural PP, most Teflons and many re-usable materials that irradiation cannot, and cellulosics (paper and so on) that oxidising agents cannot sterilise.
- It can sterilise and re-sterilise many surgical procedural trays that many other methods cannot.
- It is not recommended for inactivating prions; for these, only steam is recommended.
- It can sterilise sealed encapsulated drugs and substances (e.g., antibiotics, antiseptics and so on) that irradiation would penetrate and destroy, and that very deep vacuum processes such as H_2O_2 with plasma could burst, as part of surgical operation tray.

Table 1.6 Resistance of some materials to EO and some other methods		
Material	Processing	
	EO	Other methods
PA 6,6	Excellent	Excellent – for autoclave and radiation
PA 6,12	Excellent	Good – for autoclave and radiation
Polyoxymethylene	Excellent	Good – for autoclave but not for radiation
PC	Some gas mixtures cause microcracks	Good – for autoclave, fair for radiation
PP	Excellent	Good – for autoclave, poor for radiation
Polyacrylic	Good, but temperature limited	Bad - for autoclave, fair to good for radiation Melts and yellows with radiation, fair for H_2O_2
PS	Good, possible sticking between adjacent surfaces, crazing	Poor - for autoclave, excellent - for radiation, poor for ozone
PVC	Very good, but varies	Fair – for autoclave, yellowing with radiation and blooming of plasticisers on surface
Polybutylene terephthalate	Excellent	Excellent - for autoclave and radiation

1.1.14 Some General Considerations of the Ethylene Oxide Technique

The EO sterilisation technique uses multiple conditions in validation and routine processing such as temperature (mild heat), moisture (non-condensing humidity), pressure changes (vacuum and pressurisation), and exposures to %RH and concentrated EO with or without its non-flammable diluents, degassing (purging) and aeration. Product and packaging should be designed to allow for removal of air and penetration of steam and EO. Consideration should be given to the potential physical and chemical effects of these conditions and the formation of residuals. During an EO sterilisation process, products can be subjected to environmental stresses such as vacuum and pressure changes and pulses, heat (mild temperature changes), air/gas changes, and changes in humidity. The product may also react with EO and/or diluent gases used, but not like irradiation or oxidising agents. The product design should ensure that functionality and safety are not compromised by exposure to the anticipated range of sterilisation conditions, or multiple re-sterilisations. Furthermore,

a high moisture content and changes in pressure may affect the strength of package seals with a consequent loss of integrity.

1.1.15 Some Material Compatibility Considerations

EO sterilisation is very gentle on polymers compared to other sterilisation methods. Limitations related to material compatibility typically relate to a polymer's EO adsorptivity and some sensitivity to humidity, such as hydrophilic coatings. Users also need to be careful with EO sterilisation when applying polymers as carriers for drug delivery, because it is reactive to most drugs. Some drugs such as Taxel based-formulations cannot withstand high temperatures and high humidity EO cycles. EO will sterilise most polymers for medical devices.

Some polymers that are compatible with EO [1] are:

- Acetal.
- Elastomers – silicones (peroxides and platinum cured - no significant crosslinking), thermoplastic elastomers styrene-ethylene-butylene-styrene, thermoplastic polyolefin, natural isoprene, ethylene-propylene-diene terpolymer, urethane, nitrile, butyl and styrene-butadiene.
- Fluoroplastics - PTFE and fluorinated ethylene propylene, polyvinylidene fluoride and polychlorotrifluoroethylene.
- 'High-end' engineering resins, polyether ketone, polyether ether ketone and polyetherimide.
- PA, especially aromatics, PA12, PA11, PA6/12 and PA6/10.
- Polyethylene, low-density polyethylene < linear low-density polyethylene, high-density polyethylene and ultra-high molecular weight polyethylene (UHMWPE).
- Polyesters and polyethylene terephthalate glycol.
- PC and its alloys.
- Polysulfone.
- PVC - flexible and semi-rigid, coloured, plasticised and unplasticised.
- PU (8 chemical varieties).
- PP (unstabilised).

- Polypropylenes (stabilised) and their co-polymers and polymethylpentene - radiation stabilised.
- PS and its copolymers (ABS and styrene-acrylonitrile).
- Polyacrylics (PA, polymethylmethacrylate and polyacrylonitrile).
- Silicone - prostheses.
- Thermosets - epoxies, phenolics, polyimides, PU and polyesters.

Because EO is deemed to be a potential human carcinogen and reproductive toxicant, its use is becoming increasingly limited and controlled. Post-sterilisation evaluation for toxic residuals (EO and ethylene chlorohydrin) must be performed before release or validation of product. Long exposure times and post-sterilisation aeration times as well as post-processing BI testing may reduce the use of this process on a practical basis.

There are not many materials that can be damaged by EO except for some enzymes, some bioabsorbables, and a few polymers such as styrene, which can possibly be crazed by the action of EO. Many of these damages can be overcome by changing cycle parameters and systems.

EO sterilisable materials and items must be stable to EO and some humidity moisture. Select the polymers and materials that best fit the EO sterilisation temperature and process that is to be used by reviewing material properties of the polymers to be sterilised. Many more polymers (e.g., acrylics) can be sterilised by EO at lower temperatures (e.g., <55 °C) for example than at 65 °C.

In many ways, EO has been a victim of its own successes. For example, EO is most often characterised by its overkill conditions to inactivate spores. It uses extremely high gas concentrations (e.g., >400 mg/l) to inactivate highly resistant non-pathogenic mesophilic spores and more recently, extremely resistant *Pyronema domesticum*, that other sterilisation methods cannot destroy.

Lower gas concentrations <300 mg/l may be acceptable and validated, but materials and polymers must be compatible with a %RH in excess of 90% at times. Some super-humidification (slightly less than 100% RH) sterilisation is tolerated at elevated temperatures.

At lower sterilising temperatures and %RH, more polymers and items are tolerable of EO: for example, acrylics, some enzymes and electronic devices. However, these polymers and items may be sterilised or processed only once, not repeatedly.

In designing healthcare products, manufacturers need as many as possible heat

stabilised materials because of the growing complexity of products such as medical device and drug combinations, and reprocessing.

Compare the rate of microbial inactivation and the time to sterilise to rate of material damage (e.g., enzymes). There are 'more' non-liquid materials that can be EO sterilised than not sterilised. EO sterilisation of healthcare products, drugs, pharmaceuticals, medical devices, and polymeric materials is a specialised field requiring an interfacial area of investigation, discipline and information.

1.1.16 Safety and Regulations for Using Ethylene Oxide

Use of EO has been a stable trend in the sterilisation field for over 25 years, but it is increasingly less used in hospitals, and industry because of health and safety regulations. EO is toxic, hazardous, flammable, explosive, carcinogenic and highly regulated. Workers may be at risk of death or serious injury from explosions if safe operating procedures are not established and followed in large-scale industrial processes that use EO for sterilising medical devices and other products. Also people in a nearby commercial areas (e.g., malls, schools) may be affected by it. EO is not only hazardous. It is recognised as a known human and animal carcinogen.

EO is a gas not liquid and gases are more difficult to handle than liquids. EO is explosive, if not made inert. No static charge or leaks are allowed, for 100% EO. If EO is inadvertently 'overfed' into the emissions control device at rates or concentrations higher than the device can safely handle, concentrations of the gas may reach flammable levels. If that occurs, heat sources in the emissions control device may trigger an explosion. Between 1994-1998, ten explosions of EO were associated with industrial sterilisation facilities and also at EO repackaging plants where EO is transferred from large drums to small tubes or canisters for later use in small sterilisation units at hospitals. In one such explosion, a worker was killed and 59 others were injured.

To get approval to use EO is a challenging and lengthy process. If another EO facility exists within the same area, no approval for use is given. There are increased regulations and scrutiny for:

- Special controls - security and alarms.
- Monitoring.
- EO scrubber.
- Waste removal.

- Local nuisance, e.g., not in my back yard.
- Increased insurance at populated site.
- OSHA.
- EPA.
- State and local regulations
- Special EO training.
- Special personnel.
- Frequent audits.
- Potential complaints and lawsuits.
- Proposition 65, a Californian law with very low EO limits, and those who report excesses are rewarded as bounty hunters.
- Potential future community response and publicity to a facility which uses a known carcinogen.
- Facilities within the same facility, which may result in toxic, flammable and explosive levels of EO.

Other significant considerations when using EO are:

- EO is reactive with acids, bases, amines, water, and some metals. It is harder to handle as a gas than as a liquid.
- EO sterilisers are easier to install and start up, than irradiation facilities. Very small sterilisers of less than 10 litres are used.
- Highly regulated.
- Lower TWA in future from 1 ppm OSHA *versus* 0.5 ppm (National Institute for Occupational Safety and Health (NIOSH)).
- Immediate danger to life or health (IDLH) – can be less dangerous to health in small facilities.
- Low quantities allowed in some states (e.g., <2,267 kg).
- Future EO residual criteria will be likely reduced by 50%.
- Apply EO standards according to ISO 11135 [8].

- Applying the same criteria to parametric controls and reduced BI incubation, may limit release time.

Other considerations when using EO are:

- Its compatible limitation with drugs/devices. It is a strong alkylating agent and its by-product, ETG may have some additional considerations when using it with drugs, as well as local disposal from the scrubber.
- Once sterilisation is completed, EO is vented at a controlled rate through closed ductwork to an emissions control device to meet environmental emissions limits, where the EO is either burned off or converted to water and CO_2 through either heat or catalytic conversion.
- Despite all the regulations, toxicity, and safety requirements of EO sterilisation, it is still possible to install and qualify this process.

Note: Small size sterilisers and small EO usage, have less regulations, and safety issues to deal with.

1.1.17 Process Improvement and Enhancement

With recent drug/device combinations, there has been a mini resurgence in EO processing. The specific types of EO sterilisation processes can vary significantly to be compatible with device/drug combinations and various products and polymers.

EO has been used to sterilise some pharmaceutical formulations. Its use for this application is somewhat limited because the EO process may alkylate or hydrolyse chemically reactive species, and the relatively long times at temperatures of 40-60 °C may cause some thermal degradation, and the vacuum pulses may evaporate low boiling point components of the formulation.

Some EO process types encountered are: balance pressure, standard McDonald pre-humidification cycle, dynamic environmental conditioning, air displacement, modification of evacuation to prevent packages or devices from exploding, and some degree of high humidity with post-sterilisation aeration.

EO sterilisation processes can be process controlled release or parametric released for just-in-time operations. Some products and devices can be ‘sterilised-in-place’ (SIP) during aseptic assembly or released after short BI incubation times (e.g., 4 h).

EO sterilisation has greater ‘penetrating’ capabilities that alternative technologies to

inactivate spores on products that are not cleaned, or spores within serum or salt [11].

The biggest challenge of reprocessing devices after they have been used for clinical procedures is high-level disinfection or sterilisation [12]. With a decrease in EO sterilisers and sterilisation in hospitals and clinics and increase in alternative sterilisation, e.g., H_2O_2 and H_2O, this challenge has become apparent in 2010-2011, particularly with endoscopes [12].

Lower EO sterilisation processes (e.g., <300 mg/l), are as good as higher EO concentrations (e.g., 450 mg/l), when deeper initial vacuums are drawn, and higher humidities are able to reach remote locations [6]. Experiments have been carried out with theoretical considerations to show that EO concentrations may be reduced to 50-100 mg/l [6], providing sterilisation parameters are changed to improve pre-humidification and controlled with certain theoretical considerations [6].

With such a low EO gas concentration, it may be possible to sterilise many devices without any aeration, leading to faster processing, less consumption of EO, and with less gas usage, less regulations and more choice (less cost) of polymers with or without additives. This would reduce the overall time to process the EO product, and it is possible with such reduction of EO concentrations to reduce the regulations required for its use. Furthermore, while raw material costs go up for polymers, EO begins to look less expensive because it can sterilise many more polymers without additives and special processing required for irradiation.

The low steam-formaldehyde process is an example of a process using a chemical such as EO at lower than 100 °C (e.g., 65-80 °C) with steam [13]. A steam - EO may produce ETG, a safer by-product than EO residuals or ethylene chlorohydrin.

EO may still be the 'gold' standard of gaseous sterilants because of it excellent penetration capacity through lumens, salts, serum organic encrustation, unclean devices [12] and broad material compatability, see AAMI TIR 17 *Annex B: Ethylene Oxide Sterilisation - Material Compatibility Fundamentals* [1] and while newer alternatives may be faster, and lesser regulated, traditional EO sterilisation may remain the gold standard for the next 25 years.

The next future generation of EO sterilisation processing however may be quasi-alternative traditional EO sterilisation processes that reduce EO concentration and EO residuals, reduce processing time, become more environmentally friendly, require fewer regulations, and sterilise less costly polymers and products. If regulations continue to make use of EO too difficult for continuation of EO sterilisers and sterilisation, then its near twin brother, polypropylene oxide (PPO) could be considered (see **Section 1.3**) as a means to sterilise unclean (reprocessed) devices such as endoscopes, with less

regulations, less hazards, different residuals (e.g., propylene glycol (PPG), because PPO has better penetration capability of reprocessed and other uncleaned devices than current alternatives such H_2O_2 and O_3, but it is weaker than EO.

1.2 Glutaraldehyde (Liquid) Sterilisation

Although infrequently used for 'disposable 'medical devices, liquid glutaraldehyde sterilisation does have 'future' benefits (green). It can sterilise heat sensitive tissues that other traditional (heat), irradiation methods, and H_2O_2/plasma and O_3, cannot. It has excellent material compatability. Other methods (e.g., low heat), EO and glutaraldehyde are routinely used for sterilising many high heat sensitive devices, instruments, hospital utensils, wraps, and items that can be re-sterilised and re-used. Some special differences and considerations between EO and glutaraldehyde exist.

EO is typically gaseous, while glutaraldehyde is typically liquid, except for fogging (see **Section 1.2.1**). Some general charateristics of glutaraldehyde are:

- Chemical:
    - It is liquid.
    - It is toxic and can be a sensitiser.
    - It has toxic residues.
- It is a simple sterilisation process:
    - It has few parameters (e.g., solution activation (mixing), to change the pH, colour, concentration and exposure).
    - It requires simple equipment and facilities.
    - It has limited penetration, however, it can penetrate tissues.
    - It requires safe handling.
    - It typically does not require packaging.
    - It requires environmental control and monitoring.
    - It requires a long exposure for sterilisation.
- Advantages:
    - It is simple.

- It is compatible with tissue and materials.
- It inactivates a wide range of microbes.
- It is widely available.
- It is not carcinogenic.

Methods have been devised to use glutaraldehyde in SIP applications and subsequently rinse them, without causing adventitious (accidental) contamination during post-sterilisation treatment or breach of the system with low risk, subsequent to sterilisation treatment through immersion. This is critical.

This method requires that the device be sterilised totally immersed, in glutaraldehyde, and then subsequently rinsed, to remove toxic residuals. This is a two-step procedure, that requires handling a liquid sterilant instead of terminal sterilisation within a package and then rinsing with outside handling with aseptic technique(s).

Sterile is a special term, intended to indicate freedom from all micro-organisms, including bacterial spores. Since the absence of all forms of life cannot be proven unequivocally, a validated process, remote probability of survivor, and maintenance of sterility must be used to demonstrate, beyond a reasonable doubt, that no microbes are present.

One approach has been suggested to exclude any low risk, aseptic hazard to the system.

In such an approach, it is necessary to pre-sterilise the container (for immersion and rinsing), with filters and then subsequently sterilise them again with glutaraldehyde, so there is no risk at this juncture. In place filters are pre-connected to the container to prevent further contamination after sterilisation.

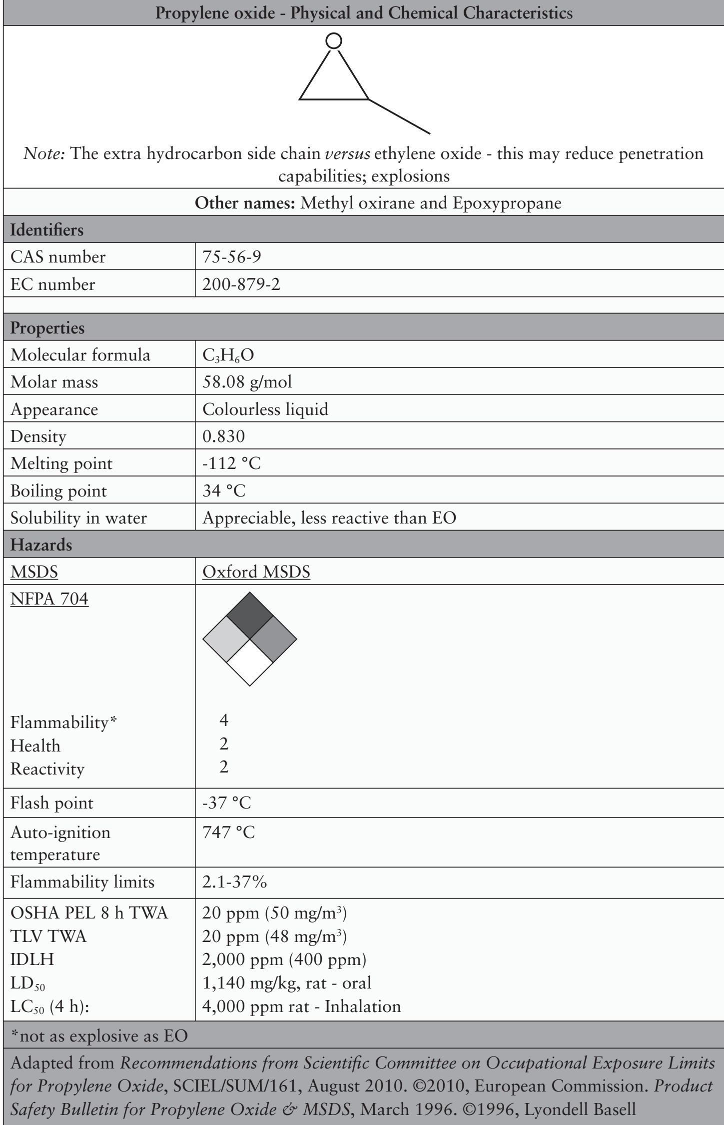
Filtration is not regarded as a terminal sterilisation method by some people but since the filters have been pre-sterilised, and SIP again with the glutaraldehyde, items treated in this way should be completely sterile. Subsequently pre-sterilised saline is used to rinse off the glutaraldehyde from the device within the confines of a container with filters on both ends. The container can have double filters to remove any doubt or skepticism that the rinse solution or device is not sterile. After rinsing the device the contents can be air filtered through the filter to remove any wetness on the device.

1.2.1 Glutaraldehyde – A Fogging or Aerosolised Method

Glutaraldehyde is typically a liquid sterilisation method, but it has been used in fogging (creation of an aerosol). Glutaraldehyde is typically only a surface contact sterilant of items or materials for fogged glutaraldehyde

1.3 Propylene Oxide

Hypothetically it would be assumed that PPO would make a good substitute for EO (see **Figure 1.4**), based upon its physical and chemical properties.



Propylene oxide - Physical and Chemical Characteristics	
Note: The extra hydrocarbon side chain *versus* ethylene oxide - this may reduce penetration capabilities; explosions	
Other names: Methyl oxirane and Epoxypropane	
Identifiers	
CAS number	75-56-9
EC number	200-879-2
Properties	
Molecular formula	C_3H_6O
Molar mass	58.08 g/mol
Appearance	Colourless liquid
Density	0.830
Melting point	-112 °C
Boiling point	34 °C
Solubility in water	Appreciable, less reactive than EO
Hazards	
MSDS	Oxford MSDS
NFPA 704 Flammability* Health Reactivity	4 2 2
Flash point	-37 °C
Auto-ignition temperature	747 °C
Flammability limits	2.1-37%
OSHA PEL 8 h TWA TLV TWA IDLH LD_{50} LC_{50} (4 h):	20 ppm (50 mg/m^3) 20 ppm (48 mg/m^3) 2,000 ppm (400 ppm) 1,140 mg/kg, rat - oral 4,000 ppm rat - Inhalation
*not as explosive as EO	
Adapted from *Recommendations from Scientific Committee on Occupational Exposure Limits for Propylene Oxide*, SCIEL/SUM/161, August 2010. ©2010, European Commission. *Product Safety Bulletin for Propylene Oxide & MSDS*, March 1996. ©1996, Lyondell Basell	

Figure 1.4 PPO - physical and chemical characteristics. EC: European Commission and MSDS: Materials Safety Data Sheet

PPO has frequently been used as a substitute for EO in sterilisation [14]. This has principally been in the food processing industry, because of its lower toxicity. It has very similar properties to EO, which may make it the next 'closest' gold standard for gaseous sterilants. A similar case history or situation occurred between very highly regulated ethanol and isopropyl alcohol (IPA), allowing IPA to be subsequently used frequently, in place of ethanol. Also, PPO residuals are not considered as hazardous as EO residuals in the food treatment area, and PPG is a considered to be safer than ETG and is used as preservative. PPO is not explosive as EO. However, there are some disadvantages [15] that need to be overcome with PPO:

- It may be much less effective than EO.
- It may diffuse relatively slowly compared to EO.
- Because of its higher boiling point, it may be more difficult to remove.
- There is some uncertainty, will its TWA OSHA inhalation level be reduced from 20 ppm?
- Recently its IDHL was reduced from 2,000 ppm to 400 ppm which is lower than that for EO at 800 ppm.

But, PPO is less explosive, less hazardous than EO, easier to handle as a liquid rather than a gas during transportation or holding and gives better penetration than the alternatives of H_2O_2 and O_3; and it is assumed that PPO would continue to decontaminate, and sterilise, after sterilisation during aeration.

However, PPO can have more than one chemical form, which is not true for EO.

It may not be carcinogenic or it may be less carcinogenic than EO. Significant changes in sterilisation procedures and equipment for PPO may make it as effective as EO and more efficacious. It has suffered as failing to penetrate organic matter, however, it is deemed to be more diffusible than either H_2O_2 or O_3. There are ways of improving its penetration for organic matter.

Steam - PPO may produce more PPG than other potential hazardous residuals. PPO may be easier to synergise with other agents to make it a more potentiated, faster, efficacious and more effective sterilant, and a quasi acceptable alternative to traditional EO sterilisaton. PPO sterilisation may be more acceptable if referred to in the future as a cold and/or hot sterilisation process, as irradiation is referred to as cold pasteurisation of certain items (e.g., foods), because of probable carcinogens they are not readily accepted. Unless PPO can be 'converted' fully into a non-carcinogenic and non-toxic PPG as a residue or by-product, it may have a difficult time being accepted in the future. If it can be converted into a PPG residual, this may be a benefit as a

preservative. H_2O_2 and irradiation are readily converted into a non-toxic and non-carcinogenic residue, radical or by-product within materials and products, and have been accepted. Early reports [16] suggest that PPO may react with organic matter (e.g., cereals) and require high concentrations to be effective, but these conditions can be overcome and improved with newer techniques to make it more acceptable as an sterilant.

1.4 *β*-Propiolactone

β-Propiolactone is unique lactone chemical with special physical and chemical properties (see **Figure 1.5**). It is a colourless liquid at room temperature and boils at 163 °C. It is not flammable at room temperature. The usual concentration for sterilisation 2-5 mg/l and it requires a high humidity (75%). It has very strong microbiological activity but has weak penetration ability, so it is essentially a surface sterilant. It is not used extensively because of its carcinogenocity, and other physiologically undesireable properties, however, its β-hydroxylpropionic acid hydrolysis by-product, is not carcinogenic and does not have its undesirable physiological properties. It is one of the most rapidly sporicidal agents. It has been used sterilise and decontaminate contaminated areas and spaces in a vapour state, as well as sterilising biological material without toxic or allergic manifestations in a liquid state.

It is 25 times more effective than formaldehyde, 4,000 times more effective than EO, and 16,000 times more effective than PPO, and 50,000 times more effective than methyl bromide [15]. How does this compare to H_2O_2, peroxide with plasma, and O_3? Like these compounds it can be converted into a non-toxic, non-carcinogenic, by-product.

However, because of its high carcinogenicity it has not been frequently used. In the recent past it was used to sterilise various contaminated areas, e.g., food processing facilities. It has a high boiling point but is converted into a non-toxic by-product, *β*-hydroxy-propionic acid, which is non-carcinogenic and has a lower toxicity [14]. It is many times faster and more sporicidal than EO. It has been used to sterilise biological materials without toxic or allergic manifestations in the liquid state [14]. It may be useful as a spray sterilant. Because of its potential detrimental properties it has been deemed ‘not’ to be suitable for hospital sterilisation.

β-Propiolactone - Physical and Chemical Properties	
IUPAC name: Oxetan-2-one 3-Hydroxypropanoic acid lactone	
Other names: Propiolactone β-Propiolactone 2-Oxetanone	
Identifiers	
CAS number	57-57-8✓
ChemSpider	2275✓
UNII	6RC3ZT4HB0✓
KEGG	D05630×
Jmol-3D images	Image 1
SMILES	
InChI	
Properties	
Molecular formula	$C_3H_4O_2$
Molar mass	72.06 g/mol
Appearance	Colourless liquid
Odour	Pungent, acrylic
Density	1.146 g/cm^3
Melting point	-33.4 °C
Boiling point	162 °C (decomposes)
Vapour Pressure	466 Pa at 25 °C
Solubility in water	Soluble, hydrolyses readily in water
Solubility	Miscible in organic solvents
Refractive index (n_D)	1.4131
Except where noted otherwise, data are given for materials in their standard state (at 25 °C, 100 kPa)	

Figure 1.5 β-Propiolactone, physical and chemical properties: KEGG: Kyoto Encyclopedia of Genes and Genomes and UNII: The Unique Ingredient Identifier

1.5 Glyoxal

Glyoxal shows good potential in the substitution of aldehydes (e.g., formaldehyde or glutaraldehyde). Glyoxal is an dialdehyde like glutaraldehyde, but has a lower bacteriocidal activity (10%). However, it can be vapourised and considered as a gaseous sterilant. It boils at 51 °C pure, or 104 °C as an aqueous mixture. Unpublished results indicate that it does not diffuse as quickly as EO through lumens, but is slower. One of the possible advantages of glyoxal, is that it is not a known carcinogen or potential carcinogen as EO and PPO are.

However, occupational allergy to glyoxal appears to have an increased incidence among employees in healthcare facilities where glyoxal is used in disinfectant solutions, but this is not necessarily true when it is used as a contained controlled gaseous sterilant.

Glyoxal is a more potent virucidal agent than BPL. Also glyoxal was less lytic for human erythrocytes than BPL in blood. Glyoxal does not have the penetrating capabilities of EO or PPO sterilants, and its residuals may be difficult to remove or neutralise. It may be useful as a spray sterilant, like BPL. However with plasma it may be synergised and may be useful in reducing glyoxal residuals.

1.6 Other Sterilants

Beyond EO and glutaraldehyde there are a number of classical sterilants (see ***Healthcare Sterilisation: Introduction and Standard Practices, Volume 1, Chapter 5),*** such as chlorine, chlorine dioxide, H_2O_2, PAA and O_3, which are not yet traditional, but alternative or novel sterilants. Of these, chlorine dioxide, H_2O_2 (without plasma) and PAA are today considered as novel sterilants, which will require further evaluation by the Food & Drugs Administration (FDA) and so on, before they can be used. Chlorine continues to be used as a decontaminant, but because of its strong oxidising and potential carcinogenic by-products it has not been readily used to sterilise hospital products, but on hospital floors, walls and other items. Methyl bromide has not been regarded as a hospital sterilant

1.6.1 Chlorine Dioxide

Chlorine dioxide is an alternative to using corrosive chlorine gas. Chlorine dioxide has been accepted as a decontaminant for bio-terrorism in facilities, but not as a standard sterilisation method as an alternative in hospitals. Most notably it was used to successfully decontaminate the Hart Office Building and Brentwood postal sorting

facility in Washington, DC, in response to their contamination with Anthrax spores. It has been approved for use as a sterilant by the EPA. A gaseous chlorine dioxide was developed in the late 1980s. Chlorine dioxide is not mutagenic or carcinogenic in humans. As the chlorine dioxide concentration increases, the time required to achieve sterilisation becomes progressively shorter. For example, only 30 min were required at 40 mg/l to sterilise with high humidity, the 10^6 *B. atrophaeus* spores at 30 to 32 °C. Its sterilising concentration, 10-50 mg/l, is low compared to that of EO which is 400-1,200 mg/l.

Nevertheless, no in-hospital, gaseous chlorine dioxide systems have been used. This may be because it has limited polymer compatibility and biocompatibility data and information, as well as some potential incompatible effects on uncoated aluminum foil, carbon steel, uncoated copper, carbon steel, unbleached paper, PC, PU and passivated stainless steel, rubber and silk. Nevertheless it is not as bad as O_3, which has been given status as an alternative sterilant, while chlorine dioxide has been given a novel sterilant status by the regulatory agencies. No corrosion has been observed when using pharmaceutical-type materials such as high-grade 316SS (stainless steel) and 304SS, Lexan (PC), and various other plastics such as Delrin (acetal), Teflon, and UHMWPE. It has been used successfully to sterilise contact lenses, industrially. It can require aeration, like EO. Residual levels may need to determined, whereas O_3 will break down to O_2. However, unlike O_3, chlorine dioxide is stable and soluble, allowing it to travel to the base of the film where it attacks micro-organisms and destroys the biofilm at its point of attachment. Because chlorine dioxide is a dissolved gas, it does not ionise to form weak acids (as chlorine and bromine do) in aqueous solutions. It appears to have better penetrating capabilities than other oxidising agents. Recent efforts have been directed to industrial applications, rather than hospitals because of its deleterious effects on a few materials. Chlorine dioxide can be explosive and flammable under certain situations, but not at low concentrations (e.g., 750 ppm) and ambient temperature with high humidity for a 12 h decontamination.

Chlorine dioxide is more compatible with polymer materials than chlorine. It is a low temperature process.

Chlorine dioxide is a novel sterilant and some of its properties and processing conditions are:

- Chlorine dioxide was investigated in the mid- to late 1980s. It has sporicidal properties and can be used at 27 to 30 °C, at a concentration of only 10 mg/l, and 80% RH.
- It cannot be shipped or stored and must be generated on site, which would increase the complexity of the steriliser design.

- Chlorine dioxide cannot be stored long and has potential to explode (degrade) into chlorine and oxygen under pressure, greater 10% in air, heat and light.
- It has an OSHA extreme limit of 0.1 ppm for an 8-h TWA and a NIOSH limit of 0.1 ppm 8-h TWA plus a 0.3 ppm STEL.
- It has an IDLH level of 5 ppm.
- Investigations are ongoing for industrial applications but not for hospitals.
- Potentially more compatible with more materials than hydrogen peroxide or ozone.
- Yellow-green gas, a true gas, not a vapour.
- Water soluble, can penetrate water.
- Boiling point 11 °C.
- Tri-atomic molecule, can be monitored real time.
- Molecular weight 67.5 g/mole.
- An oxidizer like hydrogen peroxide, peracetic acid, ozone.
- Less corrosive than peracetic acid, hydrogen peroxide, chlorine and ozone.

1.6.2 Peracetic Acid

PAA vapour (fumigation) and sterisation systems have also been described but have not seen widespread use due to its aggressive chemical action. The original sporocidal activity of PAA vapour was observed using a mixture of 40% PAA solution, 5% H_2O_2, 39% acetic acid, 1% sulfuric acid and 15% water. 40% PAA which killed spores in minutes; whereas a 20% buffered solution took about 80 min. Spore killing by fogging, depends upon the distribution of the vapour.

PAA vapour has previously been recognised as a traditional sterilant. But more recently a gaseous PAA with plasma has had a role as hospital sterilant, but not industrially. It has had some regulatory issues, and it is now regarded as a novel sterilant. Gaseous PAA has been used as a sterilising agent on a very small scale. However, it is widely used in a liquid form for disinfection/sterilising purposes. A gaseous PAA system has been developed that uses low-temperature gas plasma. The ion plasma sterilisation processes operate at relatively low temperatures by exposing PAA to either strong electric or magnetic fields. Such exposure results in the formation of an ion plasma that contains reactive radicals that are known to be reactive with almost all molecules

essential for metabolism and reproduction of living cells (e.g., DNA, RNA, proteins and so on).

This technology stimulated interest by healthcare facility personnel, because it had short turnaround times compared with EO sterilisers and it was more environmentally friendly and safer to use. The application of the PAA plasma method was limited to stainless steel surgical instruments (excluding lumen devices and hinged instruments). Also no liquids or materials that might be harmed by vacuum could be treated. The PAA process was limited by the FDA restrictions on treatment by this method, to endoscopes and other medical devices with lumens no longer than 12 inches or having a lumen diameter less than 6 mm. Cellulose, linens, and liquids may not be processed by gaseous PAA. The process requires special packaging of devices and a special tray for processing.

PAA plasma is similar to H_2O_2 plasma but has acetic acid, O_2, and water as by-products. PAA was once a non-traditional process but was subsequently reclassified as a novel sterilant with the following qualities:

- The system has been on the market and has been sold as an alternative to EO, but is off the market currently. It is considered a novel sterilant.
- Chemically, PAA is the equilibrium mixture of H_2O_2 and acetic acid.
- In concentrated form (>30% solution), it is corrosive to equipment and irritating to human tissue.
- In phase one of the process a 5% solution of PAA is introduced into the sterilisation chamber under a deep vacuum. In phase two, a non-flammable mixture of hydrogen, O_2 and a carrier gas is subjected to microwave electromagnetic energy to create the plasma.
- PAA vapour breaks down to H_2O_2 and acetic acid vapour.
- Manufacturer recommends exhaust of both phases *via* a dedicated or common outside air duct.
- Unlike EO, which is an alkalising agent and penetrates through packaging and most devices, PAA/H_2O_2 plasma is an oxidising agent and has surface contact capability only. With this system, however, up to six deep vacuums can be drawn to enhance penetration.
- OSHA exposure standard for H_2O_2 is 1 ppm for an 8-h TWA.
- OSHA exposure standard for acetic acid is 10 ppm for an 8-h TWA.

- The IDLH is 75 ppm.
- The reportable quantities (RQ) on H_2O_2 is one pound and PAA one pound under Situational awareness and response assistant Section 302 extremely hazardous substances.
- The steriliser would be classified as a Class II medical device, requiring performance standards. At this point, no performance standards are established.

Note: PAA can react with metals to form toxic metal acetates or metal oxides. While these acetates are water soluble and not a concern with liquid processing because aqueous rinses will remove them, there is not an easy mechanism to remove such by-products in the gaseous PAA process.

1.6.3 Hydrogen Peroxide (Liquid, Vapour, No Plasma)

Liquid H_2O_2 has been previously recognised as a sterilant, but gaseous H_2O_2 is relatively new. It is recognised as an novel sterilant, by FDA without plasma (likely because of residuals), but H_2O_2 has been used for a long time. It can be relatively non-toxic under diluted or low concentrations, such as the familiar 3% solutions, however, H_2O_2 becomes a dangerous oxidiser at high concentrations (>10% *w/w*). As a strong oxidant, it can destroy a wide range of pathogens and may be used to sterilise heat or temperature sensitive articles such as rigid endoscopes.

H_2O_2 produces both hydroxyl (HO) and hydroperoxy (HOO) radicals. These attack cells walls causing them to collapse.

In medical (terminal) sterilisation, H_2O_2 is used at higher concentrations, ranging from around 35%. The biggest advantage of H_2O_2 as a sterilant is a short cycle time (28-40 min), and no residues. Whereas for EO this may take up 10-15 h, with preconditioning and aeration. The use of very high concentrations or intensity of H_2O_2 will make shorter exposure times possible.

H_2O_2 can be used for terminal sterilisation of items or products in a chamber or as an low level temperature gas to decontaminate enclosed spaces or rooms or workstations, or isolation chambers. As a decontaminant it has been used to sterilise hospitals, aircraft interiors, anthrax contaminated buildings, and structures with nerve gas. Vapourised H_2O_2 has been registered by the EPA as a sterilant that inactivates spores on environmental surfaces in enclosed areas. H_2O_2 vapour may be applied to surfaces by aerosol or as a non-condensing vapour.

Aerosols are where H_2O_2 is vapourised as a fine mist with less than 50 ppm silver, less than 50 ppm phosphoric acid and less than 1 ppm arabica gum as catalysts. Over time the aerosols collapse, H_2O_2 degrades to safe conditions. This method is not used much in hospitals.

Non-condensing vapour is a four-step sequence in which an enclosed space is first dehumidified, then 35% H_2O_2 is vapourised under controlled conditions of temperature, humidity and pressure so that there is no condensation. This state is maintained for a period of hours with a super lethal concentration of H_2O_2 at several hundred ppm concentration, then the enclosure is aerated with air purging with catalytic aeration, so the concentration of H_2O_2 is below the TWA. The H_2O_2 is then converted to water, O_2 and carbon dioxide.

Condensing vapour is the same as the 35% H_2O_2 vapour but is produced in a dual axis vapour distribution system, which ensures the H_2O_2 is introduced into a room or enclosure evenly, and that the vapour is saturated. The condensing vapour forms a liquid film about one micron thick on the surfaces. This method kills a considerable number of microbes, but all surfaces must be covered, or some microbes may be missed. Applied aerosols must also be distributed, or misses will occur.

H_2O_2 vapour appears to be superior to aerosolised H_2O_2. This may be due to differences in concentrations obtained. For example, a peak H_2O_2 concentration for H_2O_2 vapour may be greater than 300 ppm whereas aerosolised H_2O_2 may be one-half or approximately 150 ppm.

In terminal medical sterilisation of items, the H_2O_2 vapour process uses very low vacuums (e.g., 133 Pa) When the vapour is fed into an enclosed area or chamber, excess water vapour is removed. This makes it a drier system.

Some synergism exists between steam and H_2O_2, and more recently has been observed between O_3 and H_2O_2 synergism.

At steam temperatures of ~100 °C, H_2O_2 concentration gradients were observed, hypothesised to be due to accelerated H_2O_2 decomposition at this elevated temperature.

H_2O_2 vapour has some disadvantages:

- H_2O_2 absorbed into polymers may be difficult to aerate in a short period of time for implantables and other healthcare product uses.
- Because it is a strong oxidant, there are material compatibility issues. For example, paper products cannot be sterilised in H_2O_2 vapour because the H_2O_2 absorbed by the paper product, destroys it.

- The penetrating ability of H_2O_2 is not as good as EO and so there are limitations on the length and diameter of lumens that can be effectively sterilised and guidance is available from the steriliser manufacturers.
- However, limited permeability was noted with polyethylene, PP, PVC, and PP-polyester composites, so without plasma, residuals require aeration.
- Kinetics of some H_2O_2 processes may still be wanting in some cases. Sterilisation is achieved by depositing an even layer of micro-condensation of H_2O_2 vapour over all the surfaces. The term micro-condensation may be defined as a microscopic film of H_2O_2 vapour, which being at a sub-micron level is invisible to the naked eye. The micro-condensation process must be achieved to ensure that the optimum conditions for biological inactivation are achieved. When the process reaches the dew point, the time required for a log reduction of activity (the D-value) is shortest. It appears that this occurs when the kill kinetic curve plotted against time is steepest. The transition between the shallow curve and the steep section coincides with the onset of micro-condensation.
- The FDA has not approved the method outright, but it is still considered to be a novel process.

While H_2O_2 offers significant advantages in terms of throughput, as with all sterilant gases, sterility is achieved through the use of high concentrations of reactive gases. H_2O_2 is primarily an irritant and the contact of the liquid solution with skin will cause bleaching or ulceration depending on the concentration and contact time. The vapour is also hazardous with the target organs being the eyes and respiratory system. Even short-term exposures can be hazardous and NIOSH has set the IDLH at 75 ppm, less than one-tenth of the IDLH for EO (800 ppm).

Prolonged exposure to even low ppm concentrations can cause permanent lung damage and consequently OSHA has set the permissible exposure limit to 1.0 ppm, calculated as an 8-h TWA (29 CFR 1910.1000 Table Z-1 [17]).

H_2O_2 may cause cancer. Thus, employers have a legal duty to ensure that their personnel are not exposed to EO exceeding this PEL. Even though the steriliser manufacturers go to great lengths to make their products safe through careful design and incorporation of many safety features, workplace exposures of H_2O_2 from gas sterilisers are documented in the FDA Manufacturer and User Facility Device Experience (MAUDE) database. MAUDE data represents reports of adverse events involving medical devices. The data consists of voluntary reports since June 1993, and user facility reports since 1991. When using any type of gas steriliser, prudent work practices will include good ventilation (10 air exchanges per hour), a continuous gas

monitor for H_2O_2 as well as good work practices and training. Further information about the health effects of H_2O_2 and good work practices is available from OSHA.

H_2O_2 has excellent antimicrobial properties against a wide range of micro-organisms including bacterial endospores. It also may inactivate prions. Under carefully controlled process conditions H_2O_2 is also safe for use with many materials. While it is compatible with many polymers, there are a few materials that are damaged by it, for example, acrylics, cellulosics (includes paper), natural rubbers, bioadsorbables such as polyglycolides and polyesters. H_2O_2 can sterilise a multitude of polymers. However, the numbers of polymers are more limited than with EO, because of the oxidising effect of H_2O_2. However, it is more attractive than EO sterilisation because of its shorter process time and lack of high residuals. It has a very short processing time and because it is not carcinogenic it makes H_2O_2 use very desirable. When designing for devices, it is best to avoid absorbers, such as PU, polyamide, ethylene vinyl acetate, and cellulosics. Low temperature H_2O_2 with plasma has less effect typically on polymers than H_2O_2 vapour without plasma, because it (plasma), destroys or gets rid of peroxide residuals rather than having to rely on aeration.

Due to the oxidative nature of H_2O_2 vapour, some materials are not recommended for instruments intended for this sterilisation method.

1.6.4 Methyl Bromide

Methyl bromide is a broad spectrum microbiocide. It is a colourless, odourless gas at room temperature, and is normally applied as a liquid under pressure that converts to the gaseous state upon release at the point of application. It boils at less than 5 °C.

Methyl bromide may have been classified as a classical sterilant, except that its use was revived after being a high O_3 depletor in 2005. It was used as a bioterrorist sterilant by the EPA. Although not initially identified by the EPA as an effective sporicidal agent following the incident in October 2001 where mail was contaminated by anthrax. Subsequent work has demonstrated the efficacy of methyl bromide as a sporicidal fumigant, capable of a 6-log reduction of a virulent *Bacillus anthracis* spores in 24 h at 30 °C on carpet. Increasing both the temperature and time of exposure improves the efficacy. The sporicidal efficacy of methyl bromide is largely dependent on both the bioburden and the surface being decontaminated. Surprisingly, non-porous glass was more difficult to decontaminate than porous carpet. This may have been due to the presence of bioburden and the methodology used during testing. A cohesive drop containing spores suspended in 5% serum was placed on the coupon and allowed to dry. On glass the spores would settle with the serum drying as a protective coating and shield. However, the carpet fibres wick away the drop allowing for a partitioning

of the spores away from the bioburden, thereby increasing their vulnerability. Methyl bromide has been shown to be sporicidal [18, 19].

Per the Montreal Protocol, a country can still use methyl bromide after 2005 by determining that a technically and economically feasible alternative with acceptable health and environmental effects is not available and that a significant market disruption would occur without use of methyl bromide. The country must take steps to minimise the methyl bromide use and emissions and conduct research to develop and deploy alternatives.

Methyl bromide has not been deemed as a hospital sterilant but was used as a soil decontaminant. It has been considered as a possible synergist for EO sterilisation by the Russians in spacecraft sterilisation. Its synergism was probably the result of facilitating improved permeation of EO through some non-polar polymers such as polyethylene. It has only about 10% of the microbiocidal activity of EO.

1.6.5 Low Steam Formaldehyde

The most widely recognised and well-established 'traditional' method of sterilisation is using high-pressure steam, but it is not sensitive to many polymers and materials. Low steam formaldehyde has many characteristics of steam sterilisation but with lower compatible temperatures (e.g., 65-85 °C), than by using steam alone.

Together steam and formaldehyde are synergistic. It is used in European countries, UK, Sweden, Holland, Germany instead of EO, however, it is being used to a lesser extent. It is however used in India and some Asian countries. Some characteristics of the low steam formaldehyde process are:

- It requires heat tolerant temperatures: 65-85 °C and higher RH.
- It is not generally used in the US (except in some unpublished applications).
- It has OSHA worker exposure levels of 0.75 ppm 8-h TWA and 2 ppm 15-min STEL.
- Formaldehyde is considered to be a potential carcinogen, particularly in California – it has an IDLH of 20 ppm.
- Formaldehyde is known to be toxic, irritating and allergenic.
- It has an RQ in case of a spill of 100 lbs under the Comprehensive Environmental Response Compensation and Liability Act.

1.7 Conclusions

EO sterilisation may be the gold standard for traditional chemical sterilisation methods. However, alternatives to it, have been identified, but in general they do not have the penetrating and material compatibility that EO has.

Hypothetically while PPO would seem to be a good substitute for EO, traditional EO sterilisation remains a gold standard for gaseous sterilants, and has been developed, and evolved with a greater capacity to penetrate through dirty, encrusted and salt protected microbes on unclean materials, devices or hospital products, than current alternative and new novel sterilisation methods. On the basis of this scenario traditional EO sterilisation will remain a useful method from the past and remain as an alternative to better future sterilisation processes, because there are no perfect means of cleaning completely, all areas or sites of manufactured or re-usable healthcare products. Most bioburden testing following current standards including aerobic and anaerobic selected media, do not reveal clear, consistent and harmonised results but widely fluctuating and volatile numbers and types over different conditions, time, seasons, and cleaning variations. Many bioburdens are hidden in cracks and crevices, mated surfaces, very narrow lumens and impacted by variable environmental conditions that most cleaning methods, and alternative sterilants may not reach or overcome.

Safer ways of using and applying PPO are still available. PPO is less hazardous and toxic than EO, but has only 25% of the microbiocidal activity of EO. For example, using lower concentrations of PPO and a high humidity but running temperatures close to boiling point may be synergistic, pulsing the gas for deeper penetration of lumens, while potentiating the gas for near surface sterilisation, and aeration. With current alternative sterilants, (e.g., H_2O_2 with plasma and O_3), there is no recycling, but PPO can be recycled and pulsed, and can be neutralised into a non-toxic by-product, which can act as a preservative: propylene glycol, which helps to achieve safe residue levels. It is assumed to have lower residue toxicity levels than EO.

Similar equipment can be used for PPO sterilisation as is used for EO sterilisation in hospitals and industry, with some modifications to improve penetration, and microbiocidal activity. It is assumed that PPO status to EO sterilisation would be as similar as IPA use is to ethanol disinfection, from a regulatory point of view. Use of PPO would have to have regulatory approval (e.g., FDA, EPA and so on). PPO may not be as microbiocidally intense or as active as H_2O_2 or O_3, but it has greater penetration capabilities, and is more compatible with materials than H_2O_2 or O_3.

Of the oxidising sterilants described, it is interesting to note that O_3, H_2O_2 and PAA all have the capability of producing breakdown products that are not toxic. For example:

- O_3 breaks down to O_2.
- H_2O_2 breaks down into water and O_2.
- PAA changes to acetic acid, water and O_2. Plasma actually improves their breakdown.
- Chlorine dioxide does not breakdown into non-toxic residuals.

Currently, use of H_2O_2 (with plasma) and O_3 are replacing EO sterilisation in individual hospitals. Also, use of high concentration H_2O_2 vapour has been accepted as an alternative for sterilisation of items in industrial healthcare. They will be discussed later in **Chapter 5** as alternative (recognised) sterilants to traditional sterilants (e.g., steam, dry heat, irradiation and EO).

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2 Traditional Liquid Chemical Sterilisation

The magic of liquid chemical sterilisation is unlike heat, gaseous sterilant, gas/plasma and irradiation methods; it is fortuitously driven by cleaning, pre-sterilisation handling, biocompatibility/safety, and aseptic technique.

Autoclaving, dry heat, ethylene oxide (EO) sterilisation, and irradiation have been traditionally accepted as effective means of sterilising billions of inanimate objects, but they typically require expensive and non-mobile equipment. This equipment also requires preventative maintenance. Some pieces of materials or product are too biologically, heat, moisture, oxidising or irradiation sensitive, that they have to be treated differently. In a healthcare setting, it is essential to be able to control infectious organisms. Sterilants and high-level disinfectants are important tools for meeting that need. But because they are necessarily toxic to living organisms, sterilants and disinfectants must be handled carefully, and their associated wastes must be managed properly, to avoid causing unintentional harm as they fulfill their intended function.

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According to previous Centers for Disease Control and Prevention data, healthcare-acquired nosocomial infections may account for ~70,000 annual deaths within hospitals. This 'number' may be greater if home-care, urgent care, and other healthcare settings, are included. Also, the increasing number of antibiotic resistant microbes in hospitals is another reason for using high-level disinfectants or liquid sterilants. Liquid chemical germicides are important tools for meeting and eliminating these needs and more. But because liquid chemical germicides are necessarily toxic to living organisms, in order to inactive or kill them, these liquid sterilants and high-level disinfectants must be handled carefully, and their related wastes must be managed properly, so as to not cause any unintentional harm.

Although, the terms, liquid chemical sterilisation and traditional sterilisation (thermal, gas, gas/plasma, irradiation) have similar meanings in terminology and semantics,

they are, however, different, as defined by the US Food & Drugs Administration (FDA). Liquid chemical germicides (LCG) may be approved as sterilants, but are typically used as high-level disinfectant. The FDA believes that sterilisation with liquid chemical sterilants does not convey or necessarily provide the same ‘level’ of sterility assurance as sterilisation as with traditional sterilisation methods such as EO, moist heat and radiation, because liquid chemical sterilisation involves a two or more part steps or process. First, devices are treated with a LCG, typically by immersion. This is then followed by a second step in which the processed devices are rinsed with water to remove sterilant and the chemical residues. There are further limitations with liquid chemical sterilisation. Although the rinse water is treated to minimise any bioburden, it may not be sterile (unless terminally filtered). Because the rinse water may not be sterile, devices rinsed with this water cannot be assured to be sterile (unless pre-treated, e.g., filtered).

Furthermore, devices may not be wrapped or adequately contained during the processing in a liquid chemical sterilant (unless the device remains within an enclosed container). This means that there may be no way to ensure or maintain sterility once devices have been processed (unless the device is maintained under a bacterial container), because after rinsing the product is typically moved to be wrapped in a package or enclosures. Consequently liquid sterilant or high-level disinfectants really can only be considered clinically sufficient.

Another concern is that there are no approved biological indicators (BI) for liquid chemical sterilants. However, chemical indicators may exist for monitoring minimum required concentrations and important parameters. BI are used in traditional methods to demonstrate a sterility assurance level (SAL) of 10^6, consequently, there is not the assurance of sterility for liquid chemical sterilants as there is for traditional thermal, gaseous, or radiation sterilants. However, irradiation for medical device manufacturers typically, requires no BI, but only dose measurement, while irradiation of drug product requires both dose and BI.

If liquid chemical high-level disinfectant or sterilants could provide a thermal (or temperature) chemical time indication, then there may be a precise calculation of SAL, rather than just chemical concentration monitoring. Typically high-level disinfectants are chemicals that can act as sterilants if an increased exposure time or temperature is utilised.

Another concern is that, although liquid sterilants can sterilise with extended exposure times, items to be sterilised (e.g., endoscopes) may not be repeatably exposed with prolonged immersion times, without damage [1].

Among the advantages of liquid sterilants are their convenience, there is no need for high capital equipment such as for gaseous sterilisers or irradiators. For many applications, in which the sterilant may evaporate or be rinsed away when its action is incomplete, they may be inexpensive. Hypochlorite and peracetic acid (PAA) are particularly rapid acting sterilants but still several minutes of treatment are required to produce a sterile surface, but traditionally they can be corrosive to many materials.

The rate of liquid sterilant effectiveness is typically dependent upon its concentration and temperature. In practical terms many liquid sterilants halve their exposure time, by doubling their concentration. Similarly, some chemicals may halve their exposure time or lethality by increasing their temperatures significantly (e.g., >10 °C), depending upon what the temperature coefficient (Q_{10}) value is for the sterilant. A Q_{10} temperature coefficient is a measure of the rate of change of a biological or chemical system as a consequence of increasing the temperature by 10 °C. Q_{10} is a factor by which a rate changes, and is a useful way to express the temperature dependence of a liquid sterilant process. For most biological systems, the Q_{10} value is ~2-3. But this is not always true, as the Q_{10} value for phenol is 4 and for ethanol is 48. The coefficient suggests that, for example, phenol activity would be increased by a factor of 4 by an increase of 10 ºC (from 20-30 ºC). The Q_{10} for EO is ~2. While Q_{10} values are usually in the range of 2 to 3, a slightly wider range of Q_{10} values may be found for ozone (O_3) and chlorine dioxide (ClO_2) (>3). Q_{10} can be used to determine the temperature difference required to cause a 10-fold change in the decimal reduction/death value (Z-value). A Z-value = 10 °C/Log Q_{10}.

With most liquid sterilants it is a cold sterilisation process, which typically is one applied at a temperature of less than 50 °C [2] but is sometimes carried out at 55-60 ºC.

The number of liquid sterilants which don't cause any damage are very few and limited in their action. Classically formaldehyde with or without alcohol was an historical one. However, because of its high irritancy, odour, and carcinogenicity it is rarely used anymore. Another reason for its non-use in alcohol (e.g., methanol) was that it eventually polymerises or through autoxidation forms formic acid. In applications for interplanetary spacecraft or electrical connections it causes an undesirable corrosive condition [2].

Liquid EO in water or methanol converts slowly over several months to ethylene glycol and or ethylene glycol monomethyl ether, unless there is a synergist such as acid or high temperature. Unlike formaldehyde's reaction product (e.g., formic acid or polymerised formaldehyde), the reaction by-products of EO such as ethylene glycol or ethylene chlorohydrin are often not corrosive to metals, but they have solvent properties different from those of the mixtures of the reactants [2].

Glutaraldehyde has predominantly replaced formaldehyde, and other agents such as chlorine, halogens, phenolics, which were not able to sterilise polymers, without polymer damage, toxic residuals, and which were ineffective against microbial spores.

There are number of other agents that are disinfectants such as alcohol, 3% hydrogen peroxide (H_2O_2), low-level iodine (50 ppm), phenols and quaternary ammonium compounds (Quat), which are not always considered to be sporicides, unless specially treated with another agent. Hypochlorites at high concentrations (ppm) can kill spores but are typically too corrosive to be used or applied to healthcare products. High concentrations of iodine are less sporicidal than hypochlorite or chlorine, and may not inactivate dry spores, and certain *Pseudomonas* microbes. For example *Burkholderia cepacia* was found to be 'viable' in iodophore solutions. It is unknown what the diatomic iodine (I_2) concentration, pH, or non-free iodine levels were. There was some suspicion of organic matter, causing the problem. But iodine at a slightly higher temperatures than ambient may be active and effective. Iodine may also stain fabrics and tissues. Use of iodophores can reduce the staining, and iodine stains can be neutralised with bisulfites. *Note:* Iodophore at elevated temperatures (e.g., 50-60 °C with ultrasonics can be a very effective sterilising agent for surgical and dental instruments [3]). While its chemical reactivity is low, it may be, by far the best sterilising agent approaching the speed and effectiveness of glutaraldehyde [3], and without the toxic residuals of glutaraldehyde.

The choice of a LCG will depend upon a variety of factors and considerations and no single LCG is ideal for all purposes or practices. Consequently there are new agents being developed. New liquid sterilants are available [e.g., buffered PAA and higher percentage H_2O_2, performic acid, and *ortho*-phthaldehyde (OPA)] and these will be discussed later or under alternative or novel methods. High-level disinfectants or sterilants that act by generating active forms of oxygen (O_2), such as H_2O_2 or PAA, typically create fewer by-products than compounds relying on other active elements. They also can persist in the presence of organic matter while other agents may not, while iodine without organic interference may have a longer use life. Glutaraldehyde has been used as a sterilant for the past 35 years and is considered to be a traditional liquid sterilant, and continues to be used globally.

2.1 Glutaraldehyde Sterilisation

Glutaraldehyde is commonly used in place of formaldehyde and frequently as a liquid high-level disinfectant, used for quick turnaround devices. Glutaraldehyde is frequently used as a disinfectant for heat-sensitive equipment such as dialysis instruments, surgical instruments, suction bottles, bronchoscopes, endoscopes, and ear, nose, and throat instruments. Glutaraldehyde has also been used as a tissue fixative in histology and

pathology laboratories and as a hardening agent in the development of X-rays. It is also used as a sterilant of animal tissues (e.g., porcine valves and collagens).

2.1.1 Characteristics of Glutaraldehyde

It is a sterilant that is useable for heat sensitive, medical devices with long thin lumens that alternative gaseous sterilants such as H_2O_2, plasma, and O_3 cannot reach. Some interesting characteristics of glutaraldehyde are:

- It is used in aqueous solutions, but needs pH activation. There have been alcohol solutions of it.
- It is a five carbon dialdehyde with less toxicity than formaldehyde, but it is a stronger sporocide.
- It is a high-level disinfectant or sterilant (if left in solution for the specified time).
- It is used for unwrapped items only, unless it is used within an enclosed filter system that can flush the residuals out.
- It is used for sterilising animal tissues and enzymes.
- It is a cause of hazardous residuals.
- Is a mucus membrane irritant – it can cause contact allergies.
- It is not a carcinogen like formaldehyde and yet it is inexpensive.
- A new American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value ceiling limit (TLV-C) of 0.05 ppm was set in 1997.

The biocidal activity of glutaraldehyde is through its alkylation of sulfadryl, hydroxyl, carboxyl, and amino groups, which alters ribonucleic acid (RNA), deoxyribonucleic acid (DNA) and protein synthesis within micro-organisms. The biocidal data for glutaraldehyde products varies with different formulations.

Glutaraldehyde is a unique dialdehyde compound with the formula $CH_2(CH_2CHO)_2$ (see **Figure 2.1**), which is not carcinogenic as many other aldehydes (acetaldehyde, formaldehyde) are.

Other names for glutaraldehyde are glutaraldehyde, glutaric acid dialdehyde, glutaric aldehyde, glutaric dialdehyde and pentanedial, 1,5-pentanedial.

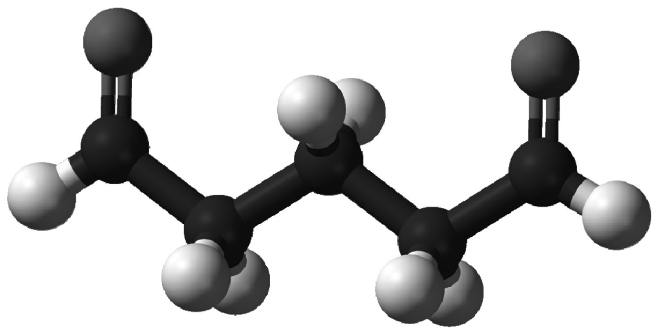


Figure 2.1 Molecular structure of glutaraldehyde

2.1.2 Properties of Glutaraldehyde

Some of the unique properties of glutaraldehyde are shown in **Table 2.1**.

Table 2.1 Some properties of glutaraldehyde	
Property	**Results fitting property**
Molecular formula	$C_5H_8O_2$
Molar mass	100.12 g/mol
Appearance	Clear liquid
Density	1.06 g/ml
Melting point	–14 °C
Boiling point	187 °C
Solubility in water	Miscible

Glutaraldehyde is a colourless, oily, liquid with a pungent odour, but not as malodourous as formaldehyde but nevertheless pungent. It has a propensity to polymerise with increased pH (alkaline) over time. It is more stable at an acidic pH, but less bacteriocidal at an alkaline pH.

2.1.3 Some Advantages and Disadvantages of Glutaraldehyde

The major problem associated with glutaraldehyde is its known respiratory, and dermal irritancy, respiratory sensitisation, and adverse health effects that may occur with exposed workers, without any safeguards.

The advantages of glutaraldehyde may be summarised as:

- Glutaraldehyde is broadly antimicrobial. It inactivates viruses, all vegetative microbes, *Mycobacterium* organisms, and spores, but not necessarily prions.
- It is active in the presence of organic matter [3].
- It is a widely used disinfectant for heat sensitive devices, and sterilisation of biological tissues.
- It can show synergy with plasma, ultrasonic cavitation, temperature, pH, formaldehyde, dimethoxane (2,6-dimethyl-1,3-dioxan-4-yl-acetate), 2-(decylthio) ethanamine, dodecylguanidine hydrochloride or phenols.
- Buffered solutions are not deleterious to cements of various lenses of devices.
- Buffered solutions do not interfere with the electrical conductivity of rubber anesthesia equipment.
- It does not affect markings on clinical thermometers and so on.
- Its low surface tension permits easy penetration and permits easy rinsing.
- It is non-corrosive to numerous metals.
- It does not affect rubber or plastic articles [2].
- It does not coagulate blood, making it easy to clean blood covered instruments [2].
- It does not affect the sharpness of cutting instruments [2].

Some of its disadvantages are:

- It takes a long time to sterilise (e.g., 10 h), but not for disinfection. It is a relatively rapid, high-level disinfectant (e.g., 45 min).
- It is toxic and an irritant, a respiratory sensitiser and more, but it is not carcinogenic (see **Section 2.1.4**).

- It has limited stability at an alkaline pH - once activated its use is between 14-28 days.

2.1.4 Health Risks

The results of breathing and or exposure to glutaraldehyde include:

- Throat and lung irritation.
- Asthma and difficulty in breathing.
- Contact and/or allergic dermatitis.
- Nasal and ocular irritation.
- Sneezing.
- Wheezing.
- Burning eyes and conjunctivitis.
- Hives.

Regarding its carcinogenicity, glutaraldehyde is not a carcinogen, nor teratogen. It has equivocal results with the AMES mutagenicity test, but no reported carcinogenicity in the Chinese hamster ovary cell test, or with *Drosophila* or in dominant lethal assays in mice. There have been no reports of carcinogenicity for glutaraldehyde.

However, its use requires respiratory equipment, gloves and apron. The major problem with glutaraldehyde is its known adverse health effects, such as respiratory and ocular irritancy, and sensitisation, as a result there are numerous health and safety requirements for its use. For example the time weighted average (TWA) for glutaraldehyde is 0.05 ppm and its permissible exposure limit for a 15 min period is only 0.05 ppm. Because it is an acute sensitiser there is no safe level that can really be set.

Glutaraldehyde residuals do not get the attention that EO residuals do because of their lack of carcinogenicity, nevertheless, glutaraldehyde residuals must be rinsed away. For example, adverse biological reactions to glutaraldehyde have been limited to infrequent contact dermatitis and to biocidal effects which are exploited in chemical sterilisation media. In one study of a glutaraldehyde-tanned (crosslinked) collagen sponge, the presence of glutaraldehyde was correlated with cytotoxic effects upon fibroblasts in tissue culture and foreign body, giant cell reaction to bio-implants of the sponge.

Some rinse instructions indicate a 2 min rinse or rinse thoroughly, but the manufacturer's instructions must be followed.

2.1.5 Sterilisation and High-level Disinfection

Glutaraldehyde can inactivate all micro-organisms including many toxins (bacterial by-products), but not pyrogens or prions, and it may require from 3-12 h depending upon the formulation, with soaking followed by rinsing or washing with sterile water or filtered water, and then drying. Products should be stored in such a way as to prevent contamination. Liquid sterilants and processes have a much lower SAL (with a higher risk) than physical chemical traditional sterilisation agents such as steam, radiation and EO, because they can sterilise within packages, whereas liquid chemicals typically cannot.

To achieve glutaraldehyde sterilisation requires making contact with all the areas of a component or device to be sterilised, and then an elevated or extended period of exposure time is necessary. If inadequate contact, concentration and time are not achieved, then a 10^{-6} sterility assurance cannot be made [4]. This is a concern of most liquid sterilants that they cannot reach all areas in complex devices or surfaces.

Overcoming barriers for liquid sterilant contact to microbes is important to achieve adequate sterilisation, but it may be difficult, if there are tight parts, small capillaries, non-smooth surfaces (e.g., dental burs, screws) or mated surfaces to be penetrated.

Some materials such as Teflon may require surface acting agents for liquid glutaraldehyde or other sterilants to make adequate surface contact. Teflon has a material surface that is difficult to wet. Sterilisation by glutaraldehyde requires longer exposure times and temperatures than by EO at the same temperatures, for sporicidal effectiveness. It may kill all vegetative microbes, acid fast *Mycobacterium* (except *Mycobacterium chelonae* in tissues), pathogenic fungi and viruses within a period of 5-20 min.

The most resistant viruses are Poliovirus type 1, Coxsackie virus type B1, and echovirus type 6. These viruses require concentrations of greater than 1% glutaraldehyde to kill them.

2.1.6 Formulations

There are numerous glutaraldehyde formulations.

Glutaraldehyde products are marketed with a variety of brand names, formulations and are available in a variety of concentrations (solution concentrations may range from 2.3-3.4%), with and without surfactants.

For example, there are 2.3% or high concentration formulations (e.g., 2.4%, 2.5%, 2.6%, 3.4% of glutaraldehyde, but caution should be exercised with all glutaraldehyde formulations when further in-use dilution is anticipated). There is a combination of glutaraldehyde (1.12%) with 1.93% phenol/phenate, and another composed of 3.4% glutaraldehyde with 26% isopropanol, and others.

The effects of formulations will vary due to various factors such as:

- Acidic glutaraldehyde (e.g., pH 4) shows poor lethality at ambient temperature but this pH lethality increases with temperature (>45 °C) and ultrasonic cavitation. However as a vapour or gas, it is equivalent to alkaline glutaraldehyde.
- Glutaraldehyde stability increases under acidic conditions rather than alkaline.
- Glutaraldehyde under ambient conditions tends to be more active under alkaline conditions than acid conditions, but it will be more active at a higher temperature (60 °C) and under potentiated acidic conditions (e.g., 60 min).
- Acid glutaraldehyde tends to be more corrosive than alkaline formulations, also, acid formulation(s) at ambient conditions are less effective than alkaline formulations on dry spores.
- Glutaraldehyde lethality will increase with increase in glutaraldehyde concentration.
- A highly fortified glutaraldehyde formulation may consist of a Quat, glutaraldehyde, *para-tertiary*-amylphenol, citric acid, isopropyl alcohol, and water to form a concentrated formulation, which may be diluted as requested.
- A glutaraldehyde-based disinfectant containing a Quat is good as a specially formulated a long lasting disinfectant for poultry and cattle sheds. It may remain active in the presence of a high organic content.
- Glutaraldehyde interacts strongly with the outer layers of spores. This interaction may reduce lysis induced by peroxides.
- It is possible but debatable that acidic glutaraldehyde interacts with the surface of spores and remains at the surface, whereas alkaline glutaraldehyde may penetrate (more) deeply into spores.

While glutaraldehyde sterilisation typically sterilises with concentrations of 1%, 2%

and 3+%, other concentrations and conditions are available such as use of surfactants and pH buffers. Typically on adjusting the pH to 7.5-8, the antimicrobial effects are greatly increased. An alkaline solution alone may take 10 h at room temperature to sterilise.

Some formulations include mixtures with phenol, phenates and so on. Another combination is a mixture of glyoxal and glutaraldehyde.

Temperature increases from 25 to 35 °C have enhanced its germicidal and sporicidal activity.

Use of ultrasonics can reduce sterilisation from hours to minutes. The germicidal activity of ultrasound is thought to be intracellular cavitation that results in micromechanical damage to cellular structures and may lead to lysis, but it also helps other agents such as glutaraldehyde to sterilise better.

Potentiated acid glutaraldehyde can sterilise at 60 °C in 60 min. A pH of 2.2 and increased temperature can reduce sterilisation from hours to minutes. The solution should be discarded after four weeks. The stability of a solution of glutaraldehyde for sterilisation lasts typically only 14 days, although some solutions last up to 28 days.

Some trade formulations of glutaraldehyde are: Cetylcide-G (3.2%); Cidex (2.4, 2.5, 3.4%); MedSci (3%); Metricide (2.5, 2.6, 3.4%); Omnicide (2.4, 3.4%); Procide (2.4%); Rapidcide (2.5%); Sporicidin (1.12/1.93% glutaraldehyde/phenol); and Wavicide-01 (2.5%).

2.1.7 Applications and Uses

There are more than 45 million surgical procedures and more invasive medical procedures performed each year that need to have pathogenic microbes eliminated from them. Glutaraldehyde is one of the ways to prevent infections.

Glutaraldehyde use continues after more than 30 years as a high-level disinfectant or sterilant method of choice in a number of hospitals and surgical centres. One of the reasons for its continued use, is because of its long tradition and because so many of the hospital sterilants developed today are more surface (gaseous) sterilants and not capable of sterilising through the heavy biological or organic wastes which accumulate from hospital applications.

Glutaraldehyde is useful for sterilising thermolabile polymers such as optical instruments, rubber and man-made products, dialysis, and in veterinary applications such as poultry, and for skin disinfection in mastitis.

Glutaraldehyde is also used as a preservative and it has been used in preparation of vaccines. It has been a valuable agent in the aseptic assembly of products.

It is less corrosive than formaldehyde solutions. There are some materials that are incompatible with it, for example, some metals, powders, and electrical components.

Glutaraldehyde is useful for disinfecting/sterilising work surfaces between patients or hospital rooms after patients have used them.

Glutaraldehyde (0.2-1%) is used for sterilising biological tissues at 32-38 °C, such as porcine valves prior to implantation in animals or humans. Its use helps to crosslink the tissue as well (porcine heart valves). Formaldehyde (3-5%) is sometimes added to improve the inactivation of the resistant *M. chelonae*.

Glutaraldehyde has also been used to sterilise glucose monitoring enzymes, and other tissues of animal origin.

Glutaraldehyde can be activated with a pH change and through increase in temperature and concentration. Glutaraldehyde has multiple uses, for example, preservation, surface inactivation, device and product sterilisation, biological tissue sterilisation, and vaporous decontamination.

2.1.8 Glutaraldehyde within Closed Systems

Sterilisation with glutaraldehyde can be performed in a sterile isolation hood or tent with glove handles, so that products that may been immersed it its solution and removed from the solution can be rinsed without any source of adventitious contamination, and then packaged within a sterile environment.

Glutaraldehyde is a dialdehyde that may be used in a closed system, to minimise contamination. It is used typically as an aqueous solution, for example at a concentration of 2% or less. It is typically considered to be a high-level disinfectant rather than a sterilant. It is used for unwrapped items only. It has a strong odour. It can have hazardous residuals, which can cause contact allergies and be irritant to mucus membranes. The 1997 ACGIH TLV-C limit is 0.05 ppm. A standard for use is 8 CCR 5155 (Californian Code of Regulations) [5]. Glutaraldehyde has been used to disinfect/sterilise all sorts of hospital items, e.g., bronchoscopes, cystoscopes and rubber anesthesia equipment. It has also been used to decontaminate working areas within closed systems. However, because of lack of *in situ* packaging sterilisation, it is difficult to achieve/maintain sterility, and its activity, and thus, its effectiveness as a sterilant has been questioned without a closed system, and an aseptic technique is required for handling treated product.

In recent years, glutaraldehyde has been used to sterilise biomaterials such as porcine heart valves under sterile environmental assembly conditions using aseptic techniques, and subsequently used as a preservative to maintain sterility. In this case the glutaraldehyde can act both as a protein crosslinker, and as a sterilant. Sometimes it is mixed with formaldehyde or other agents to improve its penetration of the organic tissue.

Its failure to sterilise porcine heart valves has resulted in growth of *Mycobacterium*. Because of its slow chemical activity, it, like EO, is able to penetrate, and to continue to diffuse into areas without being fully reacted before penetration or sterilisation. It is selective enough to inactivate some microbes without totally inactivating the enzymes that are used to monitor biological chemicals. Because it is a slow reactant chemical it can take up to 10-12 h of exposure time for it to fully sterilise materials. Its rate of sterilisation can be increased by increasing the temperature and acidity.

The major limitation of glutaraldehyde, causing its disuse, is similar to that of formaldehyde – its' extremely pungent odour and residual toxicity to patients and objects. Glutaraldehyde is a mutagen, and possibly a carcinogen. If glutaraldehyde is used in a closed system, many of its disadvantages and limitations can be overcome.

Another approach is to sterilise within a container with two filters at the end of the container. The item being sterilised can be rinsed subsequent to exposure, by flushing fluid through the filters. Modifications of this approach may also be designed.

2.1.9 Two-part Sterilisation Process using Glutaraldehyde

A liquid chemical sterilisation process such as glutaraldehyde is best applied as a two-part process:

- Devices are treated (immersed) with a LCG.
- The processed devices are rinsed or flushed with water to remove the chemical residues.

There are the limitations with liquid chemical sterilisation. Liquid may not seep into or penetrate all areas or surfaces of a product in the same way that a gas, high heat, vapour or steam can. Also, although the rinse water is treated to minimise any bioburden, it may not be sterile. If the rinse water is not sterile, devices rinsed with this water cannot be assured as sterile. Furthermore, devices cannot be wrapped or adequately contained during processing in a liquid chemical sterilant. This means that there is no way to maintain sterility once the devices have been processed. Consequently the use of liquid chemical sterilants such as glutaraldehyde may be

limited to reprocessing only critical devices that are heat-sensitive and incompatible with sterilisation methods such as steam, dry heat, EO, gas/vapour/plasma low temperature processes and use of a gaseous zone.

A further consideration, is that diffusion occurs easily within the product for EO because it is a small gaseous compound (two carbons with O_2) without immediate chemical charge or attraction, whereas liquid glutaraldehyde typically only sterilises by surface contact of items or materials. Liquid glutaraldehyde is a large molecule, [e.g., five carbon backbone with a dialdehyde chemical end in water or on contact with another carrier source such as alcohol or wetting agent(s)]. Liquid glutaraldehyde makes chemical bonds with water and alcohol, while EO does not. Also, besides having lack of diffusion, glutaraldehyde does not absorb itself into polymers and materials as EO does, leaving residuals. Its exposure time (e.g., 10-12 h) to inactivate spores is significantly greater than EO (e.g., 15 min to 6 h) depending upon temperature, concentration, humidity and so on.

However, a potentiated acid glutaraldehyde may sterilise in 60 min at 60 °C. For example, an activated 2.4% glutaraldehyde, sterilised in 10 h at 25 °C; and is a high-level disinfectant in 45 min at 25 °C. An alkaline activated glutaraldehyde has a 14 day maximum re-use period, a less effective acidic glutaraldehyde may be stable for a longer period, at ambient conditions (e.g., up to 4 weeks).

Various trade names and formulations of glutaraldehyde have concentrations which vary between 2.3-3.5%. A 1.2% glutaraldehyde has been mixed with a 1.9% phenol/phenate solution to sterilise in 12 h at 25 °C and to high-level disinfect in 20 min at 25 °C.

Another combination is a glyoxal/glutaraldehyde mixture, which has been used without incident at approximately 50 °C in other European countries for several years.

There is a 3% glutaraldehyde formulation that will sterilise in 10 h at 20 °C rather than at 25 °C, high-level disinfect at 40 min at 20 °C with a 28 day maximum re-use rather than 14 days.

Sterilisation by glutaraldehyde and EO occurs principally by alkylation of proteins and EO requires humidity for the alkylation to occur effectively.

2.1.10 Performance

Survival curves for liquid chemical sterilants such as glutaraldehyde may not exhibit the log-linear kinetic shape of the survivor curve and this may vary depending on the formulation, chemical nature and stability of the liquid chemical sterilant. If

glutaraldehyde does not exhibit log linear kinetics, but micro-organisms still grow or reproduce logarithmically, the net change between bioburden (contamination) and sterility assurance may be significant.

Biological indicators are not typically appropriate for monitoring the liquid chemical sterilisation process. The design of the Association of Official Agricultural Chemists (AOAC) sporicidal test used to quantify liquid sterilants does not provide the same quantification of the microbial challenge, used for gaseous sterilants. Therefore, sterilisation with a liquid chemical sterilant such as glutaraldehyde may not convey the same sterility assurance as traditional sterilisation methods. However, EO may not be effective against salt occluded microbes, whereas liquid sterilants may. This points to the need for adequate cleaning of the product to be reprocessed.

Chemical indicators are used for monitoring the minimum required concentration of most liquid chemical sterilants. Dipsticks have been supplied with some glutaraldehyde products but these give only a rough indication of the levels of active glutaraldehyde available in solutions being used.

In industry, manufacturers using liquid sterilants usually create their own liquid chemical sterilant to do the job, and they do not often share the formula publicly. International Organization for Standardization (ISO) 14160 [6] is the standard that they follow. It does not specify what should be in the sterilant, only how it should perform.

Regardless of the proprietary formulation, its performance has to meet minimum standards, which ensure that the product is sterile and safe for use on the patient. There are certain micro-organisms that may not be killed easily sterilised by a liquid chemical sterilisation (e.g., *Mycobacterium)*. These micro-organisms may also sometimes be difficult to find on a device (e.g., animal sterilant) after it is sterilised, because those micro-organisms grow slowly, and the tester could overlook them, which means that a longer incubation may be required. Glutaraldehyde may be among the liquid sterilants used for sterilisation of animal tissue.

Glutaraldehyde cannot penetrate many polymers, but it can sterilise many aqueous liquids, including water, biological tissues and enzymes without difficulty or adverse effects, such as being hydrolysed to ethylene glycol, as with EO. EO and heat can penetrate barriers, such as biofilms, tissue, and blood, to kill organisms, whereas liquid sterilants such as glutaraldehyde cannot adequately penetrate these barriers. Consequently bioburden, biofilm and so on, on the product to be sterilised by liquid sterilants have to be cleaned thoroughly first.

Like EO, liquid, glutaraldehyde sterilisation occurs primarily by alkylation but also through crosslinking, and at temperatures typically higher than freezing and can

sterilise many types of tissues, sensitive materials and polymers. The process for glutaraldehyde sterilisation requires extremely long ‘holding’ periods under moist (liquid) conditions, and generally they must be washed to remove their residuals as compared to EO sterilisation exposure and aeration. EO sterilisation typically sterilises items within packaging, while glutaraldehyde does not, so there may be less assurance of maintaining sterility.

To determine which sterilisation method (EO or glutaraldehyde) is the method of choice, identification and consideration of their sterilising qualities, principles and limitations for each method and materials is necessary. The final determination of the method of choice may include - identifying the method that appears to be more compatible to tissues, product design, materials and package, penetration, lethal activity, cost, safety/toxicity, process time, sterilisation in-line (assembly or procedure) or sterilisation-in-place, sterilisation release time, availability, and for industrial sterilisation this may require performing some feasibility studies to determine gross compatibility with the selected process, and then performance of preliminary validation studies to demonstrate product compatibility with the selected process and attainment of a required SAL.

While glutaraldehyde can kill spores, it is more typically used as a high-level disinfectant capable of killing *Mycobacterium* and some spores, and also because it is used primarily as a liquid sterilant, it does not have the same minimal risk of EO sterilisation which can sterilise most products within packages, and maintain their sterility after sterilisation. Glutaraldehyde may have some effectiveness against prions.

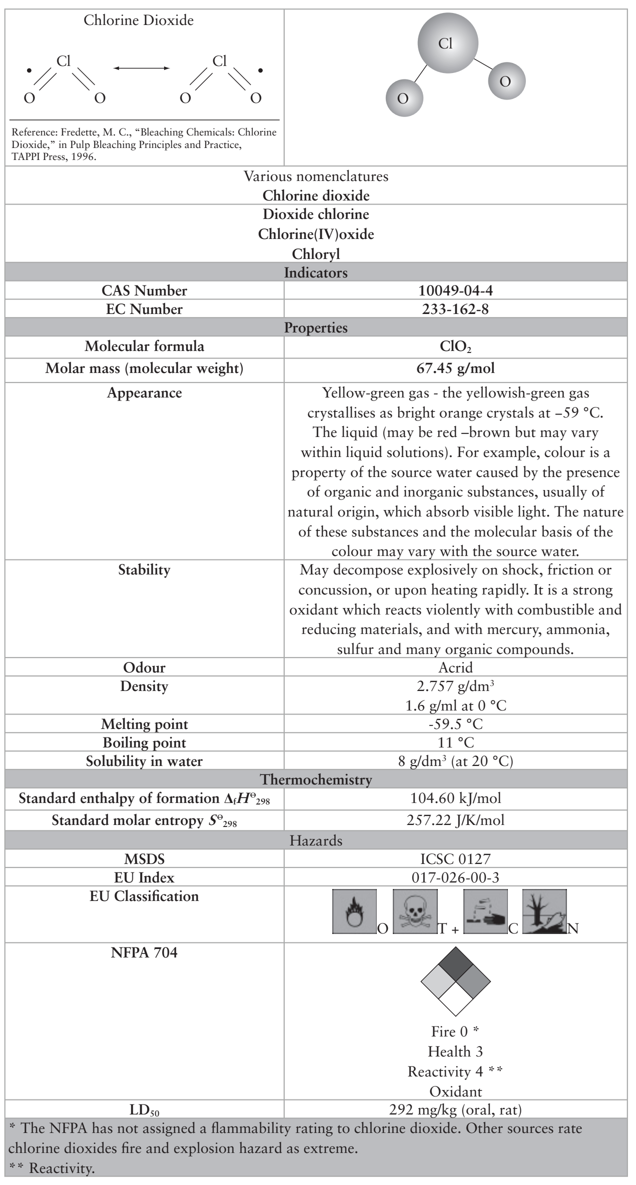
Other traditional liquid sterilants exist, e.g., ClO_2, β-propiolactone (BPL), chlorine, H_2O_2, PAA, OPA and so on.

2.2 Chlorine Dioxide

ClO_2 is a powerful oxidising agent discovered by Davy in 1811. It is a slightly soluble gas that dissolves in water that may give a green coloured solution. ClO_2 is less corrosive than chlorine and less reactive to ammonia and amine compounds. It is equivalent or better than chlorine in the inactivation of microbes.

2.2.1 Characteristics

Before ClO_2 became fashionable as a gaseous sterilant, it was approved as a liquid disinfectant - sterilant. Some of the properties of ClO_2 are shown in **Figure 2.2**.



Chlorine Dioxide Reference: Fredette, M. C., "Bleaching Chemicals: Chlorine Dioxide," in Pulp Bleaching Principles and Practice, TAPPI Press, 1996.	
Various nomenclatures **Chlorine dioxide**	
Dioxide chlorine **Chlorine(IV)oxide** **Chloryl**	
Indicators	
CAS Number	**10049-04-4**
EC Number	**233-162-8**
Properties	
Molecular formula	**ClO_2**
Molar mass (molecular weight)	**67.45 g/mol**
Appearance	Yellow-green gas - the yellowish-green gas crystallises as bright orange crystals at −59 °C. The liquid (may be red –brown but may vary within liquid solutions). For example, colour is a property of the source water caused by the presence of organic and inorganic substances, usually of natural origin, which absorb visible light. The nature of these substances and the molecular basis of the colour may vary with the source water.
Stability	May decompose explosively on shock, friction or concussion, or upon heating rapidly. It is a strong oxidant which reacts violently with combustible and reducing materials, and with mercury, ammonia, sulfur and many organic compounds.
Odour	Acrid
Density	2.757 g/dm^3 1.6 g/ml at 0 °C
Melting point	-59.5 °C
Boiling point	11 °C
Solubility in water	8 g/dm^3 (at 20 °C)
Thermochemistry	
Standard enthalpy of formation $\Delta_f H^\ominus_{298}$	104.60 kJ/mol
Standard molar entropy $S^\ominus_{298}$	257.22 J/K/mol
Hazards	
MSDS	ICSC 0127
EU Index	017-026-00-3
EU Classification	O T + C N
NFPA 704	Fire 0 * Health 3 Reactivity 4 ** Oxidant
LD$_{50}$	292 mg/kg (oral, rat)
* The NFPA has not assigned a flammability rating to chlorine dioxide. Other sources rate chlorine dioxides fire and explosion hazard as extreme. ** Reactivity.	

Figure 2.2 Properties of ClO_2. CAS: Chemical Abstract Service; EC: European Commission; EU: European Union; MSDS: Material Safety Data Sheet; and NFPA: National Fire Protection Association. Adapted from W.M. Haynes in *Handbook of Chemistry and Physics*, 91st Edition, CRC Press, Boca Raton, FL, USA, 2010 p.4; N.N. Greenwood and A. Earnshaw in *Chemistry of the Elements*, 2nd Edition, Butterworth-Heinemann, Oxford, UK, 1998, p.844; and *Pulp Bleaching Principles and Practice*, TAPPI Press, Peachtree, GA, USA, 1996,

ClO_2 has a number of chemical/physical properties and safety issues which need to be considered. These include:

- Pure ClO_2 may explode on impact, when exposed to sparks or sunlight, or when heated rapidly to 100 °C. Airborne concentrations greater than 10% may explode.
- ClO_2 can be a very unstable material even at room temperature.
- Incompatibilities: Contact with the following materials may cause fires and explosions: carbon monoxide, dust, fluoride, fluoroamines, hydrocarbons (e.g., butadiene, ethane, ethylene, methane, propane), hydrogen, mercury, non-metals (phosphorus, sulfur), phosphorus pentachloride-chlorine mixture, platinum, or potassium hydroxide. ClO_2 reacts with water or steam to form toxic and corrosive fumes of hydrochloric acid.
- Hazardous decomposition products: Toxic and corrosive gases and vapours such as chlorine gas or the oxides of chlorine may be released when ClO_2 decomposes.
- ClO_2 is a highly endothermic compound, which may decompose extremely violently when trying to separate it from diluting substances. As a result, typical preparation methods that involve producing solutions of it without going through a gas phase stage are often preferred.

Its use or recognition as a traditional liquid sterilant is limited, however, it was originally registered in 1967 under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act, but it has been recognised as a disinfectant since 1937. The Environmental Protection Agency (EPA) first registered the liquid form of ClO_2 for use as a disinfectant and sanitiser on a variety of sites such as animal farms, bottling plants, food processing, handling, and storage plants. ClO_2 can be neutralised with sodium bisulfite. After 1988, it was recognised as a (gaseous) sterilant.

2.2.2 Chlorine Dioxide Solutions

Historically, use of ClO_2 for disinfection applications has been made by one of three methods:

1. Using the sodium chlorite - hypochlorite method:

$$2NaClO_2 + 2HCl + NaOCl \rightarrow 2ClO_2 + 3NaCl + H_2O$$

2. The sodium chlorite - hydrochloric acid method (HCl):

$$5NaClO_2 + 4HCl \rightarrow 5NaCl + 4ClO_2 + 2H_2O$$

3. Electrolysis of sodium chlorite:

$$2NaClO_2 + 2H_2O \rightarrow 2ClO_2 + 2NaOH + H_2$$

All three sodium chlorite chemistries can produce ClO_2 with high chlorite conversion yield, but unlike the other processes the chlorite-HCl method produces completely chlorine-free ClO_2 but suffers from the requirement of 25% more chlorite to produce an equivalent amount of ClO_2. Alternatively, H_2O_2 may also be used efficiently in small scale applications. Very pure ClO_2 can also be produced by electrolysis of a chlorite solution.

ClO_2 in aqueous solution, has been used, when necessary, using nitrogen or air purging to remove the traces of residual gas. The major problems with ClO_2 as an aqueous solution, is that it may be unstable and corrosive. There have been recent developments for liquid ClO_2 systems. For example, there is a stabilised ClO_2 source that is a superior alternative to chlorine when considering sterilisation, odour control, and tasks such as mould and mildew elimination. With an innovative 'dry' pouch, smaller and medium size industrial and municipal operations can take advantage of all the benefits of it without the hassle and expense of producing it on-site. For example, a pouch mixed with 20 litres of water makes a solution of approximately 400 ppm of ClO_2, which can remain stable for several weeks with proper storage. The blend has many times the oxidation power of chlorine and can be 'potentially' used in many capacities including as a biocide, viricide, fungicide, sporicide, disinfectant, steriliser, and sanitising agent. It easily eliminates the following micro-organisms:

- *Amoebae*
- *Bacillus*
- *Clostridium*
- *Cryptosporidium*
- *Escherichia coli*
- *Giardia*
- *Legionella pneumophila*
- *Pseudomonas*
- *Enterococcus faecalis*

It has been used to disinfect water, and many other applications. It has not yet been approved by regulatory agencies for high-level disinfectant or sterilisation. It may

also be used as a decontaminant. It has been used as wipes. For example, Tristel's ClO_2-based wipes kill bugs on the small flexible endoscopes commonly used in the ear, nose and throat departments found in all hospitals worldwide.

ClO_2 may have some effectiveness against prions.

2.2.3 Performance

Solutions of ClO_2 are commercially available as liquid sterilants - under trade names such as Tristel, Dexit and Medicide. They can compete with glutaraldehyde and PAA solutions. Tristel, an oxidising disinfectant has good bactericidal, fungicidal, virucidal and sporicidal activity. In general ClO_2 oxidises proteins and destroys the cellular activity of proteins of microbes.

ClO_2 as an oxidant, is similar to O_3 but works in a completely different way to chlorine. It destroys cell membrane walls in seconds, thus destroying the pathogens. Laboratory and field tests have shown that lower concentrations of ClO_2 are faster and more effective at reducing bacteriological counts than higher concentrations of chlorine. ClO_2 is a 'broad spectrum biocide', and is more effective against fungi and spores than ordinary chlorine.

It is stable on storage but unstable after it is activated for use. It can be an irritant to skin and mucous membranes. It may also damage some materials and may be inactivated by organic matter, but it is sporicidal. For example preliminary, *in vitro* suspension tests with Tristel showed that solutions containing around 140 ppm ClO_2 achieved a reduction factor exceeding 10^6 of *Staphylococcus aureus* in 1 min and of *Bacillus atrophaeus* spores in 2.5 min in the presence of 3 g/l bovine albumin. The liquid chemical was used with a washer steriliser.

While the microbicidal efficacy of ClO_2 has long been recognised, there have been two problems associated with the use of liquid systems. First, the solutions are unstable, with the concentration of ClO_2 rapidly diminishing; secondly, because ClO_2 is highly oxidative, it is potentially corrosive to many materials. The development of usable solutions has therefore required formulations that incorporate stabilising agents, usually based on boron components and anti-corrosion compounds. These comprise a base solution and an activator which, when mixed, yield a solution of approximately 0.1% ClO_2, with a 14 day shelf-life. Solutions of this type are increasingly being used for sterilisation of items such as fibre-optic endoscopes.

2.2.4 Some Benefits and Limitations

A myth exists that ClO_2 is corrosive but unlike chlorine or hypochlorites, it is not as corrosive or toxic. A similar comparison exists between formaldehyde and glutaraldehyde. While ClO_2 is an oxidant, it is not a chlorinating agent, like chlorine.

Liquid ClO_2 is often considered to be more corrosive, particularly due to the various acids involved in the generation processes. Some solutions may be corrosive, but others are not. It has been used to wash plants and other biological items without adverse effects, with a moderate acidic pH. An aqueous solution is considered safe to handle, the dissolution being essentially physical. Watery solutions containing approximately 1% ClO_2 (10 g/l) can be safely stored, as long as they are protected from light and heat.

On standing in sunlight the solution slowly decomposes to a mixture of acids. In alkaline solution a mixture of chlorate(III), ClO_2, chlorate(V) and ClO_3 ions is rapidly produced. ClO_2 is paramagnetic, the molecule containing an odd electron and possessing a structure very like that of nitrogen dioxide.

Pure ClO_2 gas that is applied to water produces less disinfection by-products than oxidators, such as chlorine. Unlike O_3, pure ClO_2 does not convert bromide ions into bromate ions, unless it undergoes photolysis. Additionally ClO_2 does not produce large amounts of aldehydes, ketones, ketone acids or other disinfection by-products that originate from the ozonisation of organic substances.

The opposite of chlorine, ClO_2 is effective at a pH of between 5-10. The efficiency may at higher pH values, while the active forms of chlorine are more greatly influenced by pH. Under normal circumstances ClO_2 does not hydrolyse. This is why the oxidation potential is high and the disinfection capacity is not influenced by pH.

At 50 μg/ml, ClO_2 can kill significantly higher numbers of *Pseudomonas* cells than chlorine can, however, chlorine may be more effective in killing cells and spores of *Bacillus cereus* suspended in horse serum. A *B. cereus* biofilm can be reduced by treated it with ClO_2 or chlorine at 200 μg/ml.

ClO_2, especially in solution, is complicated by its volatility, its sensitivity to light and instability over time, and interference from related redox species. The interference of the by-product formation when using ClO_2 as a disinfectant is of great concern. Chlorite ions and ClO_3^- are always potential by-products when ClO_2 is used as a disinfectant.

2.2.5 The Future

ClO_2 as a liquid sterilant appears to be frequently overlooked [7]. The bactericidal activities of ClO_2 solutions were demonstrated as long ago as the 1940s. Compared to chlorine, ClO_2 is not as reactive, and consequently is increasingly favoured for water treatment. It is also used to remove odours. It is a powerful oxidant. Like all liquid sterilants (e.g., Expor), ClO_2 must have contact with hidden or hard to find microbes with sufficient concentration, temperature and time to be effective, or sterility will not result [4]. Data suggest that commercial liquid sterilants and disinfectants are less effective on contaminated surfaces (e.g., dental burs, screws, silicone tubing) than generally acknowledged. Items to be sterilised must have a low bioburden or be pre-cleaned to reduce the bioburden.

One of the difficulties with ClO_2 in solution is its volatility from the solution. For example, disinfection of spore suspensions with aqueous ClO_2 solution in sealed microfuge tubes can be highly effective, reducing the viable spore counts by 8 log_{10} in only 3 min. By contrast, the process of spraying or spreading the disinfectant onto surfaces may result in only a 1 log_{10} kill because the ClO_2 gas rapidly vapourises from the solutions. However, full potency of the sprayed ClO_2 solution can be restored by preparing it in 5% bleach (0.3% sodium hypochlorite), this may restore full potency and increase its stability for one week and prevent treatment failures that are caused by its volatility. ClO_2 unlike PAA may have some effectiveness against prions.

2.3 Peracetic Acid or Peroxyacetic Acid

PAA started as a liquid area decontaminant, and has been refined for use in product sterilisation. PAA breaks down into acetic acid, water and O_2 all of which have a low toxicity. It is a popular alternative to glutaraldehyde. PAA besides sterilising removes primarily protein surface contaminants from endoscopic tubing.

2.3.1 Characteristics

PAA has several interesting characteristics, including:

- It is a wet process.
- It is a strong oxidising agent produced from acetic acid and H_2O_2.
- It is an extremely reactive and consequently a very hazardous chemical.

- It is predominantly used with endoscopes but increasingly applied to other items, (e.g., reverse osmosis membranes).
- It typically requires the sterilisation equipment to be designed as a closed system, so that its finished product may be maintained and handled (aseptically) as a sterile item, at the point of use.
- Previously, after treatments products had required rinsing with a neutralising agent, but waste from the cycle is now reported to be non-hazardous.
- It has been previously approved by the FDA and the EPA for processing endoscopic equipment as a liquid PAA solution of 0.2%, and later as 0.08% plus 1.0% H_2O_2.
- As a buffered solution at 50-55 °C it is circulated through and around the devices for about 12 min. This is followed by an automated rinse to remove the sterilant from the products.
- It requires specially designed trays or containers which are used for positioning the instrumentation.
- As with any liquid process, monitoring is a problem. It needs use of *Geobacillus stearothermophilus* biological indicators, but the FDA has never approved a BI for it.

Although chemical monitoring may be an alternative, the ionic concentration of the buffer is sometimes monitored instead of the active compound:

- It has a by-product, acetic acid, which has an Occupational Safety and Health.
- Administration and a National Institute for Occupational Safety and Health exposure limit of 10 ppm 8-h TWA.
- The immediately dangerous to life or health level for acetic acid is 50 ppm.
- It has a reportable quantity of a release of one pound under situational awareness and response assistant Section 302 Extremely Hazardous Substances.

Recently liquid PAA has had a significant role as hospital sterilant, but was not used very much industrially. Low concentrations (0.2%) have been used for respiratory equipment. Concentrations of 0.l-0.5% have been used for surgical equipment. It has been used to sterilise kidney dialyser filters throughout the world.

Because it had no harmful effects it was deemed to have significant potential in sterilisation, however, in concentrations greater than 30% it is corrosive. Interestingly, human skin can tolerate 0.4% PAA, which has been the concentration used for

sterilisation. One of the advantages of PAA is that it is biodegradable into O_2, water and acetic acid.

PAA concentrations at less than 0.1% combined with a low percentage of H_2O_2 (<0.1%) have powerful oxidising capabilities to disinfect a broad range of microbes, are rapid, and are not corrosive. PAA is considered to be ineffective for destruction of prions.

2.3.2 Performance

Liquid PAA, as well as, gaseous PAA has had some regulatory issues, and they may be deemed as novel sterilants, although liquid PAA has been used significantly in hospitals. Liquid PAA is a highly biocidal oxidiser that maintains its efficacy in the presence of organic soiling. PAA or peroxyacetic acid are oxidising agents that denature protein, disrupting cell-wall permeability and oxidising sulfhydryl and sulfur bonds in proteins, enzymes and other metabolites.

PAA can remove surface contaminants (primarily protein) from endoscopic tubing. In 1983-1988, there was an automated machine using liquid PAA to sterilise medical, surgical, and dental instruments chemically (e.g., endoscopes, arthroscopes). PAA can inactivate Gram-positive and Gram-negative bacteria, fungi, and yeasts in <5 min at <100 ppm, however, levels of 200-500 ppm are required in the presence of organic matter. For viruses, the dosage range is wide (12-2,250 ppm), with Poliovirus inactivated in yeast extract in 15 min at levels of 1,500-2,250 ppm. Bacterial spores in suspension are inactivated in 15 seconds to 30 min with 500-10,000 ppm (0.05-1%). The automated machine uses a microprocessor controlled, low-temperature sterilisation method. The sterilant, begins with 35% PAA, with an anti-corrosive agent are supplied in a single-dose container. The container is punctured at the time of use, immediately prior to closing the lid and initiating the cycle. This concentrated PAA is diluted to 0.2% with filtered water (0.2 μm) at a temperature of approximately 50-55 °C. Puncturing the container causes the filtered water to dilute the acid to 0.2% at a temperature of 50 °C. This diluted acid solution circulates through the machine chamber, then pumps through the endoscope tubing for 12 min, resulting in decontamination of all exterior surfaces and accessories, including fibre optic lights, or lumens. This diluted PAA continues to be circulated within the chamber of the machine and pumped through the channels of the endoscope for the 12 min. Interchangeable trays are available to permit the processing of up to three rigid endoscopes or one flexible endoscope. Rigid endoscopes can be decontaminated by being placed in a lidded container that is plunged into the flowing sterilant, filling the lumens, or by directing the flow through the channel connectors. The connectors are available for most types of flexible endoscopes to irrigate all of the channels by

directed flow. Rigid endoscopes are placed within a lidded container, and the sterilant can fill the lumens either by immersion in the circulating sterilant or by use of channel connectors to direct flow into the lumen(s) (see next for the importance of channel connectors). The unused and undecomposed PAA is discarded *via* the sewer and the instrument is rinsed four times with filtered water to remove residuals. There have been concerns that the filtered water used may be inadequate to maintain sterility. Limited data have shown that low-level bacterial contamination may follow the use of filtered water but no data has been published recently on using a revised PAA system. Clean filtered air is passed through the chamber of the machine and the endoscope channels to remove excess water. As with any sterilisation process, the system can only sterilise surfaces that can be contacted by the sterilant. For example, bronchoscopy-related infections occurred when bronchoscopes were processed using the wrong connector. Investigation of these incidents revealed that bronchoscopes were inadequately reprocessed when inappropriate channel connectors were used and when there were inconsistencies between the reprocessing instructions provided by the manufacturer of the bronchoscope and the manufacturer of the automatic endoscope reprocessor. The importance of channel connectors to achieve sterilisation has also been shown for rigid lumen devices.

2.3.3 Biological Monitoring

Manufacturers recommend the use of biological monitors (*G. stearothermophilus* spore strips) both at the time of installation and routinely to ensure the effectiveness of the process. The manufacturer's clip must be used to hold the strip in the designated spot in the machine as a broader clamp will not allow the sterilant to reach the spores trapped under it. One investigator reported a 3% failure rate when the appropriate clips were used to hold the spore strip within the machine. The use of biological monitors designed to monitor either steam, dry heat sterilisation, H_2O_2 vapour or EO for a liquid chemical steriliser has been questioned for several reasons including spore wash-off from the filter paper strips which may cause less valid monitoring. The processor is equipped with a conductivity probe that will automatically abort the cycle if the buffer system is not detected in a fresh container of the PAA solution. A chemical monitoring strip that detects that the active ingredient is >1,500 ppm is available for routine use as an additional process control and monitor.

2.3.4 Miscellaneous

Simulated-use trials have demonstrated microbicidal activity and three clinical trials have demonstrated both microbial killing and no clinical failures leading to infection. Alfa and co-workers [8], who compared the PAA system with EO, demonstrated the

high efficacy of the system. Only the PAA system was able to completely kill 6 log_{10} of *M. chelonae*, *Enterococcus faecalis*, and *B. atrophaeus* spores with both an organic and inorganic challenge. Like other sterilisation processes, the efficacy of the PAA process can be diminished by soiling challenges and test conditions.

PAA is likely to be bacteriocidally active by denaturation of proteins and enzymes and increases in microbial cell wall permeability by disrupting sulfur (S-S) and sulfydryl (-SH) bonds.

A typical PAA automated machine is used to chemically sterilise medical (e.g., gastointestinal endoscopes) and surgical (e.g., flexible endoscopes) instruments in the United States. Lumened endoscopes must be connected to an appropriate channel connector to ensure that the sterilant has direct contact with the contaminated lumen. Some manufacturers have not listed this system as a compatible product for use in reprocessing bronchoscopes and gastrointestinal endoscopes. PAA may form toxic metal oxides or acetates. The decomposition products of gaseous PAA are acetic acid, water and O_2. At its use concentration it may not have the same effect on cellulose materials as H_2O_2 does.

2.3.5 Benefits

Using liquid PAA has the following benefits:

1. Room temperature sterilisation (18-30 °C), this allows sterilisation of heat-sensitive devices.
2. No harmful residuals, this eliminates product exposure to toxic chemicals.
3. Quick turnaround time (no aeration), this maximises supply chain flexibility.
4. Superior material compatibility, including biocompatibility after rinsing, this allows sterilisation of many materials.
5. Frequently applied in enclosed systems (e.g., a processor) to maintain and handle (aspetically) sterile items at the point of use.

In benefit 4, PAA may react with metals to create toxic metal acetates or metal oxides, which are water soluble. Consequently after rinsing items with aqueous solutions, after sterilisation, these toxic metal acetates will be removed (unlike a PAA gaseous vapour process). While PAA can be corrosive to some metals, use of additives and pH modifications, as well as rinsing, can reduce this effect. PAA is considered to be unstable particularly when diluted.

A 0.2% PAA liquid was able to sterilise (as a sporocide) in 12 min at 50-56 °C, for single use only. Contact conditions were established by simulated use testing with endoscopes and passing a modified AOAC sporocidal activity test. *Note:* PAA is sporicidal at low temperatures. The temperature coefficient is generally low over a range of 20 to -20 °C, but increases significantly at temperatures below this. Results showed an initial lag in the PAA death rates that was directly dependent on the temperature.

Use of diluted PAA does not eliminate the need for manual pre-cleaning of items using a brush. Chemicals are not re-used. Waste from each cycle is reportably non-hazardous.

2.3.6 An Improved Liquid Peracetic Acid Sterilisation Method

The PAA used comes in a dry, powder form in a single-dose container. The container of powdered concentrate is first punctured during the cycle, and the concentrate is diluted with water inside the processor chamber, creating the sterilant. The sterilant cannot be reused. The diluted for use PAA enters the sterilisation chamber and is heated at a temperature of approximately 46-55.5 ºC for 6 min, then the PAA is drained from the chamber.

Following exposure to the PAA there is a flushing cycle. The flushing consists of two post-rinses after the processing cycle. All water is filtered (using pre-filters, ultraviolet (UV) irradiation and a special filter) to ensure that processed items are not recontaminated. It is important to monitor the filters and change them periodically as recommended by the manufacturer. All filter changes should be documented. It should be recognised that the facility's water quality can affect how frequently filters must be changed.

The total cycle time of the process is 23-25 min. The manufacturer recommends that at the completion of the cycle, the user verify that the PAA container is empty because the physical monitors do not measure the presence or concentration of the PAA.

All items that are to be processed must be thoroughly cleaned first, before sterilisation. The steriliser processor is a table-top unit that has processing trays and containers to position devices such as multi-channel flexible endoscopes, rigid endoscopes, and associated instrumentation for processing. It is of paramount importance that when processing flexible endoscopes in the PAA processor, that the user must be thoroughly familiar with the processor and know the correct quick connecter to attach to the flexible endoscope. If an incorrect quick connecter were to be used, the PAA is not likely to reach all areas of the endoscope for effective processing.

The products in the system described previously are not packaged, so care (aseptic technique) must be taken to avoid recontamination after processing. So like immediate use or flash sterilisation, this PAA processing system is a point-of-use, just-in-time system. Consequently, the processor should be located as close to the point of use as possible. It is necessary to follow the steriliser manufacturer's written instructions for use. Since this is a wet system the product should be dry so that the microbes have no opportunity of growth, affixing or attaching to the product. Typically with flash sterilisation the product is so hot, any water vapour evaporates and the product is dry.

This PAA sterilant processing system must be cleared (as given in by the appropriate regulatory body) for processing of immersible, semi-critical and critical heat-sensitive medical devices, such as multi-channel, flexible surgical endoscopes. It is important to verify with the device manufacturer or supplier that the device is compatible with the PAA processor. The product must be validated for the specific processor in use, in a hospital setting.

2.3.7 Supercritical Carbon Dioxide Sterilisation with Peracetic Acid Additive

PAA is an effective agent in eliminating many kinds of harmful bacteria and moulds and may be used for sterilisation and sanitation of surfaces associated with food production and storage, such as aseptic packaging operations that bottle low-acid juices. In such applications, a hot aqueous solution of PAA is sprayed on the inner surfaces of the bottles, which are then rinsed and dried. The PAA is created by combining acetic acid with H_2O_2 in water. The H_2O_2 oxidises the acetic acid in a reversible manner and the resulting solution is an equilibrium mixture of H_2O_2, acetic acid, and peracetic acid. However, heating the PAA solution and rinsing and drying sterilised surfaces require large amounts of energy, ventilation and time. Therefore, there exists a need for alternative approaches to treating surfaces with PAA.

Supercritical carbon dioxide (scCO_2) or the fluid phase of carbon dioxide (CO_2) is made at low pressure (e.g., 7.6 MPa) and at a moderate temperature (e.g., 31.1 °C). scCO_2 maintains the ideal properties of both the liquid and gas phases of carbon dioxide.

As a liquid CO_2 it has excellent non-polar organic solvent properties. At the gas phase it has no surface tension providing unsurpassed penetration.

Use of scCO_2 may achieve a 12-log reduction in bioburden without compromising the structure and integrity of the transplanted skin, tendon, or bone. Micro-organisms can be effectively sterilised by the scCO_2 treatment at 25 MPa and 35 °C. To achieve sterilisation, combining a liquid sterilisation additive with scCO_2 forms a compound

which can act like a gas, and thus, penetrate spun polyester packaging making it possible to terminally sterilise products before use.

The additive (PAA) is used in very low levels (25-100 ppm) with the $scCO_2$. Most of the chemical sterilant (PAA) is removed from the product during depressurisation of the $scCO_2$. This gives the user immediate use of the product rather than having to wait for breakdown of PAA into acetic acid and O_2.

A portion of microbial inactivation by $scCO_2$ may be due to the generation of carbonic acid, created from CO_2 and water, but with the additive (PAA) sterilisation is effected based by both the acid and the peroxide. As an acid (PAA) it may have transport properties in the $scCO_2$ which contributes to the overall intracellular acidification. This mass transfer may enhance and facilitate the delivery and action of PAA as a sporicidal agent.

The gentle nature of this process makes it a valuable tool in xenogenic allograft sterilisation. Without it, an aseptic process is required in liquid sterilisatin, which leaves the potential possibility of post-process contamination and infection. EO and radiation have effects on the tissue or recipient. H_2O_2 plasma cannot penetrate the tissue to achieve sterilisation deep within the tissue. Additionally the peroxide plasma may leave high quantities of free radicals which may react with the materials being processed.

The $scCO_2$ with PAA is compatible with poly(lactic-*co*-glycolic acid) – polyglycolic acid; poly(ether ether ketone) (PEEK), absorbable sutures, some active pharmaceutical ingredients, some drug delivery products, fabrics, other polymers and plastics and surgical metals.

It is also an alternative to irradiation, in the sterilisation of transplant tissues with bone. The problem with high doses of gamma radiation, or harsh treatments including steam and chemicals such as EO, is that they can degrade collagen and other proteins within bone and soft tissue, compromising the strength of grafts and rendering them unusable for grafts. Irradiation is quite harsh to the bone. Even, trying to use low-dose radiation to sterilise this material while trying to avoid structural degradation, remains a challenge.

$scCO_2$ sterilisation is performed on packaging. For example after a transplant tissue has been cleaned and sealed in its final medical packaging (e.g., Tyvek) it can be sterilised. This minimises the need for the aseptic technique needed for most liquid sterilants.

2.4 Chlorine, Hypochlorite and Oxidising Agents

Chlorine, is a fast-acting oxidant, a widely available and broad-spectrum chemical germicide. It is normally provided as bleach, an aqueous solution of sodium hypochlorite (NaOCl), which can be diluted with water to provide various concentrations of available chlorine. Chlorine, especially as bleach, is highly alkaline and can be corrosive to metal. Its activity is considerably reduced by the presence of organic matter (protein).

Of all the liquid chemical sterilants, chlorine and hypochlorite 'oxidising' solutions may be the best for the following reasons. They can be very inexpensive. The oxidising reactivation of these compounds substantially reduces the protective effects of most organic substances. When they are applied in aqueous solution, some protective crystals of inorganic salts are often dissolved, thereby exposing the encrusted microbes to the sterilant [2]. *Clostridium difficile* transmission decreased significantly from a high incidence ward after changing from the use of Quat to a 1:10 solution of NaOCl (bleach).

However, to achieve sterilisation, high concentrations of chlorine may be corrosive and irritating to those using it. So they are good for use on floors or innate materials that may not be corroded, but not for items that are going to be in direct contact with the body.

2.4.1 Characteristics

Hypochlorite is a chemical compound containing OCl group, with chlorine in oxidation state +1 (**Figure 2.3**). Because of their low stability, hypochlorites are very strong oxidising agents. They may react with many organic and inorganic compounds, thereby reducing their efficacy at times.

The high reactivity of hypochlorite or chlorine is important in increasing the effectiveness and restricting the range of materials and the situations to which it may be applied. Many materials are destroyed by exposure to chlorine. Although versatile, the active component of hypochlorous acid is neutralised by high pH values, to become less effective. Above a pH value of 8.5, less than 10% of the biocide is effective. However, alkali solutions may still be powerful, rapid acting, and temperature also influences intensity and spread. In some applications it is highly effective and in others not effective at all. Hypochlorite and hypochlorous acid (650-675 ppm active free chlorine) pass a modified AOAC sporocidal activity test in 24 h at 25 °C. It may be used for a 10 min (non-sporocidal test for single use, generated on site, for contact conditions established by simulated use testing with endoscopes.

International Union of Pure and Applied Chemistry name Hypochlorite	
As an acid: hypochlorous acid	
Identifiers	
CAS number	14380-61-1
UN number	3212

Figure 2.3 Properties of hypochlorite

Hypochlorous acid and hypochlorite (400-450 ppm as active free chlorine) provides for a 10 min exposure for high-level disinfection (non-sporicidal) at 30 °C. It passed the modified AOAC sporicidal activity test in 32 h at 30 °C. Higher levels of chlorine (500-1,000 mg/l) may be more rapid, but they can oxidise and reacting with some materials. Chlorine and chlorine compounds may have an effect on the coat of the spore as well as on its cortex. They may induce dipicolinic acid leakage and remove the spore protein, although this may not affect viability. Sub-lethal concentrations may render spores sensitive to mild heating with the cortex being the major site of action. NaOCl solution containing 20,000 ppm available chlorine may be considered effective for prion inactivation of transmissible spongiform encephalopathies or bovine spongiform encephalopathy disease agents.

2.4.2 Performance and Uses

Globally, bleach (hypochlorite) may be used 77% of the time in sterilisation (decontamination) of medical equipment and surfaces. For example, a solution of one part bleach to 100 parts water can disinfect surfaces of items including re-usable thermometers, stethoscopes, needles and syringes. Emerging Third World countries may often rely on bleach as their best (and only) means of destroying harmful microscopic organisms on medical equipment.

Hypochlorites in the USA are widely used in foods and healthcare facilities in a variety of settings. Inorganic chlorine solution is used for disinfecting food surfaces and tonometer heads in healthcare, and for spot disinfection of counter tops and floors. A 1:10 to 1:100 dilution of 5.25-6.15% NaOCl (household bleach) or an EPA-registered tuberculocidal disinfectant has been recommended for decontaminating blood spills and contamination. For small spills of blood (e.g., drops of blood) on non-critical surfaces, the area may be disinfected with a 1:100 dilution of 5.25-6.15% NaOCl or an EPA-registered tuberculocidal disinfectant. Since hypochlorites as well as other germicides may be substantially inactivated in the presence of blood, large spills of blood require that the surface be cleaned before an EPA-registered disinfectant or a 1:10 (final concentration) solution of household bleach is applied. If there is a possibility of a sharps injury in hospital, there should be an initial decontamination, followed by cleaning and terminal disinfection (1:10 final concentration).

Extreme care should always be employed to prevent percutaneous injury. At least 500 ppm available chlorine for 10 min is recommended for decontamination of cardiopulmonary resuscitation training manikins. Other uses in healthcare include as an irrigating agent in endodontic treatment and for disinfecting manikins, laundry, dental appliances, hydrotherapy tanks, regulated medical waste before disposal, and the water distribution system in haemodialysis centers and haemodialysis machines.

Hyperchlorination of a *Legionella* contaminated hospital water system can result in a dramatic decrease (30-1.5%) in the isolation of *Legionella pneumophila* from water outlets and a cessation of healthcare associated Legionnaires' disease in the affected unit.

Chloramine T and hypochlorites have been used in disinfecting hydrotherapy and other equipment.

Hypochlorite solutions can be used in tapwater at a pH>8, stored at room temperature 22-25 °C) in closed, opaque plastic containers that may lose up to 40-50% of their free available chlorine level over a period of one month. Thus, if a user wished to have a solution containing 500 ppm of available chlorine at day 30, a solution containing 1,000 ppm of chlorine should be prepared at day 0. There is no further decomposition of NaOCl solution after 30 days when stored in a closed brown bottle.

2.4.3 Hazards

NaOCl is a very strong oxidising agent. Oxidation reactions are corrosive, solutions burn skin and cause eye damage, particularly, when used in concentrated forms. Solutions containing more than 40% NaOCl by weight are considered hazardous

oxidisers, whereas solutions of less than 40% are classified as having a moderate oxidising hazard.

Chlorination of drinking water can oxidise organic contaminants, producing trihalomethanes (also called haloforms), which are carcinogenic. *Note:* Iodine has none of these reactions.

Household bleach and pool chlorinator solutions are typically stabilised by a significant concentration of lye (caustic soda, sodium hydroxide (NaOH)) as part of the manufacturing reaction. Skin contact will produce caustic irritation or burns due to defatting and saponification of skin oils and destruction of tissue, but this is not so with iodophors. The slippery feel of bleach on the skin is due to this process. Trichloramine, a gas that is formed in swimming pools when chlorine reacts with organic matter can cause atopic asthma.

Sodium thiosulfate is an effective chlorine and halogen neutraliser. For example, rinsing with a 5 mg/l solution, followed by washing with soap and water, quickly removes chlorine odour from the hands.

Mixing bleach with some household cleaners can also be hazardous. For example, mixing an acid cleaner with NaOCl bleach generates chlorine gas. Mixing with ammonia solutions (including urine) produces chloramines. ClO_2 will not react with ammonia. Mixtures of other cleaning agents and or organic matter can result in a gaseous reaction that can cause acute lung injury.

Chlorine is compatible with a number of materials such as some polyesters, polyetherimide but not PEEK or polysulfone. It is compatible with flexible and rigid polyvinylchloride (PVC), and polypropylene. It is not compatible with polyurethanes, but is relatively compatible with most fluoropolymes except possibly polychlorotrifluoroethylene. It is compatible with most elastomers but not nitriles, styrene-butadiene or polysulfide rubber. It is not compatible with polyvinyl alcohol or silicone. It is poor to fair with celluloses but it is not compatible with metals such as most stainless steels.

Chlorine is also very reactive with other compounds to form for example, trihalomethanes, which cause mucus irritation, and may be regarded as potential carcinogens.

Other strong oxidants such as O_3 and PAA have similar properties, however, many materials are corroded or damaged by these strong oxidising agents.

2.4.4 Iodine

The action of iodine may be similar to that of chlorine on microbes (e.g., a sulfhydryl group (or thiol group), an SH group of an organic enzyme or protein compounds), although they may be slightly less inhibited by organic matter. Iodine can stain fabrics and environmental surfaces and is generally unsuitable for use as a disinfectant. On the other hand iodophors may stain less than pure iodine. In general iodophors and tinctures of iodine are good antiseptics. is a reliable. Polyvinyl pyrrolidone (povidone, PVP) and elemental iodine (PVP-I2) is a safe surgical scrub and pre-operative skin antiseptic. The widely known iodophor is povidone-iodine, a compound of PVP with iodine. This product and other iodophors retain the germicidal efficacy of iodine but unlike iodine, are non-staining (less staining) and relatively free of toxicity and irritancy. If there is staining, sodium bisulfite will remove the brown stain.

Antiseptics based on iodine are generally considered unsuitable for use on medical/ dental devices. Iodine should not be used on aluminum or copper, because they are reactive and the reaction can be catalysed by water. Iodine may be toxic, but is less toxic than chlorine. Organic iodine-based products (with low levels of free iodine) may be stored at 4–10 °C to avoid the growth of potentially harmful bacteria in them, or used at higher temperatures (50-60 °C) to inactive mesophilic microbes.

Iodine has been traditionally used for both antiseptic and disinfectant purposes. The discovery of iodine, like most discoveries, was a fortuitous accident. Sunker Bisey, an Indian youth, won a contest run by an English manufacturing firm in which the winner would get a full scholarship at a major British university. He boarded a ship to England, but never made it to the university. Arriving in England dying of Malaria, he was treated to the best physicians the British Isles had to offer, but to no avail, and he soon opted to die in his homeland, and set sail. At a stopover in France where firing up kilns of seaweed was now the latest craze, someone suggested treating the dying lad with iodine. Bisey recovered and finished his education in England and set sail for New York where he brought this magnificent new treatment for disease to the world.

Although less reactive, less corrosive and less sporicidal than chlorine, iodine is bactericidal, fungicidal, tuberculocidal, virucidal, also sporicidal, but also antiseptic. Aqueous or alcoholic (tincture) solutions of iodine have been used for over 150 years as antiseptics. It has been used as an emergency sterilant. However, as a liquid it has a difficult time penetrating most items, and it may stain and cause irritation.

2.4.4.1 Characteristics of Iodine

Iodine is by far the less chemically reactive than chlorine and several other oxidising

agents, and while for example *B. cepacia* has been found to be 'viable' in iodophore solutions, it still has 'great' potential. While iodine may also stain fabrics and tissues, iodophores can reduce its staining effects. It can be a better antiseptic than other oxidising agents (e.g., H_2O_2). *Note:* Iodophores at elevated temperatures (e.g., 50-60 °C) and with use of ultrasonics can be an effective means for sterilising surgical and dental instruments [3]. While its chemical reactivity is low, it may be by far the best sterilising agent approaching the speed and effectiveness of glutaraldehyde [3]. While this may be disputed, previous AOAC evaluations have shown it to be as sporicidal in effectiveness as an iodophore with 1% available iodine. The iodine must be free.

Discrepancies in reports of sporocidal activity of iodine as well as chlorine have been reported. However, without the presence of organic matter, as with clean devices, iodine has been indicated to kill wet spores within 15 min. While iodine is very effective against vegetative microbes (more so than with chlorine), it is reported to be less effective against spores than chlorine. However, in a case where a laboratory room was highly contaminated with large populations of different spores, iodine was shown to be more effective (because it sterilised contaminants) than chlorine (which did not decontaminate fully), which previously was not able to fully decontaminate the area. Subsequent (further) decontamination with an iodophore was shown to be effective.

Iodophore has been shown to be partially effective against highly resistant spores.

Since iodine has a high boiling point, it has been shown to be effective at higher temperatures where chlorine would be released as a vapour. Every 10 °C increase essentially halves the exposure time or increases it, up to a maximum temperature of 40 °C. However, above 43 °C iodine vapour may occur.

Iodine requires the smallest effective concentration compared with chlorine or bromine in water to provide a free residual. A concentration of 2% iodine can destroy the following resistant spores: *B. atrophaeus, Bacillus anthracis* and *Clostridium tetani.* Iodine is enhanced by acid conditions.

I_2 is highly bacteriocidal, whereas hypoiodous acid is less effective; and the hypoiodite ion is even less effective: typically triodide, the iodide ion and the iodate ion are inactive.

The effect of organic matter may reduce the quantity of iodine, however, this is less significant where high concentrations exist or occur, but more significant with low iodine concentrations.

Commercial iodine complexes have been shown to be a good/excellent decontaminants of a highly contaminated (BI) laboratory with resistant spores that were isolates that survived heat and other agents (overnight), that could not be decontaminated by other

sporicidal means, including chlorine, glutaraldehyde and so on. Afterwards sodium thiosulfite was used to remove stains on floors. In this case the iodine solution (an iodophor) was used an emergency sterilant.

Iodine effectiveness will vary with the amount of free iodine available. However, while iodine in iodophores may be complexed and free [1], its evaluation with AOAC testing for effectiveness may 'still' demonstrate significant sporicidal activity. While iodine or iodophore may not have the same sporocidal activity as hypochlorites, they cause significantly less corrosion and damage to materials. Antiseptic iodophores may not be suitable for disinfecting instruments, devices or environmental surfaces [1].

Iodine is compatible with a few materials and polymers such as polyphenylsulfone, polypropylene, most fluropolymers, but responds poorly with PVC. It is compatible with many elastomers but not with not with polychloroprene (Neoprene). It is compatible with fluorosilicone, and cellulose acetate. Like chlorine it does not appear to be compatible with many stainless steels, however, the corrosive properties of iodophore-type sanitisers are substantially lower than those of chlorine-type sanitiser.

As inidicated earlier, iodine should not be used on aluminum or copper, because they are reactive and the reaction can be catalysed by water.

The FDA has not approved any liquid chemical sterilant or high-level disinfectants with iodophors as the main active ingredient. However, it has accepted them as part of a manufactured product.

An iodophor is a unique combination of iodine and a solubilising agent or carrier and the resulting complex provides a sustained-release reservoir of iodine and releases small amounts of free iodine in aqueous solution, within an adequate pH range.

2.4.4.2 Performance

Some reports that have indicated some intrinsic microbial contamination (*B. cepacia*) of antiseptic formulations of povidone-iodine and poloxamer-iodine caused a reappraisal of the chemistry and use of iodophors. 'Free' iodine (I_2) contributes to the bactericidal activity of iodophors and dilutions of iodophors demonstrate more rapid bactericidal action than do a full-strength povidone-iodine solution. The reason for the observation that dilution increases bactericidal activity is unclear, but dilution of povidone-iodine might weaken the iodine linkage to the carrier polymer or cause a change in pH with an accompanying increase of free iodine in solution. The results of some studies are conflicting, however, it must be remembered that the relationship between povidone iodine and free iodine concentrations is not linear, as it forms a

bell shaped curve, which peaks at a concentration of 0.7%. Higher concentrations of povidone iodine can uniquely bind more free iodine to the carrier molecule (e.g., PVP), thereby lowering the available free iodine. Consequently iodophors must be diluted according to the manufacturers' directions to achieve their antimicrobial activity. Also increasing the temperature and reducing the pH typically improves the sterilising ability of iodophore.

A complex of iodine with, for example, alkyl phenoxy polyoxyethylene ethanol and dilution in aqueous ethanol may prevent the survival of *B. cepacia* that has been previously isolated.

Iodine can penetrate the cell wall of micro-organisms quickly, and the lethal effects are believed to result from the disruption of protein and nucleic acid structure and synthesis. However, the killing of spores by glutaraldehyde or iodophore is not due to DNA damage, and while the spore coat protects spores against killing by glutaraldehyde it does not protect them from iodophore.

The mechanisms of inactivation of Poliovirus by ClO_2 and iodine have been found to differ. Iodine inactivated viruses by impairing their ability to adsorb to HeLa cells, whereas ClO_2-inactivated viruses showed a reduced incorporation of [^{14}C] uridine into new viral RNA. The killing action of iodine on most microbes occurs quickly and is thought to be from inactivation of vital cytoplasmic substrates, which are necessary for bacterial viability. Plasma proteins can bind up to 80% of free iodine. The presence of organic matter can reduce iodine's efficacy.

Reports on the *in vitro* antimicrobial efficacy of iodophors demonstrate that iodophors are bactericidal, mycobactericidal, and virucidal but can require prolonged contact times to kill certain fungi and certain bacterial spores. Three brands of povidone-iodine solution have demonstrated more rapid kill times (seconds to minutes) of *S. aureus* and *M. chelonae* at a 1:100 dilution than did the stock solution. The virucidal activity of 75–150 ppm of available iodine was demonstrated against seven viruses.

Other investigators have questioned the efficacy of iodophors against Poliovirus in the presence of organic matter and rotavirus SA11 in distilled or tapwater. Manufacturers' data demonstrate that commercial iodophors are typically not sporicidal, but they are tuberculocidal, fungicidal, virucidal, and bactericidal at their recommended use-dilution. Follow the manufacturer's directions for mixing and contact time.

Besides their use as an antiseptic, iodophors have been used for disinfecting blood culture bottles and medical equipment, such as hydrotherapy tanks, thermometers, and endoscopes. However, antiseptic iodophors may not be suitable for use as hard-surface disinfectants because of concentration differences to ordinary iodophores. Ordinary

iodophors are probably among the most commonly used surface disinfectants. They have a low toxicity, no offensive odour, and are not irritating to the skin, however, iodine compounds are not hazard free. Toxic symptoms can result from systemic absorption. These may include nervousness, depression, insomnia, myxoedema, hypersensitivity and skin reactions. Elemental iodine (I_2) is mildly toxic if taken orally. The lethal dose for an adult human is 30 mg/kg, which is about 2.1-2.4 grams (even if experiments on rats demonstrated that they could survive after eating a 14,000 mg/kg dose). Excess iodine can be more cytotoxic in the presence of selenium deficiency. Iodine supplementation in selenium-deficient populations is, in theory, problematical, partly for this reason. Its toxicity derives from its oxidising properties, which make it able to denaturate proteins (and so consequently enzymes).

Elemental iodine is an oxidising irritant and direct contact with skin may cause lesions, so iodine crystals (concentrated) should be handled with care. Solutions with high elemental iodine concentration such as tincture of iodine and Lugol's solution are capable of causing tissue damage if their use for cleaning and antiseptics is prolonged, but not iodophores, which are less concentrated.

Iodophores do not produce a 'significant' residual effect on the treated surface (except for some staining), as many other sterilants do. Iodophors are rated by the EPA as a tuberculocidal hospital disinfectant. The FDA has not cleared any liquid chemical sterilant or high-level disinfectants with iodophors as the main active ingredient. An iodophor is a combination of iodine and a solubilising agent or carrier; the resulting complex provides a sustained-release reservoir of iodine and releases small amounts of free iodine in aqueous solution. It has a greater sustaining ability (than peroxides, PAA, glutaraldehyde, OPA, and other 'significant' liquid sterilants. It also has the potential of lasting longer on skin and organic matter than other liquid high-level disinfectants or sterilants.

Iodophors formulated as antiseptics contain less free iodine than do those formulated as disinfectants. Iodine or iodine-based antiseptics should not be used on silicone catheters because they may adversely affect the silicone tubing.

Again, unlike other oxidising agents, iodine may persist longer, continuing to have microbiocidal activity when other stronger oxidising agents may have reacted with organic matter and decompose quicker, or lost their stability (usefulness).

Iodine may combine with the amino acid tyrosine in proteins and denatures proteins for microbial inactivation.

Iodophors may mildly stain some items, but their stains are less resistant than non-iodophor iodine mixes, and yet are still removable. The presence of a stain may

indicate the possibility of some residual microbial activity with adequate water activity. Staining of skin has been misinterpreted as irritation.

2.4.4.3 Future of Iodine

For many people, iodine is most familiar as an antiseptic. Historically, mothers put iodine on children's cuts. Hospitals today use iodine-based products as antiseptics and disinfectants.

While, most people are familiar with iodine as a skin antiseptic, it is also a powerful disinfectant for surfaces. Iodine kills a wide range of bacteria, including the hard to kill tuberculosis bacteria. A very small amount (25 g) of iodine can kill all of the bacteria in 30,000 litres of water. It should be used in liquid form, because iodine gas is toxic. Whereas iodine stains surfaces and corrodes mop buckets, iodine can be neutralised. Like most disinfectants and sterilants, any organic matter needs to be cleaned from surfaces before using iodine to disinfect them and iodophors must be diluted according to the manufacturers' directions to achieve anti-microbial activity to ensure effectiveness.

Iodine may be synergistic with ultrasonic treatment. It may be used as an emergency sterilant or as a high-level disinfectant. Since iodine is an antiseptic, it may not require the two-step procedure (immersion and rinsing) required for most liquid high-level disinfectant or sterilants, which are toxic and have some by-products that require rinsing, before implanting or contacting the patient. An added feature of iodine is its long-term residual effect.

The addition of sub-gingival PVP-iodine irrigation to conventional mechanical therapy may be a means of reducing total counts of periodontal pathogens and helping control periodontal disease. For example using this irrigation with 10% PVP–I_2, a 95% reduction in pathogens has been demonstrated, without adverse effect to patients.

Despite its many successes, iodine solution has many unsuitable properties such as staining of skin, blue staining on laundry, and in presence of starch. But this is a discoloration problem that had been dealt with, with irradiation, which has been accepted with modifications. Iodine can be an irritant but this can be reduced with reduction of the concentration of the iodine.

An enzyme-based iodine (EBI), a high-level disinfectant that continuously generates free molecular iodine in a controlled fashion has been developed and evaluated for use in disinfecting flexible fibre optic endoscopes. EBI starts as a powder concentrate that produces free iodine from sodium iodide and calcium peroxide when catalysed by

horseradish peroxidase. The horseradish peroxidase has been used to generate iodine formulations that are principally free molecular iodine. The concentration of free molecular iodine in these enzyme-based compositions ranged from 44 to 63% of the EBI; this is substantially higher than the corresponding value for the povidone-iodine preparation formulation. The biocidal efficacy of these compositions are proportional to the concentration of free molecular iodine.

After dissolving the powder in water, it delivers relatively high concentrations of free molecular iodine (>15 ppm) at relatively low concentrations of total iodine (~30-40 ppm). It demonstrates the ability to function as an effective low-level iodine disinfectant by rapidly inactivating bacteria, fungi and viruses, but inactivating spores slowly. A unique feature of this EBI system is its ability to re-oxidise reduced iodine, which results in a constant level of active (free molecular) iodine during use. EBI inactivates *Mycobacterium tuberculosis var bovis* more rapidly than a 2% glutaraldehyde formulation. Its sporicidal activity, however, was found to be slower than the aldehyde formulation. It is, however, more effective than PVP-I_2 on spores.

Iodine compositions with relatively low total iodine concentrations but high-levels of free molecular iodine (20-175 ppm) can kill *S. aureus* and spores of *B. atrophaeus* more rapidly than the concentrated iodophore formulation. Within this new class of iodine-based topical formulations it will not stain skin but provides over 100 times more biocidal iodine than traditional iodophors. An essential rationale for the use of molecular iodine to treat skin diseases is the observation that molecular iodine readily penetrates into skin and remains biocidal while it diffuses in.

The qualification of EBI for use as a practical disinfectant has been shown by its negligible toxicity in dermal, ocular, oral and inhalation studies on animals, which is attributed to the low level of total iodine in the solution. At low concentrations it has relatively no staining problems.

Redox-iodometry is a simple, precise, and time-saving substitute for the more laborious and expensive iodometric titration method, which, like other well-established colorimetric procedures, is clearly outbalanced at low concentrations; this underlines the practical importance of redox-iodometry. This is a new iodometric method for quantifying aqueous solutions of iodide-oxidising and iodine-reducing substances, as well as plain iodine/iodide solutions. It is based on the redox potential of said solutions after reaction with iodide (or iodine) of known initial concentration. Calibration of the system and calculations of unknown concentrations can be performed on the basis of developed algorithms with simple GW-BASIC programmes. The method is recognised by its short analysis time (2-3 min) and a simple instrumentation consisting of pH/mV meter, platinum and reference electrodes. In general the feasible concentration range encompasses 0.1-10^{-6} mol/l, although it can go down to 10^{-8} mol/l

(0.001 mg Cl_2/l) for oxidants such as active chlorine compounds. The calculated imprecision and inaccuracy of the method has been found to be 0.4-0.9% and 0.3-0.8%, respectively, resulting in a total error of 0.5-1.2%. Based on the experiments, the average imprecisions of 1.0-1.5% at oxidising concentration $>10^{-5}$ M, 1.5-3% at 10^{-5} to 10^{-7} M and 4-7% at $<10^{-7}$ M were found. Knowing the amount of free iodine is a means of monitoring the effectiveness of iodine disinfectants without need for BI.

2.5 *β*-Propriolactone

BPL has been used to sterilise biological materials as a liquid without toxic or allergic reactions in the liquid state. It has been used to decontaminate contaminated areas (e.g., food facilities). BPL has been used to sterilise tissue grafts, surgical instruments, and enzymes, blood plasma, water, milk, and nutrient broth, and as a vapour-phase disinfectant in enclosed spaces. BPL is a heterocyclic ring compound, a colourless pungent liquid at room temperature and boils at 163 °C. It is not flammable at room temperature and it has very strong microbiological activity. Of great importance is the fact that, in aqueous solutions, BPL is rapidly and completely decomposed into hydracrylic acid, a molecule differing from lactic acid only in the position of the OH group.

BPL appears to be a superior sterilising agent for biologicals because of its ability to inactivate a wide variety of bacteria, fungi, and viruses. At the same time, this agent has a minimal effect on proteins, avoiding final neutralisation, and produces relatively non-toxic end products. Its rapid penetration of tissues allows safer and more homogenous tissue grafts of arteries, bone, and cartilage. For example, blood fractions may be sterilised with 0.25% BPL and UV light for about 1 h at a pH of ~7.2.

The effectiveness of BPL as sterilant for liquid microbiological media was studied. Preliminary tests against heavy suspensions of *Bacillus coagulans* in various media confirmed the sterilising levels found by earlier investigators. In actinomycin fermentation tests, BPL was found to sterilise effectively at concentrations up to 0.5% without significant effects on the growth-promoting qualities of the medium. But at the higher concentrations required to sterilise large numbers of spores (0.5–1.0%) however, inhibitory effects were seen on the fermentation. Interesting BPL was less damaging than EO in this sterilisation experiment.

It is not used extensively because of its carcinogenicity, and other physiologically undesirable properties. The BPL molecule remains stable only in the absence of water. In this state it is a caustic vesicant and is capable of producing chemical burns in the tissue in which it is placed, however, its β-hydroxylpropionic acid hydrolysis by-product, is not carcinogenic and does not have its undesirable physiological and

toxic properties. It is one of the most rapidly sporicidal agents. It has been used to sterilise biological material without toxic or allergic reactions in a liquid 'aqueous' state, and there are no toxic residuals. It is used as an attenuating agent for vaccines.

BPL has been used extensively to inactivate viruses for both human and veterinary vaccine production. Human vaccines against influenza, and rabies have been successfully developed and safely administered using BPL-inactivated forms of whole viruses associated with each disease. Experimental candidates for poliomyelitis, severe acute respiratory syndrome, and human immunodeficiency virus (HIV) have additionally been produced with this inactivation technology and are currently being evaluated in animal models and human clinical trials. Although effective inactivation processes have been devised for each of these vaccines, the scale at which the BPL inactivation process has been performed remains a limiting factor. It is a novel operation yielding complete viral inactivation while retaining antigenicity and immunogenicity of the viral proteins. Additionally it has been used to sterilise blood plasma, tissue grafts, surgical instruments, and enzymes. For example, 1% BPL has been shown to be capable of sterilising animal tissues which have been artificially infected with vegetative and spore-forming microbe before treatment.

Another example, biological cardiac valve substitutes have been sterilised at room temperature using BPL with a 0.5 vol% solution within 120 min, with a 1.0 vol% solution within 90 min, and with a 1.5-2 vol% solution within 60 min. The physico-chemical properties of the graft material could be influenced by an appropriately chosen concentration and sterilising period. Enlarged procedures for decontamination were not necessary at the time, because of the rapid dissolution of aqueous BPL solutions. *Note:* Both BPL and PAA are sporicidal at low temperatures, with PAA the more active. The temperature coefficients of the two chemicals are generally low over a range of 20 to -20 °C, but increase significantly at temperatures below this. Results showed an initial lag in the PAA death rates that was directly dependent on the temperature. BPL did not show this lag time.

2.6 *Ortho*-phthalaldehyde

OPA is another dialdehyde. It has a high-level disinfectant status, which means it kills *Mycobacterium* as well as some spores. It received FDA clearance in October 1999. It contains ~0.55% 1,2-benzenedicarboxaldehyde. The OPA solution is a clear, pale-blue liquid with a pH of 7.5.

2.6.1 Microbial Characteristics

Primary studies on the mode of action of OPA suggest that both OPA and glutaraldehyde interact with amino acids, proteins, and micro-organisms. Unlike glutaraldehyde, OPA is a less potent crosslinking agent. This is compensated for by the lipophilic aromatic nature of OPA that is likely to assist its uptake through the outer layers of *Mycobacteria* and Gram-negative bacteria. OPA appears to kill spores by blocking the spore germination process.

Studies have demonstrated the excellent microbicidal activity of OPA *in vitro*. For example, OPA has superior mycobactericidal activity (5-$\log_{10}$ reduction in 5 min) to glutaraldehyde. The mean times required to produce a 6-$\log_{10}$ reduction for *M. bovis* using 0.21% OPA was 6 min, compared with 32 min using 1.5% glutaraldehyde. OPA showed good activity against the *Mycobacteria* tested, including the glutaraldehyde-resistant strains, but 0.5% OPA was not sporicidal with 270 min of exposure. However, increasing the pH from its unadjusted level (about 6.5) to pH 8 improved the sporicidal activity of OPA. The level of biocidal activity was directly related to the temperature. A greater than 5-$\log_{10}$ reduction of *B. atrophaeus* spores was observed in 3 h at 35 °C, rather than in 24 h at 20 °C. Also, with an exposure time <5 min, biocidal activity decreased with increasing serum concentration. However, efficacy did not differ when the exposure time was >10 min. In addition, OPA is effective (>5-$\log_{10}$ reduction) against a wide range of micro-organisms, including glutaraldehyde-resistant *Mycobacteria* and *B. atrophaeus* spores.

The influence of laboratory adaptation of test strains, such as *Pseudomonas aeruginosa*, to 0.55% OPA has been evaluated. Resistant and multi-resistant strains increased substantially in susceptibility to OPA after laboratory adaptation ($\log_{10}$ reduction factors increased by 0.54 and 0.91 for resistant and multi-resistant strains, respectively). Other studies have found naturally occurring cells of *P. aeurginosa* were more resistant to a variety of disinfectants than were sub-cultured cells.

2.6.2 Advantages

OPA has several potential advantages over glutaraldehyde. It has excellent stability over a wide pH range (pH 3–9), is not a known irritant to the eyes and nasal passages, does not require exposure monitoring, has a barely perceptible odour, and requires no activation. OPA, like glutaraldehyde, has excellent material compatibility. It has shown superior mycobactericidal activity compared to glutaraldehyde with less contact time.

2.6.3 Disadvantages

Like other liquid sterilants it is typically used without sterile packaging, and requires aseptic technique to handle products or items it treats.

One possible disadvantage of OPA is that it stains proteins gray (including unprotected skin) and so must be handled with caution. However, skin staining would indicate improper handling that requires additional training and/or personal protective equipment (e.g., gloves, eye and mouth protection, and fluid-resistant gowns). OPA residues remaining on inadequately water-rinsed transoesophageal echocardiogram probes can stain the patient's mouth. Meticulous cleaning, using the correct OPA exposure time (e.g., 12 min) and copious rinsing of the probe with water should eliminate this problem. The results of one study provided a basis for a recommendation that rinsing of instruments disinfected with OPA will require at least 250 ml of water per channel to reduce the chemical residue to a level that will not compromise patient or staff safety (<1 ppm). Personal protective equipment should be worn when contaminated instruments, equipment, and chemicals are handled. In addition, equipment must be thoroughly rinsed to prevent discoloration of a patient's skin or mucous membranes. In April 2004, the manufacturer of OPA disseminated information to users about patients who reportedly experienced an anaphylaxis-like reaction after cystoscopy where the scope had been reprocessed using OPA. Of approximately 1 million urological procedures performed using instruments reprocessed using OPA, 24 cases (17 cases in the United States, six in Japan, one in the United Kingdom) of anaphylaxis-like reactions have been reported after repeated cystoscopy (typically after four to nine treatments). Preventive measures include removal of OPA residues by thorough rinsing and not using OPA for reprocessing urological instrumentation used to treat patients with a history of bladder cancer. A few OPA clinical studies are available. In a clinical use study, OPA exposure of 100 endoscopes for 5 min resulted in a >5-$\log_{10}$ reduction in bacterial load. Furthermore, OPA was effective over a 14 day use cycle of 100 endoscopes. Manufacturer data shows that OPA will last longer in an automatic endoscope reprocessor before reaching its microbial effective concentration (MEC) limit (MEC after 82 cycles) than will glutaraldehyde (MEC after 40 cycles). High-pressure liquid chromatography confirmed that OPA levels are maintained above 0.3% for at least 50 cycles. OPA must be disposed of in accordance with local and state regulations. If OPA disposal through the sanitary sewer system is restricted, glycine (6.5 g/litre) can be used to neutralise the OPA and make it safe for disposal.

The high-level disinfectant label claims for OPA solution at 20 °C vary worldwide (e.g., 5 min in Europe, Asia, and Latin America, 10 min in Canada and Australia, and 12 min in the USA). These label claims differ worldwide as do the formulations, because of differences in the test methodology and requirements for licensing. In

an automated endoscope reprocessor with an FDA-cleared capability to maintain solution temperatures at 25 °C, the contact time for OPA is 5 min.

A typical 0.55% OPA formulation may sterilise in 32 h at 20 °C, and give high-level disinfection within 12 min at 20 °C, with a only a 14 day maximum re-use. There is a registered OPA concentration of 5.75% (with a 0.05% in use solution) that may high-level disinfect in 5 min at 50 °C; and sterilise at 32 h at 50 °C.

There is little human toxicological research on the health effects of OPA. It is 6,500 times more toxic to aquatic life than gluataraldehyde requiring neutralisation with glycin before disposal to the drains. There have been allergic reactions to patients from instruments cleaned with OPA. It is a potent skin sensitiser. The state of California indicates on the label of one of the brands of OPA that is a hazardous waste. It requires neutralisation with glycine. There is no established air monitoring method or exposure limit. A product safety alert cautions against use for cystoscopes in patients with bladder carcinoma. Although it is not classed as a hazardous chemical typically, it would be prudent to handle it with the same precautions as glutaraldehyde.

2.7 Liquid Hydrogen Peroxide

In contrast to unformulated hydrogen peroxide (H_2O_2), which may have some weak and slow microbicidal activity at a concentration of 3%, stabilised and accelerated formulations have very good bactericidal activity with *Mycobacteria*, and good fungicidal and virucidal activity, however they still may only act slowly against bacterial spores. A H_2O_2 concentration of 7.5% can sterilise in 6 h at 20 °C. Although the FDA has approved products containing 7.5% H_2O_2 as a high-level disinfectant/sterilant, it has not been found to be compatible with most flexible gastrointestinal endoscopes.

These preparations are slight irritants but are not allergenic and are stable on storage. They may exist in various forms: stabilised and 'accelerated' with appropriate detergents and acids, alkalis, or combined with PAA. They may also be useful for endoscope surfaces and for hygienic hand washes.

H_2O_2 can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets, and stronger solutions may be suitable for disinfecting heat-sensitive medical/dental devices. The use of vapourised H_2O_2 or PAA for the decontamination of heat-sensitive medical/surgical devices requires specialised equipment. H_2O_2 and peracids can be corrosive to metals such as aluminum, brass, copper and zinc, and can also decolorise fabrics, hair, skin and mucous membranes. Articles treated with them must be thoroughly rinsed before contact with eyes and mucous membranes.

H_2O_2 is safer than chlorine to humans and the environment. H_2O_2 is provided either as a ready-to use 3% solution or as a 30% aqueous solution to be diluted to 5–10 times its volume with sterilised water. However, such 3–6% solutions of H_2O_2 alone are relatively slow and limited as germicides. Products now available have other ingredients to stabilise the H_2O_2 content, to accelerate its germicidal action and to make it less corrosive.

Accelerated H_2O_2 (AHP) is a synergistic blend of commonly used, safe ingredients that when combined with low levels of H_2O_2 produce exceptional potency as a germicide and performance as a cleaner.

AHP is composed of H_2O_2, surface acting agents (surfactants), wetting agents (a substance that reduces the surface tension of a liquid, causing the liquid to spread across or penetrate more easily the surface of a solid) and chelating agents (a substance that helps to reduce metal content and/or hardness of water). The ingredients are all listed on the EPA and Health Canada Inert lists and the FDA Generally Regarded as Safe List. All chemicals used in the formulation of AHP are commonly found in commercial and industrial cleaners and disinfectants.

Among some of the AHP products are:

- Virox™ - acidic (pH 1.3).
- Hvèzda SCH™ - Alkali (pH 12.5), preparation contains 20% stabilised H_2O_2.
- Metrex Compliance™ - combined with PAA (7.35% H_2O_2 + 0.23% PAA).
- Cidex PATM - 1% H_2O_2 + 0.08% PAA.

These combinations can be corrosive.

A formulation of 7.5% H_2O_2 with 0.23% PAA can sterilise in 180 min, and high-level disinfect in 15 min at 20 °C. It only has a 14 day maximum re-use.

A formulation of 8.3% H_2O_2 with 7.0% PAA can sterilise in 5 h, but high-level disinfect in only 5 min, but there is only a five day maximum re-use.

The accelerated H_2O_2 product referred to previously (Virox), is a colourless and odourless solution, with a pH of 1.3. It contains H_2O_2 at a final concentration of 7%, food grade acids and detergents. The combination of these ingredients also makes it non-corrosive. All its components meet the requirements for food contact surfaces. Its germicidal action is believed to be based on its activity as a strong oxidising agent. For testing its bactericidal and virucidal activities, a 1:16 dilution of the product was prepared by adding one part of it to 15 parts of water with a standardised level of

hardness. The dilution was prepared immediately prior to each test and the diluted product was used only once. In certain cases, the product was tested with an anti-foam to determine if this could, in any way, interfere with its germicidal activity.

2.8 Alternative or Novel Liquid Sterilants

Other opportunities to replace traditional glutaraldehyde may include OPA, buffered PAA, high percentages of H_2O_2, O_3 and performic acid, have also been considered [9].

While liquid EO in water or methanol slowly converts, over several months, to ethylene glycol and/or ethylene glycol monomethyl ether, and was less corrosive to metals, than formaldehyde [2], the use of propylene oxide would have been an improvement but it has different solvent properties and toxicity to those of EO, and could self-sterilise the liquids they are within, such as isopropyl alcohol.

A 5% solution of freshly distilled monomeric formaldehyde in methanol is also a useful sterilant and is compatible with a large variety of materials. In methanol the formaldehyde is less corrosive and when freshly prepared leaves no deposit of paraformaldehyde, and this is an important consideration in spacecraft applications [2].

Other newer (oxidising) possibilities exist such as monopercitric acid, and super-oxidised water.

Monopercitric acid is a new peroxygen compound that is virucidal within 0.5-1 min at 0.5% against Poliovirus and at 0.1% against adenovirus. In qualitative suspension tests it has been shown to be sporicidal at 1%. It is not known if it is an irritant or if it is stable.

Super-oxidised water is a novel approach to disinfection where the disinfectant is produced in or near the location where it will be used. It is sporicidal, but only moderate against *Mycobacterium* and non-enveloped viruses. Users can buy or rent the production machine. The disinfectant is produced from a sodium chloride solution by electrolysis and contains a variety of oxidising agents, mainly hypochlorous acid at low pH (2.3-6.5), and has high redox potential (>950 mV). There are various electrolysis systems such as Super Oxseed alpha 1000™ (Janix Inc., Japan) producing a pH of 2.3-2.7 or the Sterilox 2500™ (Sterilox Medical Ltd., USA) producing a pH of 5.0-6.5.

It is microbiocidal against all forms of micro-organisms with short application times (0.5-10 min), however, depending on the equipment used, the age since production of

the super-oxidised solution is important. It should be used shortly after production.

Super-oxidised water is neither toxic nor harmful for tissue and skin but may damage certain instrument surface materials. It is inactivated by organic matter and not stable during storage. It can be used for instrument disinfection, particularly in endoscope washer-disinfectors.

Another interesting sterilant is performic acid. Performic acid is a quick-acting sporicide that was incorporated into an automated endoscope reprocessing system. The system's using performic acid was never FDA cleared.

2.9 Summary

In summary, an ounce of prevention is worth a pound of cure. Without heat sterilisation, infectious diseases would exist everywhere in a hospital or in healthcare facilities and elsewhere. It is debatable that antibiotics could ever control an onslaught of infections everywhere without the concurrent practice of sterilisation. In review, heat sterilisation methods have tremendous value to medicine, healthcare, control of infections (including blood borne diseases) and this will continue even more in the future.

2.10 Recommendation(s)

For the reasons stated in the introduction of this chapter, the FDA recommends that the use of liquid chemical sterilants be limited to reprocessing only critical devices that are heat-sensitive and incompatible with traditional sterilisation methods such as EO, steam or irradiation. Some exceptions to this may exist such as the initial sterilisation of tissues of animal origin and enzymes. In addition to any two part systems for liquid chemical sterilants, it is essential to perform thorough cleaning before subjecting any device or product to high-level disinfection and sterilisation because inorganic and organic materials that remains on the surfaces of instruments, interferes with the effectiveness of these processes. **Table 2.2** shows the differences between the types of liquid chemical sterilants.

Cleaning or *decontamination* removes pathogenic micro-organisms from objects so that they are safe to handle, use, or discard, prior to sterilisation. Unlike other liquid sterilants, BPL does not have to have a liquid two stage sterilisation process, but will degrade into a non-toxic by-product, through hydrolysis. BPL has been accepted by the FDA to attenuate viruses to create vaccines.

Do no harm -which liquid sterilant does the least harm and is most effective? BPL is not actually effective against prions.

Table 2.2 Some comparisons of several liquid chemical sterilants

Agent	**Microbial activity**							
	Bacteria			**Viruses**		**Critical characteristics**		
	Vegetative	Spore	*Mycobacterium*	Envelope	No envelope	Interact with organic matter	Irritant	Corrosive and stable
Glutaraldehyde (2%)	++	Slow	+	++	++	No	Irr, A	+
OPA (0.55%)	++	-***	++	++	++	No	Irr, slightly	++
PAA (0.2%)	++	++	++	++	++	Slighty	Irr	-
ClO_2, Cl_2 releasers	++	++	++	++	++	Yes	Irr, mixed[a]	Mixed
Iodophore (1%)*	+ to ++	Slow	++	++	++	Slightly[c]	Very slight but can be A	+ to ++
BPL**	++	++	++	++	++	No	Irr, slightly	Mixed[b]
Accelerate hydrogen peroxide	++	Slow	++	++	++	No	Irr, slightly	++

++ : Very good
+ : Moderate
- : Poor
Irr: Irritant
A: Allergenic
*: Stains protein
**: Carcinogenic
***: There are 0.55% concentrations that will sterilise in 32 h
[a]: Chlorine dioxide is typically less corrosive and more stable than chlorine, hypochlorus or hypochlorite
[b]: BPL can polymerise and hydrolyse with moisture
[c]: Iodophors can stain, but their stains are removable to certain extent
Stability: Stability of in-use sol
Irritant: Possible irritant and allergenic
Corrosive: Corrosive damage

Every liquid chemical sterilant must be what its own qualities determine. An ideal liquid chemical high-level disinfectant or sterilant should have the following qualities:

- It should have a wide spectrum of antibacterial activity, including against spores. It should be tuberculocidal, and effective against hepatitis B, and HIV.
- It should be quick acting, as should the associated activities (e.g., pre cleaning).
- It should be effective in the presence of bioburden, organic matter and debris.
- It should be compatible with soaps and other chemicals.
- It should be compatible with products and materials, and non-corrosive. It should not corrode instruments nor cause deterioration of rubber, plastics, metals or other construction materials such as elastomers.
- It should be non-staining.
- It should have a low residual effect.
- It should be odourless or have a good fragrance.
- It should be registered with the appropriate regulatory body (e.g., EPA, FDA and so on) and have been sufficiently tested and evaluated.
- It should have ease of use – it should not require a lot of training to use it.
- It should have a prolonged re-use life - it should be able to be used repeatably over an extended period of time.
- It should have unrestricted disposal. It should have no requirements for special disposal (e.g., requirement for collection or neutralisation prior to disposal).
- It should be cost effective and economical. It should have a reasonable cost per cycle or process. Associated equipment should be economical to use.

Unfortunately the ideal high-level disinfectant or sterilant does not exist as all products have limitations. For example, despite all the above high-level disinfection and sterilants: the ECRI institute (formally the Emergency Care Research Institute) has listed cross contamination from flexible endoscopes as the fourth most common health technology hazard in 2012!

Is it possible that those high-level disinfectants as well as liquid sterilants approved by the FDA and EPA are not sufficient or adequate for the job. There appears to be an excuse that products are not cleaned enough, and these 'present' agents are not strong enough (high enough concentration) to get through organic or inorganic matter that may protect them. It appears something stronger and longer lasting is needed.

Oxidising agents such as PAA and chlorine-based agents are generally better biocides

than the alkylating agents but have a poorer materials' compatibility. Alkylating agents such as glutaraldehyde, OPA, and formaldehyde are generally not as good a biocide as are the oxidising agents but they have a better materials' compatibility.

The choice of specific liquid sterilant or high-level disinfectant or sterilant is largely a matter of judgment, guided by product label claims and instructions and government regulations. A single liquid chemical sterilant might not satisfy all disinfection requirements in a given situation. In some cases, manufacturers have not yet approved the use of EO alternatives for sterilisation of their products, components or accessories. Such limitations may vary by vendor and are not specific to one instrument or medical device product type. For example, where alternatives have been investigated for EO, but may still requires the use of EO on several instruments such as angioscopes, choledocoscopes, surgiscopes, and hysterectoscopes.

Under any circumstances, when alternative sterilants and high-level disinfectants are considered for a medical device, there should be a check with the original equipment manufacturer or possible supplier(s) for any specific warranty restrictions on the use of specific materials, polymers or methods of high-level disinfection or sterilisation.

Realistic use of liquid chemical sterilants depends on consideration of multiple factors, including the degree of microbial killing required, the nature and composition of the surface, item, or device to be treated, and the cost, safety, ease of use of the available agents, and product/material compatibility. Does the sterilant have to be activated? Is the correct concentration used? Will the sterilant reach its target microbes? Has the sterilant not been brought in contact with all surfaces? Sterilants should be chosen that have passed all appropriate tests, standards and reproducible procedures. Selecting one appropriate product with a higher degree of potency to cover all situations might be more convenient.

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3 Aseptic and Filtration Sterilisation

Trying to be perfect is where synchronicity of sterilisation magically appears. Aseptic processing is not a mystery. It is the disseminating wand of where environmental control and terminal sterilisation methods come together.

Aseptic and filtration processes are not typically considered to be terminal sterilisation processes but elimination or removal of most microbes with a limited guarantee of sterility: however, they are considered as classical and traditional methods and are briefly discussed here.

These methods of sterilisation are not frequently discussed because they are not the most preferred method of sterilisation. In general, it is preferred to terminally sterilise products in their final configuration and packaging, in order to minimise the risk of microbial contamination. Products designed for aseptic and filtration processing may consist of components or substances that have been previously sterilised by one of the previously discussed terminal sterilisation methods (see ***Healthcare Sterilisation: Introduction and Standard Practices, Volume 1,*** **Chapters 6**, 7, **8**, and **Chapters 1** and **2** of this volume). So ultimately, one must not only know how to aseptically assemble, process, or filter a final product, but also know other associated terminal sterilisation methods and techniques, because if any of the latter terminal methods fail to sterilise, aseptic assembly, processing, and final fill will subsequently fail.

3.1 Aseptic Assembly

This is a means of putting together sterilised parts, components, products or packaging under a strict controlled environment. It is process that requires perfection. It typically requires personnel to wear sterile outfits (e.g., gowns, bunny suits, gloves and so on) and/or the use of isolation hoods and laminar flow benches. It uses high efficiency particulate air (HEPA) filtration, microbial filtration of liquids and gases when applicable, and self-sterilising items. It provides of a means assembling products that cannot be terminally sterilised as a finished product in a package. Aseptic processes can be a very complicated, using a combination of sterilised parts and packages or sterilisation-in-place (SIP) methodology (e.g., infrared tunnels), and

filtration to remove microbes from the environment, and then the parts are assembled aseptically and finally packaged. Aseptic processing can incorporate other methods of terminal sterilisation such as liquid sterilisation, dry heat, ethylene oxide (EO), irradiation, and steam sterilisation. Germicides such as liquid alcohol chlorine dioxide, glutaraldehyde, hydrogen peroxide (H_2O_2), iodine, peracetic acid (PAA), phenol and/or even formaldehyde have been used. Ultraviolet (UV) light has been used as a surface decontamination agent, sterilising tubes, vials, and flasks for special surfaces for cell cultures.

3.1.1 Aseptic Assembly Requires Environmental Control

Aseptic assembly in health care must of necessity exert environmental control over microbes and items to be sterilised and to be kept sterile. Aseptic assembly inherently includes many of the applications discussed in ***Healthcare Sterilisation: Introduction and Standard Practices, Volume 1***, **Chapter 4**. *Note:* environmental control is not the same thing as environmental monitoring. Environmental control exists at all times, monitoring is only a snapshot of the control.

Microbes are found nearly everywhere - they are ubiquitous. They live in the air, in the water, in oil, in many liquids, in dust, in food, on animals, on plants, on clothing, on appliances, in ducts, in buildings and homes, on particles, on the ground and on surfaces. Even healthy and clean individuals have microbes. They are on skin, in the mouth, ears, nose, hair, nails and within the intestine, they are expelled from the lungs when we breathe or cough. To have environmental control must of necessity eliminate all of these microbes from these sources.

Humans are the potential largest source of contamination. For example:

- People virtually generate ‘millions’ of particles every hour from touching, breathing, coughing, talking, hair, skin, body movements, clothing and so on.
- There may be upwards of 1.2 million aerobic bacteria per m^2 on the surface of the head and neck region.
- There may be 0.9-3 million per m^2 on hands, arms, skin and much higher numbers of viable anaerobes (*Propionibacterium acnes* or similar species). This organism is possibly the best indication of environmental contamination. If you do not test for anaerobes, fastidiously, you will not know if contamination exists or not.
- A fully gowned person sitting in a cleanroom may release ~ 15,000 particles per minute.

- A walking person may release approximately 157,000 particles per min.
- The ratio of total particles >0.5 μm and viable aerobic organisms may be 600-7,000 to 1, if not filtered. This will vary, however, depending upon amount of dirt and source.
- Humans may release 600-1,300 total particles per hour in the >0.5 μm size range with ~40 colony forming units (CFU) viable aerobic organisms among these.
- However, a properly gowned, cleanroom worker may contribute 10-100 CFU of viable aerobic organisms to the environment per hour. With a worker with acne (anaerobic *Propionibacterium*), the CFU count may even go higher.

Therefore, the main considerations of environmental control are:

- Good training, which includes asepsis, cleaning and disinfection;
- Discipline;
- Microbiological or management overview; and
- Keeping the number of people in environmental areas under control.

Other sources of contamination are:

- *The atmosphere*: Air alone is not a natural environment for microbial growth, however, in the presence of moist air it may create a growth environment if nutrients are available, but if it's too dry (absence of moisture and water activity) it will desiccate some microbes, and organisms such as *Bacillus, Clostridium, Staphylococcus, Micrococcus, Streptococcus, Penicillin* and *Aspergillus* may survive, while others will die. The degree of contamination depends on particle level, dirt, water and nutritional source. Hydrocarbons, for example, in the air will help some microbes to flourish.
- *Buildings, structures, containments*: There is the potential for mould contamination. Nutrients come from plaster, and cracks and inadequate sealing can be a cause for concern.
- *Water*: One must always be concerned about the presence of water where there are microbes like *Pseudomonas* that may simply grow in natural water.
- *Raw materials*: For raw materials one must be concerned about natural sources (e.g., corn starch and so on) which may have large numbers of microbes, but also artificial materials (e.g., medical devices) that are washed with water or manufactured under unclean situations may be highly contaminated with microbes, particulates and pyrogens.

- *Packaging*: May have mould spores, especially if there are any paper or organic sources.

Controlling the types of microbes to be sterilised may be one of the most significant considerations in many methods of sterilisation of healthcare products. Many methods have challenging microbes (e.g., *Pyronema domesticum* on cotton is a mould which is extremely resistant to EO and irradiation. Some viruses (e.g., picovirus such as porcine circovirus) are such small targets that they can survive irradiation. H_2O_2 does not have the same penetrating capability as irradiation, steam or EO through some medical devices. Consequently the agent cannot reach the microbes contaminating hidden, tight, shielded, tortuous, or matching surfaces. (*Note:* matching surfaces that rub together may generate particles). Pre-cleaning or cleaning prior to decontamination for controlling the microbes from contamination can be significant. Germ free spacecraft assembly applied this pre-cleaning step with a significant reduction of decontamination.

Environmental control is not a single cause but the combination of a variety of numerous factors that bring about the control. The life of a microbe is typically a continuing flow of chemical reactions among molecules of confounding complexities. Microbiologists have found that their dormant spores, reproductive, vegetative, ionic and enzymic state can determine their survival and reaction rate. So, anything that interferes with the continuing reactions or with the molecular complexity can limit, prevent or kill a microbe. Though the laws of physics (barriers and intensity), and chemistry (reactions) may predominate in environmental control, the biologist has found another way (the microbial influence and state) that shows that the microbe's activity, dormancy, reproductive state, water activity, ionic and enzyme states determines their survival, growth and reproductive rate. A variety of factors must always be considered in environmental control and one need to recognise what is a microbe, where it is, and what makes it resistant.

3.1.2 Environmental Precautions

It is incumbent upon hospital, healthcare facilities, and manufacturers to exercise precautions. The control of sterilisation begins with procedures, primary stages of preparation (cleaning, decontamination) and with basic materials of use. Cleaning and cleanliness is a primary consideration for sterilising items and products. Instead of *cleanliness is next to godliness*, in this situation, *cleanliness is next to sterilisation, is prior to sterilisation.* The degree of contamination is critical because of the numbers and types of microbes and their environmental state [their age and transformation (spore)], protection (barrier to sterility), occlusion, desiccation, encrustation with dirt salt, or other organic matter (e.g., blood and faeces). A

variety of factors may influence the ability to exert control over the environment and its micro-organisms.

Factors to consider and control are:

- Utilise a microbiologist whenever possible.
- Maintain cleanliness.
- Minimise use of or eliminate water, because microbes require water to grow. Water or water vapour is necessary for the continued growth of microbes. Humidity control to prevent condensation and high water activity is used in industrial pre-storage of devices for some eventual sterilisation, but not so dry as to create resistance.
- Control material and people entering an environmentally controlled area.
- Use transfer – pass through for an environmentally controlled area.
- Use HEPA filters, laminar air flow control, controlled (clean room) clothing.
- Perform sanitisation on clean environmental surfaces, medical equipment surfaces, and housekeeping surfaces such as table-tops, walls, sinks and floors. Fumigation of the air and the environment may be necessary.
- Update materials and preventative maintenance on equipment. Make surfaces of materials and design that are easy to clean and maintain, and do not rub together.
- Calibrate measuring equipment.
- Have materials coming into an environmental area in packages, double pouched if possible, and remove upon transfer the package and wipe off material or remaining package as it comes out.
- Perform microbiological and non-viable (particulate) testing.
- Establish microbial limits and types.
- Establish environmental control for viable and non-viable organisms.
- Establish environmental limits (e.g., temperature, humidity, and materials). Rate of growth of microbes is influenced by both temperature and humidity. Low humidities and temperatures will keep people from shedding more microbes. Do not allow materials that may be suitable for growth of undesirable microbes. Keep out food and wastes.

- Personal hygiene is essential. Hygiene is a critical part of cleanliness (e.g., provide clean clothing to workers, keep out sick workers, and provide washing facilities and enforce their use.

3.1.3 Controlled Clean Room Areas

When a product is incorporated into manufacturing, the control, lethality and statistics of sterilisation begins with exertion of control of the manufacturing environment (**Table 3.1**) and to minimise and to control micro-organisms and bioburden on the incoming materials or components or products through production. This may require cleaning or other pre-treatment steps. In some cases, manufacturers will wipe down devices and components with isopropyl alcohol (IPA) or other agents (e.g., H_2O_2 and so on), before packaging and sterilisation. Care must be taken that the IPA does not become contaminated with spores through use and reuse of the containers or IPA, because alcohol does not kill spores.

Table 3.1 Airborne particulate cleanliness classes*

Class name		**Class limits**	
		Particles equal to and larger than 0.5 µm	
		Volume units	
SI	**English (US customary) units****	**(m^3)**	**(ft^3)**
M1	—	10.0	0.283
M1.5	1	35.3	1.00
M2	—	100	2.8
M2.5	10	353	10.0
M3	—	1,000	28.3
M3.5	100	3,530	100
M4	—	10,000	283
M4.5	1,000	35,300	1,000
M5	—	100,000	2,830
M5.5	10,000	353,000	10,000
M6		1,000,000	28,300
M6.5	100,000	3,530,000	100,000
M7	—	10,000,000	283,000

*Adapted from the US Federal Standard (US FED STD) 209E - *Airborne Particulate Cleanliness Classes in Clean Rooms and Clean Zones*, 1992.
** For naming and describing the classes, SI names and units are preferred, however, English (US customary) units may be used.
US FED STD 209E was officially cancelled by the General Services Administration of the US Department of Commerce on November 29, 2001, but may still be used.
ISO 14644-1, *Cleanrooms and Associated Controlled Environments – Part 1: Classification of Air Cleanliness*, 1999, is the replacement for US FED STD 209E.

Other aspects of control of sterilisation are through control of the equipment, product and process, so that repeatable sterilisation can be achieved. This will include a cleaning procedure and schedule of the controlled environmental area.

Cleaning and disinfection can be confusing at times. For example, 70% IPA may be used as a cleaning agent as well as a disinfectant. Cleaning is important because:

- Cleaning typically uses a detergent before disinfection.
- Detergents are typically cleaning agents and are used to remove soiling (such as dirt, dust and/or grease) from a surface.
- Detergents typically work by penetrating soiled areas and reducing the surface tension to allow for its removal.
- The removal of soiling or dirt is important prior to disinfection because it will subsequently allow the disinfectant to penetrate.

Typically the cleaning agent is a detergent. Important factors to consider when selecting the appropriate cleaning agent are:

- Cleaning agents must be compatible with each other.
- The detergent typically should be a neutral and non-ionic solution.
- The detergent should be non-foaming.
- The detergent or its residuals should be compatible with the disinfectant.

When cleaning, use appropriate cleaning techniques such as:

- Sweeping, wiping or mopping.
- Ensure that the detergent has dried.
- Remove disinfectant residues by wiping with water or 70% IPA.
- Detergents used on a surface may be applied using the double or triple bucket system to avoid cross contamination. Buckets should be autoclaved.
- Clean from top to bottom, from the cleanest area to the dirtiest.
- Know the formulation of the materials to be used for cleaning and the subsequent disinfectant, to check for compatibility, and that they are not particulate shedding.
- Cleaning may include use of IPA, a disinfectant as well as a non-toxic residual cleaning agent.

Disinfection typically occurs after cleaning. A disinfectant is typically a chemical and is used to reduce or eliminate a population of microbes. Typically a disinfectant will not eliminate all spores, which are resistant.

Understanding the type of disinfectant to be used is important:

- Know the difference between disinfectants such as those that will penetrate, or not evaporate before disinfection is complete.
- Know which ones will dissolve or penetrate organic matter or salt encrustation.
- Know which ones will kill spores and those that will not.
- Disinfection will be periodically performed with 70% aqueous IPA, a quaternary, phenolic, oxidising agents (e.g., PAA, H_2O_2, chlorine, chlorine dioxide, iodine), or other acceptable regulatory (e.g., Environmental Protection Agency), approved germicide.

Disinfectants that do not leave a residue or can be subsequently cleaned up are favoured. It is important to rotate cleaning and disinfectants (at least every two weeks) to minimise or prevent buildup (biofilm) of resistant microbes. For example *Burkholderia cepacia* has been found to be 'viable' in iodophore solutions. Some iodophores may inactivate low levels of spores, while alcohols will not. Residuals of iodine and phenols may remain, while H_2O_2 will degrade quickly, and alcohol will evaporate rapidly from hard surfaces.

Selecting the appropriate disinfectant agents is important:

- Consider using two disinfectants in rotation. One of the agents could be sporicidal.
- The disinfectants considered should have a wide spectrum of microbial activity, and be capable of inactivating non-sporulating microbes and possibly *Mycobacterium*.
- The disinfectant should have a rapid activity - the speed will depend upon contact, concentration and so on.
- Residuals from detergents should not interfere with the disinfectant.
- Disinfectants used in high grade cleanrooms (see International Organization of Standardization (ISO) 14644 classes 5 and 7 [1]) must be clean and sterile (e.g., no contaminants or spores).
- Disinfectants must be used at the temperature of the specific clean room.
- The surface must be kept wet with the disinfectant until the contact time has elapsed.

- The disinfectant should not damage the materials that it comes in contact with. For example, chlorine will oxidise or damage materials such as iron or stainless steel.
- The Disinfectant should be safe for operators as well as for the product being manufactured in a controlled environment.
- Selected disinfectants should be always available, cost effective and easy to handle in the form to be used (e.g., ready to use, diluted concentrates, or trigger spray bottles and so on).
- Know what factors will affect a disinfectant's efficacy such as concentration, time, the numbers and types of microbes to be inactivated, temperature and pH criteria. The disinfectant must have been tested prior to use by an appropriate regulatory body or by controlled testing and validation.

Periodically the cleanliness of the area is monitored by taking particle readings to see that the HEPA filters are working. Microbial surface and air monitoring is also performed and may be described as 'target, alert or action levels':

- Alert level is the quantity of viable environmental organisms, which when exceeded signals a potential drift from predetermined operating conditions. An assessment and corrective action will address potential deteriorating bioburden and/or environmental conditions.
- Action level is an environmental microbial level, which is reached and an action must be taken. For example when microbial levels (e.g., bioburden or environment) exceed predetermined and specified level, an action must be taken. When an investigation exceeded is triggered, a corrective action is determined based upon the investigation.

Each facility or company, have individual situations and levels must consider variables specific to each location. Depending upon the assessment or investigation, levels will be interpreted and reviewed. Frequently an alert level will be the average number plus two standard deviations, and an action level is frequently the average number plus three standard deviations. Because statistics can be misleading at times, a level from a standard may be applied as a benchmark. Some observations typically made in a controlled environment or controlled cleanrooms are given next.

Environmental control, gowning, cleaning, pre-sterilisation and related procedures, should be revised as needed:

- Include a list of materials and equipment needed, and standards as needed.

- All materials and equipment needed should have a part number.
- Add gloves to the gowning procedure.
- Keep procedures updated - have and maintain a manufacturing change control system.
- Employees must communicate any possible contaminating or contagious conditions, sickness and blood injuries to the supervisor of the area.
- Coming into a controlled environment, personnel must go through a procedure for gowning: don garments from head to foot: cap, beard or face cover, gown, shoe covers. Place shoe cover on one at time, cover first foot and place in controlled area, and then cover the second foot and place it in the controlled area, and then continue as follows: wash and scrub hands and forearms and allow to dry, put sterile or clean gloves on last, after washing hands.
- Training is critical to all employees in the controlled or clean area.
- Sampling is to be done by a trained microbiologist or employee. Results must be reviewed by a qualified microbiologist or trained personnel.
- Minimise contamination or bioburden - by handling incoming material aseptically or whenever feasible by wiping or disinfecting (alcohol) components and so on.
- Controlling the manufacturing environment.
- Controlling any water and compressed air.
- Personnel training and controlling manufacturing process changes.
- Filter and/or rinse alcohol, disinfectant or any solvent containers between uses to minimise buildup of contamination or residues. Do not use alcohol or solvent containers after a certain period of time (manufacturing department to decide).
- Apply part numbers to items to be used, and specify items that will reduce contamination, (e.g., sterile gloves *versus* non-sterile gloves). Use part numbers for gowns, covers, gloves, hair nets, cleaning materials and other equipment.

Analyse trend data and assess the differences:

- Perform a running average on all data points.
- Look for differences.
- Assess significant differences between data points and averages.

- Periodically perform microbial identification or characterisation on predominant colonial type microbe(s), and when extremely unusual and high count levels occur.
- Trend analysis can be related to time, season, shift, facility area and so on.

Establish the validation of the controlled environmental area, and their action and alert levels:

- Calculate the average values and their standard deviations on the first six or more data points.
- Alert levels may be determined as average of +2 standard deviations, or by other statistical means.
- Action levels may be determined as average of +3 standard deviations, or by other appropriate means.
- Another useful level is the target level, which is the average +1 standard deviation.
- The microbiological levels should be benchmarked or compared to values indicated in the United States Pharmacopeia (USP) or ISO standards or other benchmarked levels as applicable.
- Ensure timely approval of environmental certifications and reports. Approve certifications and reports within a pre-determined period (or as established by the management, and the microbiologist), because delays can lead to high bioburden or microbial levels, uncleanliness and an out of control environment.
- Evaluate the level of particulates in the finished devices. Designate work areas as semi-critical where parts or surfaces can come in to contact with the circulatory or compromised tissues of the users. Perform particulate matter testing on any blood contacting device.

Some ways to improve and enhance cleaning and control are:

- Meet with cleaning representatives or trained microbiologists. Periodically perform a cleaning inspection, even qualify cleaning. Periodically review the environmental area and practices.
- Update procedures to indicate appropriate use and handling of the ‘cleaning or other’ agents.
- Store all primary cleaning agents, materials, and equipment within a storage cabinet.

- Primary agents, material, and equipment part numbers must match the cleaning procedure. There is always a tendency to use another agent without approval.
- Periodically, (e.g., weekly, monthly or quarterly), inspect the area for signs of gross contamination.
- Add an addendum to the procedure for the selection and preparation of cleaning agents.
- Periodically perform housecleaning of the controlled area, to remove non-work and non-critical items out of the work area zones.
- Put gowning and other instructional messages within view of the personnel. Place written placards at about 1.7 m above the floor, or easily accessible to the onlooker.

Revise the gowning procedure as appropriate for those working in a semi-critical area that may need to use clean gloves:

- Do not wear long or false nails with gloves.
- Wear 'clean' gloves when working in areas where device materials or surface can come in contact with blood. 'Clean' gloves can be sterile gloves.

Some miscellaneous observations on controlled environmental areas are:

- Handling of alcohol and containers often requires more control. Unfiltered alcohol, disinfectants and reused containers can accumulate spores, without inactivation.
- Environmental procedures, as well as other procedures, may be improved by including a description of materials and equipment that are needed to carry out the procedure and operation.
- Part numbers should be included for purchased useables, (e.g., gloves, shoe coverings and gowns).
- Where the gowning procedure did not have gloves included, the highest contamination can be from the hands.
- Often there is no inclusion that personnel who are ill, not just contagious, or *vice versa*, should not enter a controlled environmental area.
- What is a suitable disinfectant? This should be defined, e.g., be regulatory approved, which will require the level of disinfection, direction for use, stability and so on. Disinfectant must be evaluated by standardised tests and so on.

- Assess six or more environmental monitoring data points that may provide for a validation report of the facility's controlled environment. Less than six can lead to excessive volatility of data (e.g., bioburden) in a determination of variance.
- Perform accumulative averages and assessments which are useful for watching the trends from environmental monitoring.
- When one area has significantly higher microbial counts than another, try to determine why.
- Procedures should be improved with a list of working standards, e.g., ISO, American National Standards Institute, American Association of Medical Instrumentation (AAMI), or other applicable standards, as necessary.
- Evaluation of particulates on devices as well as in the air should be considered, particularly for areas of the device that enter the circulatory system. USP, ISO as well as other compendia have recommended 'particulate' limits.
- Written requirements for the gowning area, on a door or wall should be placed high enough and in areas and locations so that all may see them.
- Outward positive airflow should be apparent when opening the gowning room to the outside, or the gowning room to the inner controlled environment. Gowning room pressure should be positive to the outside area (uncontrolled area), and the inner core should be a controlled environment with a positive air pressure to a lesser controlled area (e.g., gowning areas).
- There should be a control or mechanism so that the gowning door and the controlled environment doors cannot be opened simultaneously.
- Everyone inside the clean room should wear gloves, when working in a semi-critical area, but often the procedure does not indicate this. Those coming in contact with the product should wear gloves, because contamination by hand can result in the highest bioburden.
- The goal of the clean room is to keep the bioburden as low as possible, and below 100 CFU and under control, because this level can influence the outcome of the overkill sterilisation validation approach.
- Gloves may be available within the gowning room, but they may not be sterile. Non-sterile gloves can be heavily contaminated, and if so they could be a primary source of product contamination. Therefore, the bioburden on the gloves purchased must be determined, to ascertain if the contamination is high, (e.g., greater than 100 CFU), or if the glove is clean (very little bioburden, such as less than 10 CFU).

- Have all the cleaning workers been trained for cleaning the gowning and clean rooms? Can they read English, or the language that the procedure is written in?
- Sometimes, no disinfectants or germicides are found within the cleaning cabinet. Always provide extra containers of disinfectants and germicides.
- The procedure may not tell the cleaning workers which disinfectant or germicide to use and for what location. Provide enough detail for the work areas involved.
- The procedure does not indicate how to prevent residual build up from specified germicides.
- Disinfectant or cleaning agents that were in the cabinet were not on the list of approved chemicals for cleaning. Periodically inspect the cabinet and verify that the cleaners are using the agents that have been specified.
- There were no part numbers or expiration dates for any of the useable gowning items – consequently these items may become inadequate and unclean over time.
- Employee bins can also collect dust. Often employees will clean areas they are asked to, but not their own areas.
- The water tap of the washing sink was dripping or water was pooled in the sink. The procedure did not include cleaning and wiping down the sinks after cleaning.
- Water is a source of contamination and can cause the growth of many types of bacteria, including Gram-negative organisms such as, *Pseudomonas* species. Minimisation of water presence or build up is essential to prevent contamination. Microbial growth is often an aquatic phenomenon.
- Check that magnehelic gauges were operating just above 0.05 cm of water, but less than 0.1 cm of water.
- Check for dust on the top of bulletin boards, and behind the soap dispenser.
- Certification of the clean room was specified – if it was not approved until later then if there were an irregularity, it could go unnoticed for some months.
- The solvent label may state non-chlorofluorocarbon (CFC) solvent, yet it contains dichlorofluroethane, a CFC. It is important to read labels.
- Pre-sterilisation procedure needs to be updated from old standard to new standard.
- Keep tape and quality control (QC) supplies, (e.g., special pens and special papers), away from work areas.
- Use of regular paper or fabric towels should prohibited

- Time should always be set aside by the QC and production personnel for just cleaning the area.
- Periodically check all the items in the clean room to be cleaned, to determine new items to be added and old items that need to be removed from the cleaning list.
- Instructions on walls need to be periodically reviewed.
- A list of employee and cleaners' telephone numbers needs to be available and updated.
- Masks and goggles may be appropriate during operations that can cause splashing.
- If there is possibility of blood borne injury, then a blood borne injury procedure should be implemented and put in place.

3.1.3.1 Training Personnel

There is a significant and immense need to select and train controlled environmental or cleanroom personnel. The personnel selected must have good habits, discipline, be willing to learn, and can be motivated. They must not have diseases, afflictions or conditions that may interfere with working in a clean, controlled environment (e.g., allergies, respiratory disease, skin disease, nervous conditions, mental conditions). Human skin shedding is one of the highest levels of microbial contamination in a controlled environment, particularly the anaerobe *Propionibacterium*.

When training personnel, speak clearly and loudly, talk at a reasonable speed, present information in a logical sequence, give examples, explain why activities are done, be consistent, use the same terms throughout, answer all questions honestly, avoid discrimination, and all must attend and be committed to the project. Prior to training, develop a company controlled environment document.

3.1.3.2 Practical Considerations - Biocontamination

Some practical considerations for control over microbes while working in clean rooms and/or controlled environments are:

- Obtain training and certification.
- Remove all make up before entering the changing room or controlled areas.
- Follow all gowning and de-gowning procedures.

- Perform normal work duties only at designated workstation areas.
- Use only approved cleanroom/environmental controlled tools or fixtures when working with parts.
- Carefully handle and place parts and components in appropriate fixtures and approved 'clean' containers.
- Use only approved and labelled clean containers for IPA, acetone or other solvents.
- Use only approved cleaning procedures to clean parts, tools and fixtures.
- Use only approved hair covers, face masks, beard or mustache covers, smocks and gloves.
- Wear face masks when handling products and/or near production work areas.
- Be alert to anything that may be contaminating. Pay attention to visible particles, hairs, metal debris, pink water stains, or oily residues. Report any contamination problems to the supervisor or lead immediately.

Some don'ts of cleanroom and controlled environments, are:

- No drinking, eating, or smoking.
- No brushing, combing, grooming of hair or facial hair.
- No scratching, or touching of uncovered areas.
- No cosmetics, hair styling products, perfume or cologne to be used.
- No exposed finger jewellery.
- No shorts, miniskirts and/or hats.
- No sitting on the workbench or on equipment.
- Do not touch parts, equipment tools or work area with bare hands.
- Do not touch bare skin with a gloved hand.
- No aerosol cans or use of spray bottles.
- No foam wipes, or foam swabs to be used unless required, for special cleaning and then only use them on items, which generate a low level of particulates.
- No horseplay.
- Do not pull down face mask to talk.

- Do not use or equipment surfaces as a workbench.
- Do not use unauthorised office supplies.
- Do not use unauthorised tools from the hardware or any store, unless authorised.
- Do not stack parts in multiple layers unless approved.
- Do not set tools on top of parts to be assembled or used.
- Do not use unauthorised solvents (e.g., Lysol, Windex, Freon and so on).
- Do not touch the surface of clean parts with a vacuum hose.
- Whenever possible, do not place parts on any bottom shelves (near the floor) or near trash cans.
- Do not use any nitrogen or an air gun that does not have a filter.
- Do not wash cleanroom gloves with tap water.
- Do not wear cleanroom garments outside the cleanroom.
- Do not use finger cots, cotton wood swabs, lens paper, or tap water to do a final clean.

The control, lethality and statistics of sterilisation begins with exertion of control over the manufacturing environment, to minimise and to control biocontamination from the environment that affects or potentially contaminates incoming materials or components or products through preparation or production. A sterilisation scientist never forgets the environment of the product from the beginning (design and/or development) to the end (final sterilisation and sterility).

A biocontamination control involves evaluation of microbes from cleanroom air, walls, floors, ceilings, products, process equipment, raw materials, process liquids and gases, furniture, storage containers, personal attire and protective clothing. Sampling frequency, site location, sample identification, culturing methods and evaluation criteria must be part of this formal system for biocontamination control.

3.2 Standards for Biocontainment

Standards for evaluation and controlling biocontamination exist and are useful and helpful.

3.2.1 ISO 14698-1, Cleanrooms and Associated Controlled Environments - Biocontamination Control - Part 1: General Principles and Methods, 2003 [2]

This ISO standard describes the principles and basic methodology for a formal system to assess and control biocontamination in cleanrooms. Good hygiene practices have become increasingly important in modern society. As we increase international trade in hygiene-sensitive products, there is a strong requirement for stable and safe products, particularly in the healthcare field.

To achieve this stability and safety requires good control of biocontamination in the design, specification, operation and control of cleanrooms and associated controlled environments.

ISO 14698-1 provides guidelines for establishing and maintaining a formal system to assess and control biocontamination in these special environments. It is not a general standard covering all aspects of biocontamination control. It is specific to cleanrooms and associated controlled environments.

A formal system of biocontamination control will assess and control factors that can affect the microbiological quality of a process or product. There are a number of formalised systems to achieve this, such as hazardous analysis critical control point, fault tree analysis, failure mode and effect analysis and others.

ISO 14698-1 is concerned only with a formal system to address microbiological hazards in cleanrooms. Such a system must have the means to identify potential hazards, determine the resultant likelihood of occurrence, designate risk zones, establish measures of prevention or control, establish control limits, establish monitoring and observation schedules, establish corrective actions, establish training procedures, and provide proper documentation.

A formal system requires a sampling procedure for the detection and monitoring of biocontamination in risk zones. Monitoring can include:

- Airborne - by use of nutrient agar plates, slit samples and electronic counters.
- Surfaces - by Rodac plates, swab rinse and so on.
- People including clothing, gloves and so on.
- Utilities such as water, compressed gases and clean steam.
- HEPA filters.

- Liquids.
- Monitoring should by location and time, duration of sampling, surface area, written procedures, frequencies and limits or levels.
- Environmental monitoring under aseptic conditions or operations is typically more involved, more frequent and in more locations than if the finished product will be terminally sterilised.
- Air and surface sampling should be taken under static conditions (no people, no work) as well as during active (actual working conditions).

Biocontainment may include the laundering of cleanroom textiles such as garments and wipes.

Target, alert and action levels must be determined for a given risk zone. Such levels will determine the required remediation effort. All of these impact on product quality. A formal programme should be established.

ISO-14698-1 provides a foundation for developing a formal system for biocontamination control in cleanrooms. It provides detailed guidance on how to measure airborne biocontamination, how to validate air samples and how to measure biocontamination of surfaces, liquids and textiles used in cleanrooms. It also provides guidance for validating laundering processes and how to provide proper personnel training.

According to the US Food & Drugs Administration (FDA) the following personnel training, qualification and monitoring should be performed:

- 21 Code of Federal Regulations (CFR) 211.22(a) [3] states that 'There shall be a quality control unit that shall have the responsibility and authority to approve or reject all components, drug product containers, closures, in-process materials, packaging material, labeling, and drug products, and the authority to review production records to assure that no errors have occurred or, if errors have occurred, that they have been fully investigated. The quality control unit shall be responsible for approving or rejecting drug products manufactured, processed, packed, or held under contract by another company.'
- 21 CFR 211.22(c) [3] states that 'The quality control unit shall have the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product.'
- 21 CFR 211.25(a) [4] states that 'Each person engaged in the manufacture, processing, packing, or holding of a drug product shall have education, training,

and experience, or any combination thereof, to enable that person to perform the assigned functions. Training shall be in the particular operations that the employee performs and in current good manufacturing practice (CGMP; including the CGMP regulations in this chapter and written procedures required by these regulations) as they relate to the employee's functions. Training in CGMP shall be conducted by qualified individuals on a continuing basis and with sufficient frequency to assure that employees remain familiar with CGMP requirements applicable to them.'

- 21 CFR 211.25(b) [4] states that 'Each person responsible for supervising the manufacture, processing, packing, or holding of a drug product shall have the education, training, and experience, or any combination thereof, to perform assigned functions in such a manner as to provide assurance that the drug product has the safety, identity, strength, quality, and purity that it purports or is represented to possess.'

- 21 CFR 211.25(c) [4] states that 'There shall be an adequate number of qualified personnel to perform and supervise the manufacture, processing, packing, or holding of each drug product.'

- 21 CFR 211.28(a) [5] states that 'Personnel engaged in the manufacture, processing, packing, or holding of a drug product shall wear clean clothing appropriate for the duties they perform. Protective apparel, such as head, face, hand, and arm coverings, shall be worn as necessary to protect drug products from contamination.'

- 21 CFR 211.28(b) [5] states that 'Personnel shall practice good sanitation and health habits.'

- 21 CFR 211.28(c) [5] states that 'Only personnel authorised by supervisory personnel shall enter those areas of the buildings and facilities designated as limited-access areas.'

- 21 CFR 211.28(d) [5] states that 'Any person shown at any time (either by medical examination or supervisory observation) to have an apparent illness or open lesions that may adversely affect the safety or quality of drug products shall be excluded from direct contact with components, drug product containers, closures, in-process materials, and drug products until the condition is corrected or determined by competent medical personnel not to jeopardise the safety or quality of drug products. All personnel shall be instructed to report to supervisory personnel any health conditions that may have an adverse effect on drug products.'

- 21 CFR 211.42(c) [6] states, in part, that 'operations shall be performed within specifically defined areas of adequate size. There shall be separate or defined areas

or such other control systems for the firm's operations as are necessary to prevent contamination or mix-ups during the course of the following procedures: Aseptic processing, which includes as appropriate: A system for monitoring environmental conditions.'

- 21 CFR 211.113(b) [7] states that 'Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilisation process.'

3.2.2 ISO 14698-2, Cleanrooms and Associated Controlled Environments - Biocontamination Control - Part 2: Evaluation and Interpretation of Biocontamination Data, 2004 [8]

This standard gives guidance on basic principles and methodology requirements for all microbiological data evaluation obtained from sampling for viable particles in specified risk zones in cleanrooms.

ISO 14698-2 is designed to be used in conjunction with ISO 14698-1 [2]. It provides guidelines for how to estimate and evaluate biocontamination data from microbial monitoring of risk zones. To determine the presence and significance of biocontamination is a multi-step task. Sampling techniques, time factors, culturing techniques, analysis method (qualitative or quantitative estimates) all have to be carefully planned. Target, alert and action levels have to be determined for each risk zone based upon an initial biocontamination data collection and evaluation plan.

Each enumeration technique must be validated considering the viable particles involved.

Good data collection and evaluation documentation is necessary to determine trend analysis and the quality of risk zones. Out-of-specification results require verification - 'Did we have a true result or is it a laboratory error?' - ISO 14698-2 provides the guidance for answering this question accurately.

3.2.3 ISO Draft International Standard (DIS) 14698-3, Cleanrooms and Associated Controlled Environments - Biocontamination Control - Part 3: Measurement of the Efficiency of Processes of Cleaning and/or Disinfection of Inert Surfaces Bearing Biocontamination Wet Soiling or Biofilms, 1999 [9]

This document gives guidance for a laboratory method for measuring the efficiency of cleaning an inert surface.

3.2.4 Standards for Controlled Environments

There are additional standards for controlled environments, which consider various physical factors and tests, typically used or applied in industry or manufacturers of healthcare products.

Some of these ISO documents for controlled environment are described in the next sections.

3.2.4.1 ISO 14644-1, Cleanrooms and Associated Controlled Environments - Part 1: Classes of Air Cleanliness, 1999 [1]

This standard defines the classification of air cleanliness in cleanrooms and associated controlled environments exclusively in terms of airborne particles in sizes from 0.1 µm to 5.0 µm. This document contains some of the only mandatory criteria called for in these new ISO Cleanroom Standards. All other information provided is for guidance only. This document defines the new international classes of air cleanliness measured in number of particles per cubic meter in six different particle sizes.

There are nine major classes of air cleanliness (see **Table 3.2**), which can be further divided into 1/10th increments from ISO Class 1 to ISO Class 9, thereby providing 81 separate classes for fine tolerance clean space design.

Table 3.2 ISO 14644-1 cleanroom standards

Class	Maximum particles/m^3						FED STD 209E equivalent
	≥0.1 µm	≥0.2 µm	≥0.3 µm	≥0.5 µm	≥1 µm	≥5 µm	
ISO 1	10	2	-	-	-	-	-
ISO 2	100	24	10	4	-	-	-
ISO 3	1000	237	102	35	8	-	Class 1
ISO 4	10,000	2,370	1,020	352	83	-	Class 10
ISO 5	100,000	23,700	10,200	3,520	832	29	Class 100
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293	Class 1,000
ISO 7	-	-	-	352,000	83,200	2,930	Class 10,000
ISO 8	-	-	-	3,520,000	832,000	29,3000	Class 100,000
ISO 9	-	-	-	35,200,000	8,320,000	293,000	Room air

For example, ISO Class 7.4 would allow up to 1,760,000 particles (0.5 µm and larger) per cm^3. This would be comparable to a Class 50,000 under ISO 14644-1 [1] and ISO 14644-2 [10].

Under ISO 14644-1, air cleanliness can be determined in three different occupancy states – 'as built,' 'at rest' and 'operational.' ISO 14644-1 requires that air cleanliness be reported by ISO class number, by occupancy status and by specific particle size or sizes. Reported data must read as: ISO Class 5, 'as built' at 0.2 and 0.5 µm.

There are further provisions for defining air cleanliness based upon particles larger than 5.0 µm. These are called macro particles or M descriptors. Macro particles are necessary for defining relatively dirty clean environments where powders or heavy dusts are present as part of a controlled manufacturing process.

There are also provisions for particles smaller than 0.1 µm. These are called ultrafine particles or U descriptors. As certain research and manufacturing processes tend toward nanometer dimensions, U descriptors can be utilised to qualify and quantify clean space.

M descriptors and U descriptors cannot be used to define airborne particle cleanliness classes. However, they may be used independently or in conjunction with specific airborne particle cleanliness classes as listed in Table 1 in the Standard.

The basic document, which includes scope, definitions, classification of air cleanliness and demonstration for compliance, is all normative. Recommendations given in annexes are typically informative.

Since 2000, there are further revisions and changes proposed for ISO 14644-1 and 14644-2. For example, in 2000, ISO 14644-2 was published, which began the process of cancelling the US Federal Standard 209E [11]. On 29th November 2001, the document was cancelled and superseded by ISO 14644-1 and ISO 14644-2.

3.2.4.2 ISO 14644-2, Cleanrooms and Associated Controlled Environments - Part 2: Specifications for Testing and Monitoring to Prove Compliance with ISO 14644-1, 2000 [10]

This document specifies the requirements for periodic testing of a cleanroom or clean zone to prove its continued compliance with ISO 14644-1 classification of airborne particle cleanliness.

ISO 14644-2 draws its understanding and strength from ISO 14644-1, which was published first. ISO 14644-2 spells out the mandatory and non-mandatory tests

that must be performed in order to prove compliance with ISO 14644-1. This short document, only 8 pages long, is extremely important.

The three mandatory tests that need be performed in order to prove compliance with ISO 14644-1 are:

- Classification of air cleanliness
- Pressure difference
- Airflow (either volume or velocity)

Tables 1 and 2 from ISO 14644-2 spell out the mandatory time interval between tests and also refer to the proper test methods from ISO 14644-3, *Clean Rooms and Associated Controlled Environments – Part 3: Test Methods* [6].

ISO 14644-2 also spells out four owner optional tests that are non-mandatory. However, use of some or all of these tests may be appropriate for evaluating clean space performance. These additional four tests are:

- Installed filter leakage
- Airflow visualisation
- Recovery time
- Containment leakage

Generally, ISO 14644-1 and ISO 14644-2 require fewer sample locations for air cleanliness classification than was the case with US Federal Standard 209E [11] thereby providing cost savings at no sacrifice to air cleanliness quality.

The final DIS version of ISO 14644-2 is significantly different from the DIS version. The time intervals between tests have a new flexibility not available with the DIS version or with US Federal Standard 209E. The monitoring plan option based upon risk assessment allows for user-friendly flexibility, but such a plan must be carefully and thoroughly thought out.

11.2.4.3 ISO 14644-3, Cleanrooms and Associated Controlled Environments - Part 3: Test Methods, 2005 [12]

This standard specifies the metrology and testing methods for characterising the performance of cleanrooms and clean zones.

ISO 14644-3 places emphasis on the 14 recommended tests used to characterise cleanrooms and clean zones. These tests are:

- Airborne particle count for classification
- Airborne particle count for ultrafine particles
- Airborne particle count for macro particles
- Airflow
- Air pressure difference
- Installed filter system leakage
- Flow visualisation
- Air flow direction
- Temperature
- Humidity
- Electrostatic and ion generation
- Particle deposition
- Recovery
- Containment leak

As identified in ISO 14644-1 and ISO 14644-2, some of these tests are mandatory but most are voluntary. The key controlling factor is the quality levels the cleanroom owner desires and what measurements are necessary to help achieve that level.

The overall emphasis of all these tests and their metrology is performance. Clean space is built and operated to specific performance criteria in order to achieve a quality standard determined by end-user needs. Deutsche Institüt für Norms European Norm (EN) ISO 14644-3 does not specifically address measurements on products or processes in cleanrooms. Rather it covers the cleanroom performance characteristics that lead to the ability to measure product and process quality levels desired by the cleanroom owner.

Of the 14 recommended cleanroom qualification tests, choice of which tests will apply to a particular cleanroom is by agreement between buyer and seller, that is, customer and supplier.

There are three major annexes to ISO 14644-3. Annex A lists all the recommended tests and provides a means of defining the sequence in which these tests are to be utilised in classifying and qualifying a cleanroom or clean zone.

Annex B details the individual test methods so there can be no misunderstanding between customer and supplier. How the test is conducted, any test limitations, and how the test data are reported are given in this annex.

Annex C of ISO 14644-3 lists all the test instrumentation used by the 14 recommended tests. The performance parameters for each instrument are given: the sensitivity limits, measuring range, acceptable error, response time, calibration interval, counting efficiency, data display and so on.

3.2.4.4 ISO 14644-4, Cleanrooms and Associated Controlled Environments - Part 4: Design, Construction and Start-Up, 2001 [13]

This standard specifies the requirements for the design and construction of cleanroom facilities.

ISO 14644-4 covers all aspects of the design and construction of cleanrooms and is a primer to intelligent cleanroom design and construction. It starts with requiring a clear definition of the roles of the primary parties involved in a cleanroom project, i.e., the customer and the supplier as well as ancillary parties such as consultants, regulatory authorities and service organisations.

3.2.4.5 ISO 14644-5 Cleanrooms and Associated Controlled Environments - Part 5: Operations, 2004 [14]

This standard specifies the basic requirements for operating a cleanroom.

This document covers all aspects of operating a cleanroom no matter what class of cleanliness or type of product is produced therein. It is a reference document for smart cleanroom operation.

There are 6 major areas of concern. This first is ‘operational systems’ where attention is focused on establishing a framework for providing quality products and processes in a cleanroom environment. This covers such factors as contamination risk assessment, training procedures, mechanical equipment operation and maintenance, safety, and proper documentation to prove that appropriate procedures are in place and being followed.

The second major area is 'cleanroom clothing.' Who wears what? How is it put on? When should it be replaced or laundered? What type of fabric is appropriate to which situation? It is recognised that the primary function of cleanroom clothing is to act as a barrier that protects products and processes from human contamination. The degree of enclosing an individual is process and product dependent. It could be done by a simple lab coat or a totally enclosed body suit with self-supporting breathing device.

The third major area is 'personnel.' Only properly trained personnel should be allowed to enter a cleanroom. To do otherwise is to create additional risk. Personal hygiene, cosmetics and jewellery can cause contamination problems. What is the policy in these areas? How should people enter and leave clean space? What is the personnel emergency response procedure?

Fourth is the concern for the impact of 'stationary equipment.' How clean should this equipment be before it is placed in a cleanroom? How should it be moved into this space and set in place? What kind of maintenance will be required? What types of ongoing support services will be needed? What will be the impact of these factors on control of contamination?

The fifth major area of concern covers 'portable equipment and materials' that is, items easily transported in and out of the cleanroom. What procedures are needed for control of these items in a cleanroom? Do some materials require protective storage and isolation? How is this done? How are waste materials collected, identified and removed from a cleanroom? Should there be a separate set of tools kept in the cleanroom? What items require sterilisation? What items in the cleanroom have out-gassing properties? What items cause static? Because all consumable items in a cleanroom are potential contamination sources, what do you do to control them from entry through use to disposal? Answers to these questions are unique to specific operations.

The last area of concern is 'cleanroom cleaning,' otherwise known as 'housekeeping.' Outlined are detailed cleaning methods and procedures along with personnel responsibilities. Here, again personnel training is important. How do you clean properly, how frequently and what contamination checks are required? Do you have an assessment system in place for evaluating your housekeeping? What special requirements are required, particularly in areas of risk due to hazardous material, hazardous equipment, equipment location and so on? For example, How aggressive are your cleaning compounds? How do you avoid adding contamination by your own cleaning procedures?

3.2.4.6 ISO 14644-6, Cleanrooms and Associated Controlled Environments - Part 6: Vocabulary, 2007 [15]

This document describes all the terms and definitions in all the ISO 14644 cleanrooms and associated controlled environments standard specifications.Vocabulary is an important document for any contamination control professional.

3.2.4.7 ISO 14644-7, Cleanrooms and Associated Controlled Environments - Part 7: Separative Devices (Clean Air Hoods, Gloveboxes, Isolators and Mini-Environments), 2004 [16]

This standard specifies the minimum requirements for the design, construction, installation, testing and approval of separative enclosures in the areas, where they differ from cleanrooms.

A separative enclosure is a cleanroom without any people inside. It is usually relatively small in size, but not necessarily so. Examples are clean air hoods, glove boxes, isolators and mini-environments - terms that, in many cases, are industry specific. For example, what the healthcare industry refers to as an isolator, the micro-electronics industry refers to as a mini-environment. However, the healthcare user quite often has to sterilise his enclosure, whereas the micro-electronic user does not. This leads to significant design and construction differences.

By way of clarification, prior to May 2000, ISO 14644-7 was referred to as '*Enhanced Clean Devices*.' The writers of this ISO document were not satisfied with its title, and it was changed to the current term '*Separative Enclosures*' because this term is more descriptive and definitive of these types of clean environments.

The term '*Separative Enclosures*' is generic, as is the subject matter covered in ISO 14644-7. Separative enclosures encompass a wide range of configurations from open unrestricted air over-spill to totally contained hard wall containers. They provide the appropriate level of protection from unwanted particle, microbiological, gaseous and liquid contamination, as well as worker safety and comfort. They provide for special atmospheres and bio-decontamination, as well as remote manipulation of enclosed manufacturing processes.

3.2.4.8 ISO 14644-8, Cleanrooms and Associated Controlled Environments - Part 8: Classification of Airborne Molecular Contamination, 2006 [17]

This standard covers the classification of molecular contamination in terms of

airborne concentrations of specific compounds or chemicals and provides a protocol that includes test methods and analysis for concentrations between $10\text{-}10^{-12}$ g/cm^3.

ISO-14644-8 is the base document for controlling molecular contamination in cleanrooms and associated controlled environments. It includes the special requirements of separative enclosures (see ISO 14644-7) such as mini-environments, isolators, glove boxes and clean hoods.

Airborne molecular contamination is the presence in a cleanroom atmosphere of chemicals (non-particle) in the gaseous, vapour or liquid state, which may have a deleterious effect on a product, process or analytical instrument.

Surface molecular contamination in a cleanroom is the presence on the surface of a product or analytical instrument of chemicals (non-particle) in the gaseous, vapour or liquid state, which may have a deleterious effect.

Outgassing occurs when is gaseous products are released from a material under specified conditions of temperature and pressure.

3.2.4.9 ISO 14644-9 Cleanrooms and Associated Controlled Environments - Part 9: Classification of Surface Cleanliness by Particle Concentration, 2012 [18]

This ISO document describes the classification of the particle contamination levels on solid surfaces in cleanrooms and associated controlled environment applications. Recommendations on testing and measuring methods as well as information about surface characteristics are given in informative annexes.

It applies to all solid surfaces in clean rooms and associated controlled environments such as walls, ceiling, floors, working environments, tools, equipment and products The surface particle cleanliness classification is limited to particles between 0.05 and 500 µm. It doesn't include microbial particals as such.

3.3 Automation and Robotics

The best results for aseptic assemble and processing will include automation and robotics with the elimination of personnel from strictly controlled environmental areas. An alternative to terminal sterilisation, aseptic processing enables the sterility of pre-sterilised components and products during assembly allowing the final product to be sterile in its final container, resulting in a terminally sterilised product.

3.4 Aseptic Processing

Aseptic processing may include: filtration, lyophilisation (drying process), clean-in-place technology (CIP), SIP and the isolater system used to maintain aseptic conditioning.

Aseptic filling of product will include the use of filtration. Typically such filtration begins with a terminal or near terminal sterile solution or suspension, but filtration can provide near terminal sterile product under specific conditions and design. Sterilising filtration is typically a part of aseptic processing.

3.5 Sterilisation by Filtration

Sterilisation by filtration is a significant form and type of aseptic processing. Sterilisation by filtration refers to the removal of viable micro-organisms by the use of filters. Classically, filtration was found to be a means of sterilising liquid with a low contamination rate. Some materials cannot tolerate the high temperatures used in heat sterilisation or irradiation without some deterioration, therefore other methods must be devised. Filtration is one such method. For example, materials such as urea, certain carbohydrate solutions, serum, plasma, acetic fluid and others must be filter sterilised. Filtration is a means of maintaining stabilisation of pharmaceuticals, biological solutions, and other solutions.

Filtration does not terminally inactivate or kill microbes, it just removes them. Fluids that would be damaged by heat, irradiation or chemical sterilisation, can typically be only sterilised by filtration using filters. This method is commonly used for heat labile pharmaceutical solutions, liquid biologics, and heat sensitive ophthalmics in medicinal drug processing. Commonly filtering with pore sizes of 0.2 µm or less will effectively remove micro-organisms. Typically, filtration, with 0.2 µm does not remove viruses or prions that are too small. In the processing of biologics, however, viruses must be removed or inactivated. A nanofilter with smaller pore can be used with sizes of 20-50 nm (nanofiltration). However, the smaller the pore size, the lower the flow rate. To achieve higher total throughput or to avoid premature blockage, pre-filters might be used to protect small pore membrane filters, and pressure can be applied. In some cases it has been shown that prions can be removed or reduced by filtration.

Filters are typically divided into four basic types:

- Screen - with holes drilled or punched out of the material.
- Depth filters consisting of thick beds of granular or porous materials.

- Cake filters – cakes of insoluble particulate materials.
- Porous or membrane filters are porous and graded to a size to remove organisms of greater size. These are sometimes referred to as absolute filters.

3.5.1 Definition of Filter and Sterility

A filter is a device using a medium to selectively separate certain substances from a given environment. The process of a filter is a filtration. Filtration is the movement of material through a semi-permeable membrane under a mechanical force. However, when it comes to terminal sterilisation and being sterile, it is a state of itself.

In the 1990s, the European Committee for Standardisation (CEN) established the standard EN 556-1 [19] *Sterilisation of Medical Devices—Requirements for Medical Devices to be Labeled Sterile*. CEN determined that it would not be acceptable to ascribe two different interpretations [i.e., 10^{-3} and 10^{-6} sterility assurance level (SAL)] to the term 'sterile'. An SAL of 10^{-6} was chosen for EN 556-1, with the provision that a greater probability of non-sterility could be permitted under special circumstances.

EN 556-1 applied only to terminal sterilisation of medical devices but did not address sterilisation by filtration or aseptically processed products labelled 'sterile'. Filtration is not a typical method of sterilisation used in the central supply of hospitals, however, it is used in the pharmacy.

Today a 'sterile' medical device or healthcare product is one that is free of viable micro-organisms. Sterility of a medical device can be achieved through:

- A terminal sterilisation process.
- Sterilisation of components, followed by sterile filtration of the final liquid formulation and aseptic filling into sterilised containers, or
- A combination of chemical/physical sterilisation and aseptic processing.

3.5.2 Aseptic Processing and Filtration

Aseptic processing does not inactivate micro-organisms, as is the case of terminal sterilisation, but prevents the introduction of micro-organisms during manufacture. Some products or components, such as liquids or gases that cannot withstand the chemical/physical intensity of certain sterilisation processes or have particles that must be removed, may be sterilised by filtration. The effectiveness of sterilisation by filtration is based on the ability of the filter to remove a known quantity of micro-

organisms of a known size under the specified filtration conditions. In addition, the subsequent filling, sealing or assembly of the filtered liquid must be conducted aseptically in accordance with quality system requirements to prevent microbial d contamination of the sterile product.

Table 3.3 summarises the clean area air classifications and recommended action levels of microbiological quality.

Table 3.3 Air classifications[a]

Clean area classification (0.5 µm particles/ft^3)	ISO Class designation[b]	Particle size designation (>0.5 µm particles/m^3)	Microbiological active air action levels[c] (CFU/m^3)	Microbiological settling plates action levels[c,d] (diameter – 90 mm; CFU/4 h)
100	5	3,520	1[e]	1[e]
1000	6	35,200	7	3
10,000	7	352,000	10	5
100,000	8	3,520,000	100	50

[a]: All classifications based on data measured in the vicinity of exposed materials/articles during periods of activity.
[b]: ISO 14644-1 designations provide uniform particle concentration values for cleanrooms in multiple industries. An ISO 5 particle concentration is equal to Class 100 and approximately equals EU Grade A.
[c]: Values represent recommended levels of environmental quality. It may be appropriate to establish alternate microbiological action levels due to the nature of the operation or method of analysis.
[d]: The additional use of settling plates is optional.
[e]: Samples from Class 100 (ISO 5) environments should normally yield no microbiological contaminants.

Aseptic processing or assembly requires the pre-sterilisation of all product parts or components that are in direct contact with the aseptically filled product. The products are processed in a controlled environment where microbial counts are maintained at or below defined levels and human intervention is minimised. Manufacturers use validated systems, trained personnel, controlled environments, and well-documented systematic processes to ensure a sterile finished product. One acceptance criterion for validating the sterility of products manufactured by aseptic processing is the maximum contamination rate as determined in microbial growth media fill experiments, where bacteriological media is substituted in place of product to demonstrate if microbial growth under simulated conditions can grow or show sterility (non-growth).

3.5.3 Types of Filters (Filtration)

Filtration is in part regarded as not being 'terminal' sterilisation, but sterilisation by a lesser means, because it only removes microbes rather than destroying them. Filtration is considered to be less efficacious and effective than terminal sterilisation, because there is always a remote possibility of not removing all the microbes. Filtration has been around since Egyptian times, when they used fabric to filter wine. There are hundreds of materials that have been used for filtration, but only a few have been adequate for sterilisation. However, at times filtration may even worsen some situations. For example, if a material is forced through a layer of suspended matter it may build up a substantial bacterial population. In such cases filter cloths, mesh strainers or pre-filters should be used and renewed frequently to remove the build up. Some examples are depth filters and absolute membrane filters.

Depth filters remove microbes through torturous path, and absolute filters remove microbes by having pores smaller than microbes. General examples of filters for healthcare products are:

- Screen filter
- Depth filters – removal through torturous paths
- Membrane filters with pores smaller than microbes
- Cake filters

Initially 0.45 μm was considered adequate as an absolute filter until >1,000 CFU *Brevundimonas diminuta* were found to pass through but were restricted by a 0.22 μm filter. A liquid or gas to be sterilised can be passed through a filter with a porosity sufficient to remove any micro-organisms in air or suspension e.g., (0.4 or 0.22 μm) or through other means, e.g., a depth filter. For example, HEPA not cotton becomes a means of sterilising air. For liquids, and so forth, a variety of filters are available, made of materials such as cellulose nitrate (Millipore filters). This method is very useful for sterilisation of liquids containing heat-labile components. Hydrophobic filters are often used for sterilising air, because moisture could wet a non-hydrophobic (membrane) filter to create a pressure, causing loss of filtration, or it may simply decrease its retentive efficiency. Microbial efficiency may be measured by retention of *B. diminuta* at 10^7 CFU/cm^2 of an effective filter area. It can also be measured as a log reduction value (LVR) which is determined by the total number of micro-organisms used as a challenge to the filter. Examples of LVR may be >10^{10} *B. diminuta* retained with a 0.22 μm filter or retention of 10^7-10^8 for a 0.45 μm filter, for example.

3.5.4 Advantages and Reasons for Using Filters

Sterilisation by filtration refers to the removal of viable micro-organisms by the use of filters; however, it also removes adverse particles as well. Sterilisation by filtration is often used where terminal sterilisation processes such as heat, EO, radiation or oxidising agents would damage or ruin a product. It is also used in combination products such as a drug within a device.

It can also be used to sterilise material that would be inoculated or impregnated on a product where very low or sterile bioburdens are needed in order to sterilise the final product by a low concentration, low intensity or low dose sterilant without destroying the product.

Sterilisation by filtration is a practical, yet last resort method of sterilising liquids or drugs, because it borders on being a non-terminal sterilisation method and is difficult at times to assure a SAL of even a 10^{-3} probability of survivor, because of the general way it is used and applied.

Sterilisation by filtration is commonly used in a pharmaceutical area for sterilisation of drugs that would be adversely affected by steam heat.

Sometimes filters are used to evaluate sporicides. For example spores are placed on filter and exposed to the sporicide, and then the filter is washed to remove any traces of the sporicide, and finally put on a recovery agar medium to see if spores are inactivated, totally killed or survived.

Filters (e.g., HEPA) are also commonly used in sterilisation of air for clean rooms and other spaces. When laminar flow of air is passed through an HEPA filter, the collection efficiency of both viable and non-viable particulates is increased. Additionally the filter acts as a diffuser lowering air velocity even further. The method is also used in some devices as a means of assuring against adventitious or accidental contamination during use. The method may be used in producing contact rinse solutions. HEPA filter does not necessarily deliver sterile air. Laminar conditions eliminate the chances of airborne particles dropping into the product, but it is not absolute. A laminar flow hood should not be regarded as a ‘sterile area’ but only as a micro-environment in which chances of contamination are considered reduced.

Filtration can also be performed by the phenomena of reverse osmosis and ultrafiltration.

3.5.5 Classes of Filters

The types of filtration may be further delineated by the types of filters used such as porous (membrane) filters, depth (probability) filters, or charged or absorptive filters.

Examples of porous (membrane) filters are:

- Cellulose acetate
- Cellulose nitrate
- Polyamide 6,6
- Polypropylene
- Polysulfone
- Polytetrafluoroethylene
- Polyvinylidene fluoride

Examples of depth filters are:

- Seitz - asbestos
- Chamberland - quartz sand and kaolin
- Berkefeld filters - Celite asbestos
- Sintered glass, compressed polymer glass or metal fibres

Examples of cake filters are:

- Cakes of insoluble diatomite

Examples of screen filters are:

- Metal or plastic screen with holes in them
- Charged or absorptive filters
- Chemically inactivating filters
- Reverse osmosis filters

Sterilisation by filtration can be described by the filter size, rating, or grade, for example, membrane filters may be 0.45, 0.22 and 0.1 μm in size. Depth and

membrane filters are the main filters used in the filtration of liquids for healthcare products.

Some advantages of using depth filters are:

- They retain a large percentage of contaminants smaller than their nominal size.
- They exhibit a high flow-through capacity, particularly of dirt-like particles.
- They often exhibit a high dirt handling capacity.

Some disadvantages of using depth filters are:

- There is a tendency of depth filters to slough off filter materials during filtration.
- Slow grow microbes trapped in the matrix present a problem on long filtration runs.
- With no meaningful pore size, depth filters do not provide limitations of size of particles or microbes that might pass through them.
- Large volumes of liquid product can be retained, and if expensive, this presents a potential problem.
- Depth filters have limitations in their use, unlike membrane or porous filters.

Some advantages of membrane or porous filters are:

- Membrane or porous filters are considered absolute filters.
- Absolute rating implies that filter efficiency is independent of flow rate pressure differentials.
- Filters do not permit passage of particles or microbes larger than the rated pore size even at high pressure differentials.
- Practically no product is retained in the membrane filter.
- Large flexible particles can form a coarse mat on membrane filters and act as depth filter, even retaining smaller particles than the pore size. Such a coarse mat of large flexible particles formed on the membrane can act as a depth filter by retaining particles smaller than the membrane pore size, thereby enhancing the efficiency of the membrane filter.
- Membrane filters do combine the desirable features of both depth and screen filters.

- Sintered silver plate materials are good, because silver inhibits microbial growth.

Some disadvantages of membrane filters are:

- Because of their surface retention, their flow capacity can be decreased.
- Not all particles smaller than the pore size will pass through them.
- In the dry state they can be easily be damaged since they are thin and brittle.
- Their performance can be increased or improved with an appropriate pre-filter.

Using a combination of filters has been considered. Since depth filters can retain a large amount of fine particulates, they can be used as pre-filters before using membrane filters. Different sized membrane filters can also be used, a 0.45 μm filter may be used prior to a 0.22 μm filter to improve flow rates and prevent blockage of the filter with smaller pores. This may be referred to as serial filtration.

HEPA filters have an efficiency of ~ 99.99%. HEPA filters are used for filtering 'air' (not liquids), in laminar flow, control of air flow patterns helps to control the microbial contamination of the environment.

Reverse osmosis means hyperfiltration of a liquid by forcing it under pressure through a membrane that is not permeable to the impurities to be removed. It is the membrane material used in reverse osmosis that stops bacteria, viruses and their residues. It is used to hyperfiltrate water, electrolytes, and other fluids.

Hollow filters improve results with cross flow techniques, because of their shape. Porous ceramics that are shaped like candles are used filters. Nucleopore membranes are polycarbonate films with etched traces formed in the film by neutron irradiation. This filter act like true screen filters.

The current accepted standard for most liquid sterile filtration is the 0.22 μm filter, but the suggested filtration level of 0.1 μm is suggested for removing *Mycoplasma* contaminants from serum and tissue culture medium. No standard methodology exists yet for testing the efficiency of 0.1 μm rated sterilising grade filters. Hydrophobic 0.3 μm filters may be used for sterilising air or gases.

3.5.6 Disadvantages of using Filters

Filtration is limited to liquids and gases, or filtration of particles. It is not commonly recognised as a terminal sterilisation process. It is a process that relies on microbial

exclusion, rather than microbial inactivation, and is therefore deemed to be outside of processes that are terminal.

Sterilisation by filtration is commonly used in the pharmaceutical area for sterilisation of drugs or items that would be adversely affected by dry heat, steam, irradiation or chemical sterilisation. In this situation it is used as method of last resort, because it borders on being a non-terminal sterilisation method. It is could be difficult at times to assure a SAL of even 10^{-6} probability of survivors, because of the general way it is used and applied. However, in a sterile fill process with filtration, sterility is achieved by microbial removal under aseptic conditions using a mechanical process, for example, filtration. The efficacy of this process can be expressed as a maximum contamination rate. Filtration can be achieved through gravity flow, pressure, and vacuum.

Pressure filtration allows for use of higher flow rates and some of its benefits are:

- It prevents leakage in the system
- It dispenses sterile, particle free filtrate
- It allows for bubble point testing
- It prevents vapourisation of solvents
- It prevents foaming and denaturation of proteins.

Some disadvantages of pressure filtration are:

- Vacuum filtration is not recommended for sterilising ultra clean particles, because particles may pass through, and some solvents and so on will evaporate, resulting in some precipitation.
- Polished stainless steel piping, sanitary fillings and stainless steel pressure vessels should be used, to minimise generation of particles.
- Threaded fittings should be avoided where possible, because they can generate particles, and may be difficult to clean.
- Surgical latex or black rubber tubing which may contain extractables that will leach out should not be used.

3.5.7 Limitations and Challenges of Aseptic Processing

Final terminal sterilisation methods are not typically used in the final step of aseptic processing, but filtration is. Aseptic processing is a method of sterilisation not discussed

because it is not deemed to be terminal sterilisation. In general, it is preferred to 'terminally' sterilise products in their final configuration and packaging in order to minimise the risk of adventitious microbial contamination. Products designed for aseptic processing generally consist of components that have been previously sterilised by one of the other terminal sterilisation methods.

3.5.8 Aseptic Processing Incorporating other Methods of Sterilisation

Aseptic processing frequently incorporates other methods of sterilisation, such as steam, EO, and dry heat, of assemblies, components, materials, packaging and so on, and in particular filtration of liquids, gases, and air. UV light can play a critical part in aseptic processing and can be used to reduce any contamination residing in a 'filtered' environmental area, or possibly of a fluid that has just been filtered. UV can reduce microbes by >90% (e.g., 98%) in laboratories and pharmaceuticals. UV is not typically relied upon for terminal sterilisation, but as an adjunct to aseptic assembly and fill and for controlling microbes in hospital rooms, on laminar flow benches and so on.

UV can also do the same in water (filtered). Any UV treatment of biologics, items, components, devices, diagnostics, and pharmaceuticals in water or not in water would need to be evaluated for compatibility. UV is typically only a surface sterilant. It does not have much penetrative capability. Like electron beam treatment, items need to have all surfaces exposed. X-rays, and gamma rays have shorter wavelengths than UV. UV in the range of 254 nm is quite bactericidal. UV carousels have been used to sterilise laboratory test tubes, flasks, containers and cups for sensitive cell culture growths. In this case the UV light is direct and intense. The biological indicator (BI) or challenge to filtration is *B. diminuta* American Type Culture Collection 19146. This organism has a small size (0.2-0.6 μm) and a width to length ratio of 1:2.

Selecting and highlighting *B. diminuta* as a challenge organism to test filters follows the same logic and rationale for choosing and selecting *Geobacillus stearothermophilus* for steam, or *Bacillus atrophaeus* for dry heat or EO sterilisation. The challengers are among the worse case microbes to challenge their respective sterilisation methods. At this time *B. diminuta* has emerged as the BI of choice for filtration. It is an analogous BI for filtration.

A 0.22 μm membrane filter is able to retain 10^7 cells per cm^2 of *B. diminuta* with a 'total' LRV upwards of 10^{10}, however, a 0.45 μm filter is only able to retain 10^4 cells per cm^2 of *B. diminuta,* very small microbes or a 'total' LRV upward of 10^7-10^8.

If this latter efficiency were expressed as a percentage the value would be 99.999999%. On a normal or routine basis, filter systems are typically tested for their integrity before and after use. This may be a bubble point test or the diffusive flow through test, or in some cases a *B. diminuta* microbial retentive test. Under select conditions or usage, other microbes such as *Mycoplasma* or a virus may be used as microbes of interest, for microbial retention tests.

Prions are smaller biological entities than *B. diminuta* and are easier to destroy by a few terminal sterilisation methods (e.g., steam, steam plus sodium hydroxide) rather than by removal.

Endotoxin testing may be required of the filtrate or filter. These are toxins given off by some microbes that may be retained, but the toxins could cross the filter. They may be minimised by not using the filters for long or prolonged times. Operation of filters should include limits for temperature, pressure, number of cycles, total throughput volume and/or time.

A sterile product by filtration and/or aseptic processing or assembly is defined by its maximum contamination rate, or decontamination level. The maximum contamination rate is a mathematical expression of the frequency of the occurrence of a non-sterile unit as determined by media fill simulation or simulation of an aseptic process.

The maximum contamination rate is normally expressed as a percentage, such as 0.1% (1/1,000), or a SAL of 10^{-3}. So if it can be demonstrated that there are no survivors out of 1,000 units in a previous or subsequent cycle or run, then it appears reasonable to indicate a 10^{-3} SAL.

Some considerations in aseptic processing *via* filtration are:

- No sterility re-testing without justification, for example, without considerable evidence of laboratory contamination.
- Microbial limits for environmental monitoring should be determined statistically.
- Media fills or assemblies testing must 'simulate' production operations.
- Increased microbial sampling is needed when returning to aseptic processing techniques.
- All configurations for aseptic processing must be validated.
- Particulate monitoring must be performed on every shift.
- Practices and policies for environmental control should be uniform and standard.

- Monitoring of personnel shall be performed on each shift and each day.
- Environmental monitoring should include maximum levels of viable and non-viable organisms.

3.5.9 Maximum Contamination Rate for Validation of Aseptic Processing via Filtration

Manufacturers or healthcare facilities using an aseptic technique should aim to achieve a contamination rate of zero. The specification of a maximum contamination rate depends on the rate that can be achieved by the particular aseptic processing technology (the process capability) and the limitations of available validation techniques. The maximum contamination rate shall be no more than 0.1%. Acceptance limits allow a maximum of 0/3,000 or 1/4,750 positive products, which provides 95% confidence of obtaining a 0.1% contamination rate (See also ISO 13408 [20], ISO 14644 [1] and ISO 14698 [2, 8, 9].

Examples of aseptically filled products that have been produced using a maximum contamination rate of no more than 0.1% include:

- Biological or biotechnology products;
- Clinical laboratory devices;
- Drug bearing devices;
- *In vitro* diagnostics;
- Lens care solutions;
- Oil based pharmaceuticals;
- Organ preservation fluids;
- Prefilled syringes;
- Radiopharmaceuticals; and
- Respiratory therapy devices.

Aseptic processing does not inactivate micro-organisms, as is the case with terminal sterilisation methods, but prevents the introduction of micro-organisms during manufacture, by removing them. ISO 13408 [20] describes control and validation for aseptic manufacturing processes. The standard details such topics as monitoring the environment, qualification of personnel, validation of cleaning, validation of

sterilisation of components, and the media fill programme. The media fill programme is considered to be a process simulation test and demonstrates the contamination rate of a particular aseptic process or part thereof. UV light is a good adjunct to filtration of air and liquids. Although it does not commonly sterilise liquid or air to 10^6, it can remove microbes and particles up to 98% to 10^3.

3.6 Lyophilisation

Lyophilisation, is another form or type of aseptic processing. It is a physical-chemical drying process designed to remove solvents or water from both aqueous and non-aqueous systems, primarily to achieve product or material stability. Lyophilisation is equivalent to the term freeze-drying. Lyophilisation typically involves freezing an aqueous system and removing the solvent, first by sublimation (primary drying) and then by desorption (secondary drying), to a level that no longer supports chemical reactions or biological growth. The result is a stable, well-formed product meant to rapidly disperse or solubilise while retaining biological or other activity. Because it is often the final step in an aseptic process with direct impact on the safety, quality, identity, potency and purity of a product, lyophilisation is a critical processing step. Where the finished lyophilised product is intended to be sterile, the product to be dried is an aqueous system that has already been sterilised. Therefore, all activities that can affect the sterility of the product or material need to be regarded as extensions of the aseptic processing of that sterilised product or material. In general, the predominant challenge in ensuring product or material sterility during lyophilisation is to prevent microbiological and particulate contamination between the filling operation and completion of the lyophilisation process. Of special, equipment-related concern, is the protection of the product or material from microbiological contamination within the chamber.

3.7 Clean-in-place

CIP is another type of aseptic assembly or processing. CIP processes allow parts of the equipment or an entire process system to be cleaned without being dismantled, reducing the need for disassembling and connections under clean conditions. For example, tanks, vessels, freeze-dryers piping and other processing equipment used for manufacture may be CIP.

The CIP process is in most instances followed by a SIP process as described in ISO 13408-5 [21]. While CIP and SIP methods differ considerably in technology, the concept of *in situ* treatment is similar. Design considerations of all systems are critical

to ensure that CIP technologies can be successfully applied to clean manufacturing equipment to the desired level of cleanliness.

3.8 Sterilisation in Place

SIP is another form or type of aseptic assembly or processing. It is, in most cases, preceded by CIP, which is described in ISO 13408-4 [22]. While methods of CIP and SIP differ considerably in technology, the concept of *in situ* treatment is similar. The most important issue to consider in establishing SIP technology is the design of the system(s) to ensure that they are able to successfully sterilise manufacturing equipment to the desired level of sterility assurance.

3.9 Isolator Systems

The isolator system is another form or type of the assembly or processing. Isolator systems typically are enclosed, rigid plastic structures or plastic bags which are used to prevent contamination by infective agents or contaminating organisms during a sterility test or aseptic transfer or assembly. But isolators are more of a 'separative enclosure', a type of clean room without any people inside. It is usually relatively small in size, but not necessarily so. Examples are clean air hoods, glove boxes, isolators and mini-environments—terms that, in many cases, are industry specific. For example, what the healthcare industry refers to as an isolator, the micro-electronics industry refers to as a mini-environment. However, the healthcare user quite often has to sterilise his enclosure, whereas the micro-electronic user does not. This leads to significant design and construction differences. By way of clarification, prior to May 2000, ISO 14644-7 [16] was referred to as '*Enhanced Clean Devices*.' The writers of this ISO document were not satisfied with its title, and it was changed to the current term '*Separative Enclosures*' because this term is more descriptive and definitive of these types of clean environments.

The term 's*eparative enclosures*' is generic, as is the subject matter covered in ISO 14644-7. Separative enclosures encompass a wide range of configurations from open unrestricted air over-spill to totally contained hard wall containers. They provide the appropriate level of protection from unwanted particles, microbiological, gaseous and liquid contamination, as well as worker safety and comfort. They provide for special atmospheres and bio-decontamination, as well as remote manipulation of enclosed manufacturing processes.

The isolator systems or separative enclosure is another part of the aseptic processing. Use of the isolator systems to maintain aseptic conditions, may include applications

for hazardous materials, such as decontaminating or sterilising agents, for example, glutaraldehyde, EO, H_2O_2, or ozone. Such systems can be like a steriliser in which components, devices, or products can be sterilised and enclosed in a sterile package before being removed. Or they could act as a steriliser of product already packaged but processed in the separative enclosure to SIP areas requiring sterilising, but other parts of the product, for example, filled vials have already been terminally sterilised or aseptically filled through filtration.

3.10 Conclusions

Healthcare products that are labeled 'sterile' are prepared by using appropriate and validated methods. When a healthcare product is intended to be sterile and cannot be terminally sterilised, aseptic assembly or processing provides an alternative. This applies to the aseptic preparation and filling of solutions, suspensions, emulsions, and solids, as well as to the aseptic handling, transfer and filling of those products, which cannot be terminally sterilised. Aseptic assembly or processing is an exacting and demanding discipline. It is essential that manufacturers make use of qualified/validated systems, adequately trained personnel, controlled environments and well-documented systematic processes to assure a sterile finished product, under aseptic assembly or processing. Once a facility or area is under control it is important to continue to clean, disinfect, train, and maintain the filter's efficacy using appropriate measuring and calibrating equipment, correct agents and monitoring at defined frequencies, validating and revalidating, using trained personnel, and maintaining discipline and control over the area, and procedures.

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4 Design, Materials, Standards and Interrelationships to Sterilisation

A magical assumption - it is believed that all products can be sterilised, without proof, but there is parenthetically more to sterilisation than is commonly imagined ...

The magic of reliable sterilisation begins with design control. While a sterilisation process must be capable of providing the appropriate statistical assurance of sterility, designs for processes for the sterilisation of devices and product criteria are critical for eventual reliable sterilisation. Generalisations are often merely open questions that need to be answered. There is assumption that all products can be sterilised. Knowing the design, characteristics and qualities of the products involved, prove useful as a means to discover how a product can be sterilised. Answers to such discoveries show how well inter-relationships can occur during the design phase.

Before any design activity, a design concept should be described in terms of the desired qualities and characteristics of the product, such as their capability to be sterilised.

4.1 Design Control and Sterilisation

The multi-functional design control process must involve many departments or disciplines to be effective. This is especially true with polymers and sterilisation [1]. Sterilisation cycles may not be designed without input from the Research & Development (R&D) and manufacturing functions, and conversely, designers may not develop a safe and effective device without input from the sterilisation department. Management with executive responsibility must play an active role in the design process by creating an environment where the concept of interdepartmental communication and co-operation will flourish. The design phase is the most significant step in the life of the product, for the inherent effectiveness, reliability and safety of the product. A design deficiency such as non-sterilisability found after the product is manufactured would be catastrophic.

All new products and processes should have a design and development plan, under design control. The steps to be considered in design control are:

- **Planning** - Identification and consideration of the product and process. A risk management plan should describe the strategic approach to identifying and controlling risk in the product and process development life cycle. This plan could be a part of the product and process development or project management plan.

- **Design input** - *Design input* means the physical and performance requirements of a device and related process (e.g., sterilisation) that are used as a basis for device design. Design input is the starting point for product and process design. The requirements which form the design input establish a basis for performing subsequent design tasks and validating the design. Therefore, development of a solid foundation of requirements is the single most important design control activity. Existing safety standards and safety requirements identified in risk assessments are the key design inputs.

- **Design output** - this means the results of a design effort at each design phase and at the end of the total design effort. The finished design output is the basis for the device master record. The total finished design output consists of the device, its packaging and labelling, and the device master record.

Specifications may be applied and may be any requirement with which a product, process, service, or other activity must conform. The quality system requirements for design output can be separated into two elements: design review and design verification should be expressed in terms that allow adequate assessment of conformance to design input requirements and should identify the characteristics of the design that are crucial to the safety and proper functioning of the device and related processes, (e.g., sterilisation). This raises two fundamental issues for developers: risk reduction measures introduced into product and process design are essential design outputs.

Design reviews - Design review means any documented, comprehensive, systematic examination of a design to evaluate the adequacy of the design requirements, to evaluate the capability of the design to meet these requirements, and to identify problems.

In general, formal design reviews are intended to:

- Provide a systematic assessment of design results, including the device design and the associated designs for production and support processes.

- Provide feedback to designers on existing or emerging problems.

- Assess project progress, and/or

- Provide confirmation that the project is ready to move on to the next stage of development.

Many types of reviews occur during the course of developing a product and process. Reviews may have both an internal and external focus. The internal focus is on the feasibility of the design and the manufacturability of the design with respect to manufacturing and support capabilities. The external focus is on the user requirements - that is, the device design is viewed from the perspective of the user.

In practice, design review, verification, and validation overlap one another, and the relationship between them may be confusing. As a general rule, the sequence is: verification, review, validation, review.

Design verification - Verification means confirmation by examination and provision of objective evidence that specified requirements have been fulfilled. In the initial stages of design, verification is a key quality assurance technique. As the design effort progresses, verification activities become progressively more comprehensive. For example, heat or cooling delivery can be calculated and verified by the air conditioning designer, but the resultant air temperature can only be estimated. Occupant comfort is a function not only of delivered air temperature, but also humidity, heat radiation to or from nearby thermal masses, heat gain or loss through adjacent windows and so on. During the latter design phases, the interaction of these complex factors may be considered during verification of the design.

Design verification should affirm that all safety requirements are covered by the risk reduction measures in the design.

Design validation - this means establishing by objective evidence that the device and related processes (e.g., sterilisation) specifications conform to user needs and intended use(s). Validation follows successful verification, and ensures that each requirement for a particular use is fulfilled. Validation of user needs is possible only after the building is built. The air conditioning and fire alarm performance may be validated by testing and inspection, while the strength of the roof will probably be validated by some sort of analysis linked to building codes which are accepted as meeting the needs of the user - subject to possible confirmation during a subsequent severe storm.

Design validation should demonstrate that all safety requirements can be consistently met with respect to intended use and user or patient needs.

Design transfer - A manufacturer shall establish and maintain procedures to ensure that the device design and related processes, (e.g., sterilisation) is correctly translated into production specifications. Each scheduled design review should address risk assessment activities, as needed, to ensure that recommended actions are assigned and monitored prior to design transfer.

The product and process life cycle does not end with design transfer. Risk management activities also should be integrated with production and distribution. Component and process controls should ensure that safety requirements are met. Non-conforming products and processes should be evaluated to make certain all risks are accounted for. Corrective or preventative action, especially in reaction to complaints, must be documented and evaluated for effectiveness.

The time it takes to draw a blueprint or build a prototype is not the major cause of design delay or failure. The design engineer is part of a development team. Many companies use a team approach to become more competitive, flexible, and responsive. Although companies have accepted the team concept in principle, sometimes these teams meet with mixed success. Working in teams can present a tremendous challenge for many design engineers who find themselves thrust into this dynamic, foreign environment. Managers dealing with these teams must know how to collaborate as team members and foster the work of others as team leaders or collaborators. They must facilitate and lead high-performance teams to demonstrate excellent team efficiency, dynamics, and innovation. Leaders need excellent interpersonal skills to communicate effectively with their peers and with internal and external customers, so that the team can develop a competitive product that will meet customer needs.

Medical device producers face the pressures of managed-care industry demands: price and value. A new medical device and related processes (e.g., sterilisation) generally carries an added value. Manufacturers generally increase prices slightly. In the future, design engineers will be under even more pressure to ensure that innovative technology is offered at affordable prices.

Medical device companies have made significant efforts to produce products that address many problems facing physicians who treat complications. It is beneficial for design engineers to understand the problem's mechanism, since they have been called upon to design a product to address it. Designers continue to explore new technologies to solve some peculiar features of plastics and processes (e.g., sterilisation). Ultimately, these new technologies should create a smoother transition of plastics, polymers and materials into medical devices.

The effectiveness of a sterilisation process for a specific medical device derives from the relationship between the robustness and the capability of the two processes. Process robustness is the ability of the process to withstand product and process variations (e.g., bioburden on a new device for radiation dose setting) while maintaining its quality attributes in this case, a minimum, validated sterility assurance level. The process capability, by contrast, is a measure of the ability of the process to reproducibly, manufacture product and effectively inactivate all microbes. For sterilisation, this means that the process for a specific device must be designed to be able to effectively

sterilise a product or product family within the expected range of acceptable product variation and process tolerances.

The primary objective of design controls in the sterilisation process is to ensure that an effective, reproducible cycle is routinely used to process a particular device or device family. Because of the importance of consumer safety in sterilised medical devices, risk management associated with sterilisation cycle development is directed toward the ultimate safety of the processed device. Risk may be considered as: the probability of occurrence of a hazard causing harm; safety as: the freedom from unacceptable risk. A 10^{-6} sterility assurance level (SAL) is the probability of one non-sterile unit out of one million units processed is generally considered an acceptable risk of non-sterility and is, therefore, used as a basis of sterilisation cycle design.

Sterilisation design also relates to the optimisation of process variables to sterilise quickly, effectively, and efficaciously without adverse effects to product quality and safety.

The design control features of the new quality system regulation are outlined in section 820.30 of the *Code of Federal Regulations - Title 21: Food and Drugs* [2] and encompass the following elements:

- Design and development planning
- Design input
- Design output
- Design review
- Design verification and validation
- Design transfer
- Design changes
- Design history file

Many organisations have historically conducted sterilisation validation studies under defined, pre-approved protocols that used worst-case challenge conditions encompassing many of the concepts outlined in section 820.30 [2]. Following the current domestic standards for sterilisation validation, for example, American Association of Medical Instrumentation (AAMI) International Organization for Standardization (ISO) 20857 [3], AAMI ISO 11135-1 [4] for ethylene oxide (EO), American National Standards Institute (ANSI) AAMI ISO 11137-1 [5] and ANSI AAMI ISO 11137-2 [6] for radiation, and AAMI ISO 17665-1 [7] for steam, ensures

that many of the elements of design control are addressed. However, the application of each section of the design control requirements may not be immediately obvious. The following discussion is intended to help clarify these requirements.

4.1.1 Design and Development Planning

This section of the regulation outlines the need for the design to be addressed in a plan prepared before starting the development process.

Some steps used in selecting or choosing a sterilisation method [8]:

- Identification of method(s) factors that appear to be compatible with product design and materials and packaging. Penetration, lethal activity, cost, safety/toxicity, process time, in line processing, release time and availability.
- List options: Costs, in house *versus* contract, regulatory issues and environmental regulations.
- Performance of feasibility studies to determine broad compatibility with selected processes.
- Performance of detailed validation studies to demonstrate product compatibility with the selected process and attainment of the required SAL.
- Identification of potential methods.

Identification of some potential sterilisation methods or related systems:

- Aseptic assembly system - used where terminal sterilisation destroys certain items, for example, some biological and pharmaceutical products - more risks, costly and time consuming.
- Dry heat - simple, typically limited for use with depyrogenation, silicone implants, glass, dental instruments, powders (barium, tantalum, and talc), orthopaedic materials, and sutures but requires heat stable materials for medical devices.
- EO - numerous polymer compatibilities, used in hospitals as well as industry; highly regulated, total processing may be lengthy and consumables are costly.
- Ionisation irradiation - common for heat moisture sensitive products, particularly in industry. Industrial sterilisation, large volume, fast, many materials with some exceptions. Limited primarily to industry because of potential extreme testing, capital costs and safety required for hospitals.

- Liquid chemicals - limited, often for biologics that may not withstand heat irradiation; sterilises some enzymes, tissues, for example, heart valves, but have applications and uses in hospitals.
- Moist heat - most common method, heat damages, but sterilises liquids, used in hospitals and industry, for heat tolerant materials and polymers.
- Ozone (O_3) – a simple process, sterilises some heat sensitive materials and O_3 delivered on site, low cost and less regulated than EO sterilisation. A replacement for EO in hospitals, and a replacement for peroxide/plasma sterilisation techniques.
- Peroxide (e.g., H_2O_2), peracetic acid and plasma – used for heat sensitive materials and others, small volume; a replacement for EO sterilisation. Plasma may be used to reduce residuals.
- Steam-formaldehyde - a combination technique, limited, may be less expensive than EO sterilisation, used for small volumes; decreasingly used and limited.

Discussion of polymers will focus on dry heat, EO, ionisation irradiation, moist heat, O_3 and peroxide/plasma.

For sterile devices, a sterilisation master plan is often prepared that meets this objective. This plan usually addresses the process to be used and the equipment, which needs to be validated. It also defines in general terms the methodology and schedule to be used, outlines the departments responsible, and defines milestones where management reviews are required. It should discuss how to handle new and saleable products prior to any validation activities. The master plan is a dynamic document that should be updated throughout the product development life cycle. Copies of the plan and its updates must be placed in a design history file.

4.1.2 Design Input

The US Food & Drugs Administration (FDA) recognises that the design input stage, sometimes referred to as the requirements stage, is the basis of a successful sterilisation validation programme. The developers must match the product and packaging specifications to the sterilisation process capabilities, taking into account such factors as gas access, material compatibility, safety, manufacturing process requirements, bioburden, and exposure. Any sterilisation process can kill or inactivate only a finite number of organisms. Monitoring the bioburden in advance ensures that the challenge to the sterilisation process is not too great to maintain an assurance of sterilisation effectiveness and efficiency. Bioburden testing can also assist in monitoring the entire manufacturing process, from raw materials, clean room procedures, to packaging.

Some broad product, material design considerations are:

- Dry heat – classically, the product/material must be able to be heated, must be non-distortable and non-melting, heat resistant, and maintain the shape of product.
- EO – this gas must be penetrable to all areas of the product, that are claimed to be sterile, have low EO residual capabilities or be capable of aeration and withstand crazing.
- Ionising irradiation - materials and products must be radiation compatible, no harmful crosslinking, scissoring, or combining within materials and between the parts and must be able to penetrate the density and the shape of the parts.
- Moist heat – materials and product must be able to be heated, must be non-distortable and non-melting, non-corrosive, and heat and moisture resistant.
- O_3 - must be able to penetrate the areas to be sterilised, the materials must be O_3, oxidising, and corrosive resistant materials, the handles' surfaces and the shape of the parts may be critical, and must be able to withstand pressure changes.
- Peroxide/plasma – the products and material must be peroxide (oxidising) and plasma resistant, the areas to be sterilised must be penetrable - typically limited to surface sterilisation; must be able withstand very deep vacuums and pressure changes. Peroxide residuals and regulations for use, may be a problem with using hydrogen peroxide (H_2O_2) alone without plasma.

If requirements are not defined in this phase, the sterilisation method selected and the subsequent validation will be inadequate.

Some further thoughts on selecting a sterilisation process are:

- Do not just assume because a similar product is sterilised by one method, it is the best method of choice and meets the product's and packaging's needs.
- Consider what sterilisation means and what must be achieved for the product or material.
- Make sure that all areas of the product deemed to be sterile are exposed.
- Select the quality or type of sterilisation needed: Commercial *versus* scientific *versus* clinical.
- Provide assurance and certainty that the sterility predictions allow for variations in or from the logarithmic order of death.
- Sterility testing (small or large percentage).

- Risk - not all processes deliver the same risk.
- Just because one method releases faster, does not imply that the method is best, compatible, and stable with all materials or designs.
- The sterility assurance level may vary with the device's use. For example a topical device may only require a 10^{-3} SAL, while an implantable device may require a 10^{-6} SAL. Achieving the SAL will vary with sterilisation method. While EO, dry heat, H_2O_2, O_3 and moist heat will typically demonstrate a SAL based upon the more or most resistant known spore, irradiation of medical devices will not. In Pharmaceuticals, a SAL based upon a more resistant spore (e.g., *Bacillus pumilus*) may be required for irradiation, while it still is not required to be based upon an extremely resistant *Deinococcus* microbe, *Bacillus sphaericus* or an extremely resistant virus. In dry heat, the SAL may be based upon significant endotoxin deactivation levels. However, for industrial moist heat, less resistant spores (e.g., *Clostridium sporogenes, Bacillus subtilis* 5230) than the extremely resistant thermophile *Geobacillus stearothermophilus* may be used.

Some basic cost considerations of various sterilisation methods:

- Dry heat - relatively inexpensive, least expensive oven cost.
- EO - expensive equipment, controls, facility, consumable costs vary depending upon volume or gas type.
- Ionising irradiation - very high initial capital costs, controls, costs vary with volume capacity.
- Moist heat - some initial capital costs - vary with complexity, relatively inexpensive, cost of heat and steam.
- O_3 - initial capital cost; consumable parts – inexpensive.
- Peroxide/plasma - capital cost for only small volume chambers; controls, consumable cost.
- The above methods vary depending upon transportation for contract sterilisation *versus* in-house sterilisation.

After design inputs are converted to specifications, those requirements of the design input stage should be further investigated and evaluated (e.g., qualification, validation). In the development of a sterilisation process, these requirements are that the product has a defined SAL (10^{-6}) and that both the product and packaging remain functional after sterilisation.

Some basic 'do's and don'ts' regarding material compatibilities are:

- Consult with material vendors.
- Don't assume that material vendors' information is complete or accurate for your specific product or packaging needs.
- Consult with contract manufacturers and sterilisation facilities.
- Don't assume that all vendors' studies are complete or all-encompassing for your specific product.

Some broad packaging issues related to different sterilisation methods are:

- Dry heat - requires heat penetrable and heat resistant packaging materials.
- EO - requires penetrable air, humidity, gas, heat, gas elution and pressure/vacuum capabilities.
- Ionising irradiation requires radiation resistant materials, odour removal and radiation stability.
- Moist heat requires packaging to be moisture and heat penetrable, heat resistant and tolerant of pressure/vacuum.
- Ozone - requires oxidation resistant materials and for them to be penetrable to air, humidity, O_3 gas, heat, gas elution and tolerant of pressure/vacuum.
- Peroxide/plasma - materials must be peroxide resistant, be able to withstand very deep vacuum and be peroxide/plasma penetrable and residuals must be removed.

Of the previously discussed methods, determine which equipment, facilities or contract services are available for product introduction. Since these requirements involve different aspects of product development, the input to a sterilisation development programme and its review are multi-disciplinary, requiring the participation of R&D personnel, manufacturing engineers, sterilisation scientists, quality and packaging engineers.

4.1.3 Design Output

The design output stage supports specification development and results in the establishment of all the product specifications. Design output includes a description of the complete specifications and provides the basis for the development of the remainder of the device master record. For sterilisation, this should include a description of any

restrictions on product or packaging sterilant limitations, temperature, moisture, or vacuum, as well as all the quality checks required for sterilisation cycle control and monitoring. It should culminate in a description of the final sterilisation cycle parameters, so that the sterilisation process to be validated should be specified prior to the introduction of a new or altered product, package, or loading pattern that include process definitions, requirements and documentation.

These include:

- Process definition activities such as exposure, dose, temperature, pressure parameters, and phase times, shall be performed in a sterilisation chamber that has undergone installation qualification and operational qualification procedures.
- Process definition may be performed in a pilot or research steriliser or in the equipment to be used to sterilise the product.
- The sterilisation process applicable for the defined product shall be established.
- Documentation and records shall support the validity of process parameters and their tolerances as defined in the process specification.
- The rate of inactivation for the process or cycle should be determined or established.
- If biological indicators (BI) are used as part of the establishment of the sterilisation process then the following shall apply:
    - Comply with Clauses 5 and 9.5 of ISO 11138-2 [9].
    - Be shown to be at least as resistant to EO, as is the bioburden of product to be sterilised.
    - Be placed within the product at location(s) where the sterilising conditions are most difficult to achieve or be placed within a process challenge device (PCD).
    - If a PCD is used for process definition, validation or routine monitoring and control, the appropriateness of the PCD shall be determined. The PCD shall be equivalent or more challenging to the process than the most difficult-to-sterilise part of the product. For information on PCD see AAMI Technical Information Report (TIR) 31 [10].
    - For information on the selection, use, and interpretation of BI, see ISO 14161 [11].

- Commercially supplied BI used in the definition of the sterilisation process should comply with the appropriate clauses of ISO 11138-1 [12].
- If chemical indicators are used as part of the definition of the sterilisation process, these shall comply with ISO 11140-1 [13]. Chemical indicators shall not be used as the sole means of establishing the sterilisation process.
- If tests of sterility are performed during the definition of the sterilisation process, they shall comply with ISO 11737-2 [14].

4.1.4 Design Review

This stage occurs after each step in the design plan. The final cycle documentation must be reviewed and approved by the appropriate individuals, who should include as a minimum: a sterilisation engineer or microbiologist, a packaging engineer, an R&D engineer, and someone from quality control or regulatory affairs. A regulatory affairs specialist is especially pertinent if the intent or claim of the sterilisation cycle validation programme is to conform to a specific domestic or international standard.

4.1.5 Design Verification and Validation

It is common industry practice to validate all sterilisation processes. Prior to validation, the sterilisaton process may be verified in a research, developmental, or even a contract facility to determine if it can be sterilised or not, and materials and packaging are acceptable. Validation however is commonly done under a comprehensive, pre-approved protocol that clearly defines the acceptance criteria of the sterilisation validation study and references a particular standard or guideline. The validation is performed under limiting conditions or worst-case operating conditions and conducted with multiple lots or batches to demonstrate reproducibility.

The results of the sterilisation validation must be detailed in a final report that is reviewed, approved, and signed. The final report and associated protocols should be permanently archived in the validation file, which should be a part of, or referenced, in the product's design history file.

4.1.6 Design Transfer and Changes

After the validation is completed, the specifications are transferred to a manufacturing function. This functional group is typically responsible for assuring that the validated sterilisation cycle parameters are accurately incorporated into the approved

specifications. Any subsequent design changes must be controlled through a formal change-control process. Any changes to the product-process specification must be subjected to the same level of controls and reviews as the initial development. That is, changes must be made under the design control requirements and reviewed and approved by individuals in the same functions and departments as those who approved the original design documentation. Changes to documents, such as correcting text or graphic errors or adding procedural text, must be made under the document controls section of the quality system regulation (820.40) the *Code of Federal Regulations - Title 21: Food and Drugs* [15].

4.1.7 Design Control Conclusion

To effectively integrate sterilisation process development and validation into a design controls programme, medical device manufacturers may need to structure their procedures to integrate the additional review and approval steps at the appropriate intervals as defined in the regulation. An example of a check list for the design considerations for a sterilisation process are given in **Table 4.1**. See **Tables 4.2** and **Table 4.3** for further information about the sterilisation process.

While at first the necessity of these numerous review and approval steps may seem overly burdensome, redundant, and unnecessary, in the long run this comprehensive review process should ultimately lead to a reduction of errors and deviations. Adherence to these concepts will provide assurance that the sterilisation cycle will be effective and will meet all quality requirements, which is, after all, the primary goal of all manufacturers.

Total sterilisation involvement encompasses an interfacial area of investigation and multiple disciplinary backgrounds. A variety of factors and functions encompass sterilisation and require understanding of environmental, physical, chemical, biological, engineering, manufacturing, quality control and assurance, regulatory, and marketing areas.

Table 4.1 Design considerations for a sterilisation process	
Design stage and considerations	**Assigned responsible party, time frame and/or check-off (completed)**
Design input	
Definition of materials, products, packages to be sterilised and end use-group in family types	TBD
Consider type of sterilisation cycle, process and/or method to be used	TBD
Consider sterility assurance level, validation approach and minimum parameters required	TBD
Consider maximum parameters effects on materials/product/ packaging	TBD
Consider product requirements international registrations, 510 k, pre-market approval, new drug approval or other regulatory approvals	TBD
Consider sterility, material safety claims- sterile fluid path, non-pyrogenic, sterility expiration dating	TBD
Design output	
Provide description, drawing, specifications of the product, packaging include: List of plastics/materials in components, package, and product Drawings Product/material/packaging design (comparison to existing device(s) Product palletising patterns	TBD
Consider what verification, validation plans, procedures, documentation need to be addressed	TBD
Consider regulatory requirements for registration, sterilisation/ material safety	TBD
Consider material/biocompatibility sterilisation information: Vendor/supplier information Literature, registration searches Historical information Material safety data sheets Sterilisation cycle specifications	TBD
Detail or revisit sterility, material safety claims	TBD
Detail or revisit environmental regulations, requirements	TBD
Select or reconsider steriliser facilities with information: Name, address Drawing of vessel Equipment Dimension, size of vessel Instrumentation Controls	TBD

Select or reconsider minimum and maximum cycle parameters on basis of expected specification	TBD
Select or reconsider maximum parameters and/or cycles to test materials/product/packaging	TBD
Select or reconsider contract laboratory	TBD
Select or reconsider manufacturer with list manufacturing performance that includes: Name, address, size Organisation Licenses, registrations, permits Environmental control performance, size Manufacturing processes Sterilisation processes	TBD
Design verification	
Perform or review biocompatibility testing	TBD
Perform or review sterilisation compatibility evaluation of component, packaging	TBD
Perform or review failure mode effect analysis (FMEA) of sterilisation of prototype product	TBD
Perform or review product stability-double sterilisation and accelerated aging	TBD
Audit or review, evaluate manufacturing, processes and environment	TBD
Audit or review, evaluate contract steriliser	TBD
Audit or review, evaluate contract laboratory - design reviews/changes	TBD
Assess biocompatibility/physico-chemical results	TBD
Assess sterilisation capabilities and processes	TBD
Assess sterilisation compatibility of component, packaging	TBD
Assess FMEA of double sterilised and accelerated aged prototype product and package	TBD
Assess product accelerated ageing	TBD
Assess laboratories capabilities	TBD
Assess manufacturer processes and environment	TBD
Assess labelling, directions for use	TBD
Design transfer	
Collect information required for transfer for sterilisation adoption or validation(s)	TBD
Collect information for transfer for contract manufacturing and sterilisation agreement(s)	TBD
Collect information for transfer of procedures for pre-sterilisation handling, sterilisation, and post sterilisation of product	TBD
TBD: To be determined by design, quality, manufacturing, and regulatory team, or by author	

Table 4.2 Steps in a sterilisation process from the start of manufacturing to product release. A global and integrated outline of various aspects of industrial sterilisation, in the manufacture of sterile healthcare product, with some related standards		
1. Pre-validation		
Category steps	**Standard**	**Reference number**
General Standards	ISO 9000	[16]
	ISO 9001 - ISO 13495	[17]
General sterilisation criteria	ISO 14937	[18]
Material characterisation, selection	ISO 10993-1	[19]
Material compatibilities	AAMI TIR 17	[20]
Packaging	EN 868-1	[21]
	EN 868-10	[22]
	ISO 11607	[23]
2. Validation of cleaning and/or control		
Environmental control	ISO 14644 – Parts 1, 2, 3, 4, 5, 6, 7, 8	[24-32]
Biocontamination	ISO 14698-1, 2, 3	[33-35],
Product microbial control	USP/NF Compendia	[36]
	ISO 11737-1	[37]
	ISO 11737-2	[14]
BI	ISO 11138 Parts 1, 2, 3, 4, 5, 6	[9, 12, 38-41]
	ISO-11461	[42]
3. Sterilisation control/validation, equipment, installation and operational qualification (differ between sterilisation methods)		
Moist heat	AAMI ISO 17665-1	[7]
	ISO TIR 17665-2	[43]
	AAMI ST 79	[44]
	AAMI TIR 13	[45]
Dry heat	AAMI ISO 20857	[3]
	AAMI ST 50	[46]
Radiation	ISO 11137-1	[5]
	ISO 11137-2	[6]
	ISO 11137-3	[47]
	AAMI TIR 33	[48]
	ISO 13409	[49]

Liquid/chemical	ISO 14160 (animal tissues)	[50]
H_2O_2, O_3	ISO 14937	[18]
EO sterilisation	ISO 11135-1	[51]
	ISO TS 11135-2	[52]
	AAMI TIR 14	[53]
	AAMI TIR 16	[54]
	AAMI TIR 20	[55]
	AAMI TIR 28	[56]
Safety/environment	ISO 14000	[57]
	IEC 1010-2-41	[58]
	IEC 1010-2-42	[59]
Laboratory examination	Product	
ISO certification/audits	Quarantine until release	
4. Method of release(s), per validation standard		
BI, overkill and process documentation	ISO 20857,	[60]
	ISO 11135-1	[51]
	AAMI ISO 17665-1	[7]
	ISO 14937	[18]
	AAMI ST 79	[44]
BI and documentation	ISO 11138 Parts 1-6	[9, 12, 38-41]
Bioburden and documentation	ISO 11737-1	[37]
	ISO 11737-2	[14]
Dosimetric release	ISO 11137-2	[6]
	AAMI TIR 33	[6]
Process control or parametric release	AAMI ISO 17665-1	[7]
	ISO 11135-1	[51]
	AAMI TIR 20	[55]

Note: for sterilisation of reusables see AAMI TIR 12 [62] and AAMI TIR 30 [63].
Other validation standards of interest can be found in references: [64-68].
Other references of interest are: [45, 46, 48, 54-56, 69].
EN: European Norm
IEC: International Electrotechnical Commission
NF: National Formulary
ST: Pre Code for AAMI standards
USP: United States Pharmacopeia

Table 4.3 Some general standards related to different sterilisation methods	
Dry heat	AAMI ISO 20857 2010 [3]
	ANSI AAMI ST63 [70]
	ISO 11607-1 [23]
	AAMI TIR 17 [20]
	ISO 10993-1 [19]
	ISO 11737-1 [37]
	ISO 11737-2 [14]
	ISO 11138-4 [39]
	AAMI ST 50 [46]
EO	ISO 11737-1 [37]
	ISO 11737-2 [14]
	ISO 11135-1 [51]
	ISO 11135-2 [52]
	ISO 11138-2 [9]
	ISO-10993-7 [71]
	ISO 11607-1 [23]
	AAMI TIR 17 [20]
	ISO 10993-1 [19]
	ISO 11138-2 [9]
	AAMI TIR 14 [53]
	TIR 15 [69]
	TIR16 [54]
	TIR 28 [56]
Ionising radiation	ISO 11737-1 [37]
	ISO 11737-2 [14]
	ISO 11137-1 [5]
	ISO 11137-2 [6]
	ISO 11137-3 [47]
	ISO 11607-1 [23]
	AAMI TIR 17 [20]
	ISO 10993-1 [19]
	ISO 10993-17 [9]
	AAMI TIR 29 [61]
	AAMI TIR 33 [48]
	AAMI TIR 35 [72]

	AAMI TIR 37 [73]
	AAMI TIR 40 [74]
Moist heat	AAMI ISO TIR 17665-1 [43]
	ISO 11607-1 [23]
	AAMI TIR 17 [20]
	ISO 10993-1 [19]
	ISO 10993-17 [9]
	ISO 11737-1 [37]
	ISO 11737-2 [14]
	ISO 11138-3 [38]
	AAMI ST 79 [44]
	AAMI TIR 13 [45]
O_3 - heat	ISO 11737-1 [37]
	ISO 11737-2 [14]
	ISO 14937 [18]
	ISO 11607-1 [23]
	AAMI TIR 17 [20]
	ISO 10993-1 [19]
Peroxide/plasma - heat	ISO 11737-1 [37]
	ISO 11737-2 [14]
	ISO 14937 [18]
	ISO 11607-1 [23]
	ISO 11138-6 [41]
	AAMI TIR 17 [20]
	ISO 10993-1 [19]
AAMI TIR 12 [62], AAMI TIR 17 [20] and AAMI TIR 30 [63] should be used for reusable sterilised or resterilisable devices.	

4.2 Compatibility of Materials and Polymers

The compatibility of materials and polymers to sterilisation processes are often too restrictive to accessibility for human use, because they are validated for a single sterilisation process and method. Too often devices are designed for a single sterilisation process where the qualification of more than one sterilisation process would make these devices more accessible for human usage, where more than one sterilisation process are available or useable.

Finding the correct polymers to use for medical devices or biomaterials requires serious consideration of design, processing and performance, including biocompatibility and functionality and ability to be sterilised. The effect of sterilisation on polymers is a key factor in device design. For example device designs with thick and absorbing dense polymers may absorb larger quantities of toxic EO residues or H_2O_2, and limit the penetration of H_2O_2, steam and the less penetrable electron beam. Consequently, polymer compatibility to the sterilisation process is a major consideration, and is the focus of this chapter. As part of the manufacture process for a device, the impact of the sterilisation process on the final biocompatibility and functionality of the device must be evaluated. Both the product biocompatibility, and physical and functionality properties provided must be validated through the intended shelf-life of the device.

Sterilisation processes may initiate deep molecular changes on polymers and such reactions may continue for many weeks to months following the process. Most sterilisation processes affect the physical and chemical characteristics of polymers. Some are gross visible changes in the polymers, while others increase the impurities that may be leached out of the polymer. Multiple sterile cycles to accommodate re-sterilisation should also be evaluated, if desired, from a material compatibility perspective. EO sterilisation is compatible with most materials, except those particularly sensitive to humidity or heat. Radiation sterilisation typically has a larger impact on materials than EO, in particular on polyacetal and unstabilised (or natural) polypropylene (PP), Teflon [e.g., polytetrafluoroethylene (PTFE)], and crosslinking of silicone, as well as other materials that require changes in formulations such as additives, antioxidants, colorants, and stabilisers to make them acceptable. AAMI has published a TIR (AAMI TIR 17 [20]) addressing compatibility of materials subject to sterilisation. Many polymers are considered in general, however, they may be limited because of sterilisation effects on function and biocompatibility. Further principles, such as validation of the shelf-life of the device, apply to any sterilisation process. The application and use of polymers in medical devices is becoming more complex. The effect of sterilisation upon these medical materials and devices is consequently of significant concern.

The number of sterilisation methods that are compatible with most polymers and medical devices are few. Furthermore, there is no ideal sterilisation method that is compatible with all materials and devices. For example, heat may deform, distort, and melt many materials and devices. Radiation may cause odours, degrade, discolour, and off-gassing from some materials and medical devices. EO is likely to be the most compatible process for medical materials and devices, however, it is carcinogenic, costly, explosive, leaves toxic residues, and may require long process times with additional pre-conditioning and aeration. H_2O_2, chlorine dioxide, oxides of nitrogen, peracetic acid and ozone are recently introduced methods and may damage and oxidise several medical materials and devices, but are safe to use with many others.

Correct, accurate and very informed material selection is critical to the manufacturer of safe and successful part design and medical devices. Because of the vast array of materials, criteria and processing methods, the task of selection may be difficult and complicated. It is important to source material information and consider numerous design factors and other device criteria. Finally understanding the compatibility of the material and the medical device to the sterilisation method to be selected is critical.

The effect of the sterilisation processes on medical materials and polymers may provide reasons why one method of sterilisation is used and why another is not considered. However, qualifying more than one sterilisation process will improve the accessibility of your device to the patient. What methods are available for your medical polymer and device is among the factors necessary for determining your sterilisation method(s) of choice.

4.3 Sterilisation Processes for Polymers

The number of methods capable of sterilising product or material without adversely affecting their product quality or material compatibility is very few. A few that are reported in AAMI TIR 17 [20] are: dry heat, EO and H_2O_2, moist heat, radiation and O_3. They can be categorised as either chemical agents or physical agents.

4.3.1 Chemical Agents

Traditional methods are EO and glutaraldehyde. Non-traditional methods may be H_2O_2 with plasma and O_3. Novel agents may be chlorine dioxide, H_2O_2 vapour, peracetic acid vapour, nitrogen dioxide and supercritical carbon dioxide.

4.3.2 Physical Agents

Physical agents include:

- Heat sterilisation: steam (moist heat) and dry heat.
- Radiation: gamma, electron beam irradiation, ultraviolet light and X-rays.

A variety of factors must be carefully considered in selecting sterilisation processes without adversely affecting polymers. For example, steam or dry heat sterilisation will melt or degrade some plastics. Chemical agents such as EO will leave toxic residues, H_2O_2 and oxidising agents will oxidise. Irradiation may discolor, cause odours, as well as damage some materials.

Manufacturers should be selective in their choice of polymers for designing components and devices. They should also become aware of how the polymers may interact with various tissues, particularly during long-term implantation. Concern for polymer compatibility will ultimately provide for longer life cycles and better cost-effectiveness for the user.

4.3.3 Ethylene Oxide Sterilisation Process

EO is a standard method that is able to sterilise many polymers, but not liquids and can cause crazing in some polymers, for example, acrylonitrile-butadiene-styrene (ABS), and it may leave toxic residues (EO) and by-products (ethylene chlorohydrin and ethylene glycol). EO is also a very hazardous material. It is highly explosive and may form toxic by-products.

EO is a process that has some penetration capabilities, but requires a long time for the overall process, for example, pre-conditioning, sterilising, and aeration. EO is an effective and soft sterilant for most medical materials, polymers and devices and re-usables. It used in 'both' hospitals and industrial manufacturing applications for the manufacture of disposables. EO will sterilise most polymeric materials. Common limitations of EO sterilisation relate to diffusion barriers, process time, and interactions. Diffusion barriers present a limitation to the efficacy of EO sterilisation if the EO gas, temperature and humidity necessary for the sterilisation process do not penetrate into all locations within the device, for example, into a stopcock or a very long, thin lumen or large dense product load. Long overall process times may be an economic limitation to the application of EO due to long pre-conditioning periods, extended exposure times, post-sterilisation aeration times and post-processing BI testing. Parametric release has traditionally been difficult to achieve uniformly with this method, but there is a growing need to reduce overall times, but its effectiveness and efficacy is limited because of diffusion barriers, process time, and toxic residuals. While parametric release is difficult to achieve uniformly with this method, faster release times may be achieved with the use of reduced, rapid BI incubation times.

Hazardous material and toxic residuals are issues since EO is regarded as an explosive chemical, potential human carcinogen, reproductive toxin and developmental toxin. It requires use of gas mixtures or special handling, robust scrubbers on gas emissions and worker exposure is a consideration. Post sterilisation evaluation for toxic residuals (EO and ethylene chlorohydrin) must be performed before release or during the validation of the sterilisation process with each device.

4.3.4 Ozone

O_3 is a very strong oxidising agent, making it an efficient sterilising agent. It is a relatively new process for medical devices, making it a non-traditional process, although it has been used to sterilise water and so on. In a gaseous or vapour form, O_3 may be used to sterilise medical products and other materials within a chamber. Because O_3 is a metastable product, it may not be stored and is therefore produced *in situ*, making it an environmentally safe and acceptable process. Its penetration may be limited or slow with organic matter. At the end of the process, the O_3 is degraded to oxygen (O_2). Because of the strong oxidising nature of O_3, materials must be resistant to oxidation. The potential disadvantage of O_3 includes its oxidising reactivity with certain polymers. It may have some penetration limitations, for example, through organic matter and non-diffusible polymers.

O_3 sterilisation has great potential as a sterilisation method and is very green, creating O_3 on site. Advantages of using O_3 are:

- O_3 sterilisation processes are particularly suited for sterilising some heat sensitive materials. O_3 sterilisation is inexpensive.
- O_3 breakdown products lead to environmental safe breakdown products- O_2 and water vapour.
- O_3 is easily produced on site, not transportation of toxic consumable. Consequently it is not hazardous outside its chamber.
- It has excellent microbial sterilising capabilities.
- It can sterilise many low temperature materials, as well as higher temperature materials. Many hospital products can be re sterilised.
- It can potentially process some cellulosics, but not all.

The principal disadvantage of O_3 sterilisation is its inability to sterilise all polymeric materials, and it does not have the penetrating capability of EO, however, it is much faster than EO, and it has the ability to sterilise more lumens and crevices than H_2O_2 vapour.

4.3.5 Hydrogen Peroxide

H_2O_2 and plasma H_2O_2 has excellent microbiocidal properties, poor penetration but is environmentally acceptable under process control. H_2O_2 is typically used in the vapour phase for medical materials and devices. While it is compatible with many polymers, there are some materials that are damaged, for example, acrylics,

cellulosics (includes paper), natural rubbers, bioadsorbables such as polyglycolides and polyesters. It does not have the same penetration as pulsating or pressurised steam, dry heat, EO or irradiation. It is a surface sterilant.

It may sterilise some lumens, but not lengthy ones. It may not sterilise some polymer materials and devices in their entirety. While its outcome is typically safe, it begins with a source of a very hazardous high concentration of H_2O_2. Plasma is effective in breaking down the H_2O_2 into water and O_2. Because H_2O_2 has a very high vapour or boiling point, it requires use of a very, very deep vacuum that may adversely affect some packaging and materials or products. Sterilisation by this process is typically achieved in small sterilisation vessels, not large chambers or facilities as with dry heat, EO, radiation or steam.

Use of H_2O_2 with plasma is not considered to be a traditional method but an alternative non-traditional method. H_2O_2 may be the method of choice for decontamination in certain applications and is widely used in hospitals, although H_2O_2 is extremely toxic and must be handled and controlled with care. Consequently systems must permit that it is safe for the environment and healthcare workers and that it leaves no toxic residues.

H_2O_2 without plasma is not considered to be an approved alternative method but is still a novel process, because it is not as microbiocidal as H_2O_2 with plasma, and it must have aeration to eliminate the toxic residues, that plasma destroys. Its principal advantages are:

- It is simple to operate, install and monitor.
- It is compatible with many medical devices and materials.
- It is environmentally safe (no toxic residues or emissions).
- Plasma breaks down H_2O_2 into reactive species, including hydroxyl radicals and atomic O_2.
- Its breakdown residues of H_2O_2 are O_2 and water vapour.
- It is a relatively rapid process - no post sterilisation aeration is required.
- It is a good candidate for many low temperature materials that tend to be hydrophobic and chemically stable, that resist oxidation and moisture.

Its principal disadvantages are:

- Unlike EO, it does not easily penetrates through packaging and lumens, crevices of many devices.

- It typically has only 'surface' contact capability, and cannot penetrate like EO.
- The number of materials compatible with H_2O_2 while extensive has some notable exceptions, for example, cellulosics (papers), natural rubber, avoidance of absorbers (polyamide (PA), polyurethane (PU), and avoidance of decomposers (silver, copper, copper alloys) and liquids.
- Plasma will affect some materials by surface modification - sometimes the effect is temporary.
- Devices should be evaluated for surface modifications and effects on functionality.
- Because of the deep vacuum required for sterilisation, the items to be sterilised must be able to withstand the pressure changes. Some devices use special venting caps for pressure equalisation.
- Some data reported suggest environmental isolates can be of significantly greater resistance than BI to H_2O_2 in isolation chambers. Also, there may be difficulties with BI outliers or rogues, which may survive a process when they are intended to be inactivated.

4.3.6 Chlorine Dioxide

Chlorine dioxide is one of the least used sterilisation methods. Chlorine dioxide is toxic, but it can be produced on site, rather than requiring shipping and it can be neutralised, however, potential residuals remain a concern. The major obstacle of chlorine dioxide is its unknown material compatibility and its toxic by-products or residuals. Consequently, chlorine dioxide may not be considered an approved alternative process, but is still a novel process.

4.3.7 Oxidising Agents

Examples of oxidising agents are: H_2O_2, peroxide plasma, halogens, O_3 and oxides e.g., NO_2. These agents however cannot sterilise ubiquitous paper, some adhesives, and some metals without concern, because of their oxidation potential or oxidising capacity. They are typically less penetrable than EO, but are considered a more environmental friendly (green) process than EO.

4.3.8 Dry Heat Sterilisation

Dry heat sterilisation is a traditional method, but it can alter, burn, damage, distort,

or melt many materials. Heat such as steam may wet, distort, soften, expand, corrode and affect product functionality. Dry heat may melt and may not sterilise aqueous exposed materials. Dry heat may sterilise some powders and heat resistant polymers. Steam and dry heat have many similarities including ease of control and monitoring, they are inexpensive, produce no toxic residues or wastes as EO or radiation may do.

Dry heat typically requires higher temperatures and the use of heat tolerant polymers for the same time as steam sterilisation. Consequently, polymers may have to tolerate higher temperatures than steam, unless it is possible to use longer exposure times at lower temperatures. Under such circumstances, dry heat may become more compatible with more heat tolerant or sensitive polymers than steam. Heat is an effective process for heat tolerant materials, devices, items and products. Many of which, have been designed to be re-sterilised. Dry heat is typically recommended only for those materials that may not be sterilised by steam or in the case of certain glass containers, oil, powders, some polymers and so on where it is undesirable to use steam. With the advent of spacecraft decontamination, the applicability of dry heat at lower temperatures has increased its use with more heat tolerant polymers. Dry heat has also been used to sterilise silicone prosthesis, and other medical devices. Use of dry heat demands that the melting limits of polymers exceed its sterilisation temperature and exposure.

Dry heat sterilisation is not commonly used in the medical device industry, but it is used for glass sterilisation and depyrogenation in the pharmaceutical industry. It is used in silicone implant sterilisation in medical devices, and has previously been used in spacecraft sterilisation/decontamination. It is applicable to heat resistant materials and can be effective as low as 105-135 °C, making it compatible with numerous polymeric materials for example, high-density polyethylene (HDPE) and PP. Also, dry heat is compatible with silicone that can be crosslinked with irradiation, residuals with EO and impermeable with moist heat. In the dental area, chemiclaves have been used for improved results of sterilising potentially corrosive instrumentation. Further future work in this area could evolve an environmental green processes that could sterilise with dry heat at temperatures lower than 105 °C. Dry heat has also been conventionally used for years for depyrogenation that other sterilisation methods cannot achieve. At extremely high sterilisation temperatures which destroy organic matter down to the elemental carbon backbone level, dry heat may be considered an absolute sterilisation method, since all life consists of organic carbon compounds, including prions.

4.3.9 Moist Heat (Steam) Sterilisation

Steam sterilisation is a popular technique and is predominantly used in the pharmaceutical industry, hospital health facilities and laboratories where re-usable materials and products are frequently re-sterilised. It is less used in the medical device industry. It is a traditional method. It is also widely used in the decontamination of infectious waste materials. The major concern and limitation of this method is the degradation or destruction of materials by heat or moisture and non-heat tolerance of polymers. In the pharmaceutical industry, the major concern may be the rate of pharmaceutical degradation relative to the rate of biological or chemical inactivation. However, moist heat can sterilise acetal, glass, liquids, PP and Teflon fibres or celluloses (papers that may be damaged by other methods. Compatibility with the high temperature and moisture resistant polymers are required for moist steam cycles. Steam sterilisation is predominantly used where re-usable materials and products are frequently resterilised. If prudently applied and controlled moist heat does not corrode metals as indicated in AAMI TIR17 [20]. Steam is frequently compatible with liquids. Steam may sterilise most metals, glass, and a large number of heat resistant plastic materials. The number of materials capable of being steam sterilised will vary considerably with the temperature of sterilisation. Standard steam sterilisation is generally carried out at 121 °C for 15 min. Steam sterilisation may be reduced, however, to as low as 105 °C, depending upon the bioburden, device design and heat resistance of the polymer material to steam heat. With recent emphasis on the environment and toxicity, ease of sterilisation of the cotton mould *Pyronema domesticum*, and sterilisation of prions, there is continuing renewed interest in steam and its compatibility with the environment (greening) and health safety.

Moist heat sterilisation is the method of choice for critical items not damaged by heat. It is the preferred method for loose instruments, packs and other items that are not heat sensitive or vulnerable to moisture. It is used in aseptic assembly and sometimes for sterilisation-in-place (SIP). Typical sterilisation is performed at 121 °C for 15 min or longer. Moist heat is applicable to moist and heat resistant materials. In moist heat sterilisation, moist heat can be effective as low as 105 °C for parenteral solutions and medical devices, where thermophilic microbes are not pathogenic. Moist heat has been the preferred method for sterilising instrumentation, heat tolerant materials, for example, fabrics, paper and for decontamination. For potential contaminated materials, and many re-useables (pans, basins, baskets, instruments, procedure trays and so on), steam sterilisers in healthcare facilities remain the work horse.

Its principle advantages are:

- It is non-toxic.

- It is simple process that can be process control released (e.g., release by process parameters without use of BI or sterility testing), with relatively short exposure times.
- It is relatively inexpensive. Typical steam sterilisation equipment costs less than one-third as much as an EO chamber system and controller. It costs less than one-quarter as much as gamma or electron-beam equipment and facilities.
- It is available and most widely used in hospitals.
- It can be used to sterilise many re-useable devices.
- It lacks toxic residues.
- It can sterilise a myriad of products, for example, foods, many drugs, and it can be used for decontamination.
- It is efficient and fast.
- It can sterilise many liquids, items, and re-useable devices.
- Steam sterilisation is used in decontamination sterilisation of instruments and devices that are potentially contaminated with prions, with higher temperatures and exposures or with processing aids such as sodium hydroxide.

Its principle disadvantages are:

- It has the potential to burn.
- Its high temperature leads to some material incompatibility (e.g., damage to ABS, acrylics, low-density polyethylene (LDPE), styrene) due to its high caloric heat and moist sensitive polymers.
- It can corrode and dull instruments.
- It is not as penetrable as EO or irradiation.
- The source of steam can be contaminated and requires a good quality water supply.
- Air can be a barrier to steam diffusion.
- It cannot sterilise powders or moisture sensitive material.
- Some types of heat sensitive materials may be better sterilised by other methods, for example, irradiation, EO, H_2O_2, O_3 or chlorine dioxide.

4.3.10 Radiation

Irradiation has been used for more than 50 years to sterilise medical devices because of its high reliability, safety applications, relative ease of validation and strong technical support. Radiation sterilisation has excellent penetration capabilities. It is a relatively short process. Radiation sterilisation is typically achieved with ionising isotopes, for example, ^{60}Co, or high voltage accelerators. Initial capital cost is very expensive. It is effective for many medical materials and devices for a singular dose, but typically poor for re-usables. It is typically not used in hospitals, but principally for industrial applications, for manufacture of disposables.

Radiation is an inherently fast and simple process (requiring only the delivered dose (e.g., 15-45 kGy), resulting in its easy application. Polymer compatibility is the major limitation for application of this method and must be overcome for its usage and application. Radiation may make deep molecular changes in polymers and require shelf-life testing to demonstrate that these changes do not continue to do damage. One limitation to the radiation treatment is the limited number of polymeric materials, which degrade because of the effect of the radiation on polymer bonds of which the materials are composed. Multiple re-sterilisation by this process is not commonly practiced, but it is applied typically to disposable (one time use) devices.

Damage of some polymers and materials by radiation will alter the molecular structure of many polymers *via* crosslinking or chain scisson or others mechanisms (**Figure 4.1**).

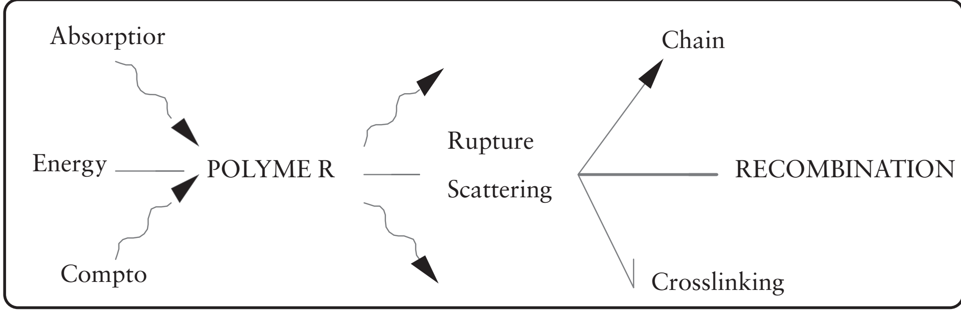

Figure 4.1 Some radiation molecular effects on polymers

Radiation may cause odours, discoloration, embrittle and degrade a few materials, or affect bond strengths, and cause changes over the shelf-life of the product. Radiation

may destroy some plastics such as acetal, unstabilised PP and some Teflons, and crosslink silicone.

However, radiation is compatible with many polymeric materials at a sterilisation dose of up to 50 kGy with a few exceptions. For example acetal, natural PP, and some Teflon material are potentially damaged by irradiation. Man-made material limitations, however, can be overcome. Radiation sterilisation typically requires qualifying a material over a long time for verification of stability.

Its principal advantages are:

- No toxic residual agents;
- Good penetration;
- Dosimetric release (quick);
- Short exposure times (fast);
- Simplicity of routine operation compared to EO; and
- It sterilises many heat and moisture sensitive materials as well as others.

Its principle disadvantages are:

- The facilities and equipment are expensive.
- There are elaborate safety criteria.
- It can generate a few by-products, for example, O_3, and leachables (plasticisers), pH shifts, 4,4'-methylenedianiline (MDA), odours, and discoloration in some materials.
- There are some changes in materials, for example, brittleness, odour, discoloration, stiffening, cracking, but many of these effects can be overcome with changes in material stability evaluations and so on.
- There are a few polymers that are not usable (natural PP, acetals, Teflon), some others may be modified to be useable (e.g., acrylics, polycarbonate (PC), polyvinyl chloride (PVC), PP, PU, silicones and so on.
- Radiation creates radical formation in water or liquid.
- It has limited application in tissue and drug irradiation.
- It cannot typically sterilise active electronics.

- Material selection may become more of an issue with radiation than with EO and require long stability evaluations.

The major green problem with irradiation lies with gamma irradiation (^{60}Co, ^{137}Cs) with its radioactive source material that requires special handling, facility controls and hazardous source disposal, but like EO, it has been possible to control and handle the source material safely.

Electron beam sterilisation is, in many respects, a sterilisation panacea. It has no toxic gases, emissions, residuals toxic disposal issues, and is extremely fast (minutes). However, it does not have such deep penetration as gamma does. Consequently it can sterilise minimum package sizes and density. There has recently been some success in sterilising tissues at cold temperatures.

X-ray sterilisation is a process with the capabilities of both electron beam and gamma irradiation, without any radioactive or hazardous source material. It has attributes of both electron beam and gamma irradiation. Like electron beam it can sterilise without any radioactive materials and it is faster than gamma irradiation. Like gamma it can penetrate deeply through dense loads and materials. But it requires very large volume capacities to be economically efficient, as either electron beam or gamma rays.

4.4 Effects of Different Sterilisation Processes on Polymers

Sterilisation processes are typically harsh on polymers, resulting in physical and chemical effects on the polymers, as well as deep molecular changes on materials that may be visible as well as affect mechanical, functional, safety and toxicity of polymers.

4.4.1 Ethylene Oxide Sterilisation Process

EO sterilisation is very gentle on polymers compared to other sterilisation methods. Limitations related to material compatibility typically relate to polymer's EO adsoptivity and some sensitivity to humidity, such as hydrophilic coatings. Users also need to be careful with EO sterilisation when using polymers as a carrier for drug delivery, and its EO residues reactivity, temperature and humidification. A pharmaceutical drug such as Taxel based-formulations may not withstand high temperature and high humidity EO cycles.

EO will sterilise most polymers and materials for many healthcare products. **Table 4.4** shows the compatibility of some polymers with EO.

Table 4.4 Compatibility of materials with EO - rating 1-5 (1 - best to 5 – worse) - for more details see AAMI TIR 17 [20]	
Material	**Compatibility rating**
Fluoropolymers	1
PTFE	1
PFA	1
PCTFE	1
PVF	1
PVDF	1
ETFE	1
FEP	1
Thermoplastics	
ABS	1-2
Polyacetals	1
Polyacrylates (e.g., PMMA)	2-3
PA (e.g., Nylon)	1
PC	1
Polyesters, saturated	1
PE, various densities	1
Polyimides (e.g., PEI)	1
Polyketones (e.g., PEEK)	2
PP	1
PP - natural	2
PP – stabilised	2
PS	2-4
PSF	1
PU	2-4
PVC acetates	4
PVC	1
PVC – plasticised	1
SAN	2-4
Thermosets	
Epoxies	1-2
Phenolics	2
Polyester, unsaturated	1
Polyimides	1

PU	2-5
PU - Aliphatic	3-1
PU - Aromatic	3-1
Adhesives	
Acrylic	2
Epoxy	1-3
Fluoroepoxy	Unknown
Silicone	1-2
Elastomers (residuals can be a problem)	
Butyl	1
EPDM	1
Natural rubber – Latex	2
Nitrile	1
Polyacrylic	3-4
Polychloroprene (Neoprene)	2-3
Silicone residuals may be a problem	1
Styrene-butadiene	1-2
Urethane will vary with formulation	2-4
Metals (some metals may cause polymerisation depending on conditions)	
Aluminum	1-2
Brass	1
Copper (EO may polymerise)	2-3
Gold	1
Magnesium (EO may polymerise)	Unknown
Nickel	1
Silver (EO may polymerise)	1
Stainless Steel	1
Tin (EO may polymerise)	
Titanium	1
Ceramics/glasses	
Aluminum oxides	1
Silica	1
Zirconium oxides	1
Liquids	
Other materials	
Bioabsorbables	

Bioabsorbables - PGA	5 - Not likely
Bioabsorbables - PLA	5 - Not likely
Cellulosics	
Esters	1
Cellulose acetate propionate	1
Cellulose acetate butyrate	1
Cellulose, paper, cardboard	1
Liquid crystal polymers	1-4
EPDM: Ethylene propylene diene terpolymer ETFE: Ethylenetetrafluoroethylene FEP: Fluorinated ethylene propylene PCTFE: Polychlorotrifluoroethylene PE: Polyethylene PEEK: Poly(ether ether ketone) PEI: Polyetherimide PFA: Perfluoroalkoxy PGA: Polyglycolic acid PLA: Polylactic acid PMMA: Polymethyl methacrylate PS: Polystyrene PSF: Polysulfones PVF: Polyvinyl fluoride PVDF: Polyvinylidene fluoride SAN: Styrene/acrylonitrile	

EO sterilisation uses several different conditions during routine processing, namely, heat, evacuation, moisture, pressure changes, and exposure to EO and/or its non-flammable diluents. Materials, products and packaging should be designed to allow for the exchange (replacement and removal) of air and penetration of steam, heat and EO. Consideration should be given to the potential physical and chemical effects of these conditions and the formation of residuals. During the EO sterilisation process, materials and products may be subjected to environmental and physical stresses such as vacuum and pressure changes, elevated temperature and changes in humidity. The materials and products may also react with EO and/or sterilising gases used. The material and product design should ensure that functionality and safety are not compromised by exposure to the anticipated range of sterilisation conditions.

Post-sterilisation evaluation for toxic residuals (EO and ethylene sterilisation) must be performed before release or validation of product. Long exposure times and post-sterilisation aeration times as well as post-processing BI testing may reduce the use of this process on a practical basis.

4.4.2 Dry Heat Process

Dry heat is typically used for sterilising oils, petroleum jellies, surgical catguts, surgical (dental) instruments, glassware, including vials for pharmaceutical drugs, powders and silicon prosthesis devices. It is used to sterilise dental instruments to minimise the corrosion of sharp items. It is commonly used in laboratories for depyrogenation of glassware. It has been used as a method of choice for spacecraft sterilisation in the United States, for sterilising electronic boards and other moist heat sensitive materials and products, however, sterilisation with steam frequently results in the wetting of materials or devices that have been sterilised. Dry heat does not have any wetting problems and it can sterilise many polymers (see **Table 4.5**)

Table 4.5 Dry heat material compatibility (rating 1 - best to 5 – worse 1-5 and N for not recommended). See AAMI TIR 17 [20] for more details

Materials	Compatibility rating
Fluoropolymers	
PTFE	1
PFA	1
PCTFE	2
PVF	2-3
PVDF	2
ETFE	1
FEP	1
Thermoplastics	
ABS (a few lower heat resistant grades, e.g., 110 °C)	3-N
Polyacetals	1-2
Polyacrylates (e.g., PMMA)	4-N
PA (e.g., Nylon)	1-4
PC	3-4
Polyesters, saturated	N
Polyesters (PE), various density	4-N
Polyimides (e.g., PEI)	1-2
Polyketones (e.g., PEEK)	1
PP	2-4
PP - natural	4

PP – stabilised	2-3
Polyphenylsulfone	2
PS	N
Syndiotactic PS (heat resistant)	1-2
PSF	4
Polysulfone	1-2
PU	4-5
Poly(vinyl alcohol) (PVA)	4-5
PVC	4-N
PVC (plasticised)	4-N
SAN	4
Thermoplastic rigid PU	1
Thermosets	
Epoxies	1-3
Phenolics	2-3
Polyester, unsaturated	3
Polyimides	1-2
PU	4-5
PU – aliphatic	5
PU – aromatic	4
Adhesives	
Acrylic	4-5
Epoxy	1-2
Fluoroepoxy	2-3
Silicone	1-3
Elastomers	
Butyl	2-4
EPDM	2-3
Natural rubber – latex	4
Nitrile	4
Polyacrylic	5
Polychloroprene (Neoprene)	4-5
PU thermoplastic elastomer	2
Silicone	1
Styrene-butadiene	3
TPPO	2

Urethane	4-5
Metals	1-4
Aluminum	1
Brass	1
Copper	2
Gold	1
Magnesium	3-4
Nickel	1
Silver	1
Stainless steel	1
Titanium	1
Ceramics/glasses	1-4
Aluminum oxides	2
Silica	1
Zirconium oxides	2-4
Liquids (aqueous – no, oils - varies)	
Other materials	
Bioabsorbables	
PLGA	**N**
PLA	**N**
Cellulosics	3-4
Esters	3-5
Cellulose acetate propionate	3-5
Cellulose acetate butyrate	3-N
Cellulose, paper, cardboard	4-5
Liquid crystal polymers	1-2

PLGA: Poly(lactic-*co*-glycolic acid)
PP: Polypropylene
TTPO: Thermoplastic polyolefin elastomer
Note: the lower dry heat sterilisation temperature is below typical 160 °C (e.g., 105-150 °C), more polymers become compatible, tolerable, and useable in dry heat sterilisation; also those polymers that are affected by moisture from moist heat sterilisation, may be useable in dry heat sterilisation.

Representative polymers may possibly be sterilised by dry heat below the melting or the degrading temperatures. The effects on the materials from dry heat sterilisation are due to exposure to elevated temperatures generally in excess of those seen in a

moist heat process. Dry heat processes typically use longer exposure times and/or repeated exposures to the conditions. Materials processed in dry heat sterilisation could demonstrate the following qualities:

- Material performance.
- Changes in physical properties, such as charring, cracking, dulling, discoloration, distortion, deformation, melting, softening or shape changes.
- Changes in chemical properties, such as decomposition, generation of gases, polymerisation, formation of toxic compounds or corrosion.
- Differences in expansion rates, which could cause damage to mated parts and stiffness.

Metals subjected to dry heat sterilisation may be affected, material fibres may be damaged and rubbers and plastics altered as temperatures reach or exceed the glass transition and melting points. It should be noted that oxidative processes occur in this sterilisation process.

Dry heat may not sterilise liquids, unless sealed, only oils and some other high boiling substances.

It may sterilise silicone without the crosslinking effects of irradiation, or the accumulation of EO residuals, and the limited penetrating and wetting problems of steam. It may sterilise acetals or PP up to 120 °C and Teflons (e.g., FEP, PCTFE) up to 170 °C whereas irradiation damages, destroys and/or embrittles some of these fluorocarbon polymers.

To achieve dry heat sterilisation requires removing significant moisture and contacting areas to be sterilised with elevated temperatures for extended periods of exposure time. Overcoming stratification of temperature and overcoming difficult to penetrate areas, for example, joints and mated surfaces is important. Transfer of heat to polymers and plastics is slow. Sterilisation by dry heat requires longer exposure times and temperature than by steam. So its damage often is due to longer exposure times at the same temperature as steam.

4.4.3 Hydrogen Peroxide

H_2O_2 is an oxidising agent that can be used as either or liquid or as gas. This section deals with sterilisation of H_2O_2 in the gas phase. H_2O_2 and oxidising agents can be used to sterilise a number of polymers and materials (See **Table 4.6**).

Table 4.6 H_2O_2 material compatibility (rating 1 – best to 5 – worse 1-5 and N for not recommended) See AAMI TIR 17 [20]

Material	Compatibility rating
Fluoropolymers	
PTFE	1
PFA	1
PCTFE	1
PVF	1
PVDF	1
ETFE	1
FEP	1
Thermoplastics	
ABS	1
Polyacetals	1
Polyacrylates (e.g., PMMA)	3-4
PA (e.g., Nylon)	1-5
PC	1
Polyesters, saturated	1
PE, various density	1
Polyimides (e.g., PEI)	1
Polyketones (e.g., PEEK)	1
PP – natural	1
PP – stabilised	1
PS	1
PSF	1
PU	2-3
Polyvinyl acetates	1
PVC	1
PVC, plasticised	1
SAN	1
Thermosets	
Epoxies	1
Phenolics	2
Polyester, unsaturated	1
Polyimides	1
PU	2-3
PU – aliphatic	2-3
PU – aromatic	2-3
Adhesives	
Acrylic	3
Epoxy	1-2
Fluoroepoxy	3
Silicone	3

Elastomers	
Butyl	2-3
EPDM	2-3
Natural rubber – latex	2
Nitrile	2
Polyacrylic	3
Polychloroprene (Neoprene)	1
Silicone	1
Styrene-butadiene	1
Urethane	2
Metals	
Aluminum	1
Brass	1
Copper	2-3
Gold	1
Magnesium	2
Nickel	1
Silver	2-3
Stainless steel	1
Titanium	1
Ceramics/glasses	
Aluminum oxides	1
Silica	1
Zirconium oxides	1
Liquids	No
Other Materials	
Bioabsorbables	2-4
PGA	2-4
PLA	2-4
Cellulosics (virtually no compatibility)	
Esters	4-5
Cellulose acetate propionate	4-5
Cellulose acetate butyrate	4-5
Cellulose, paper, cardboard	4-5
Liquid crystal polymers	1
Note: some materials that are compatible may have higher peroxide residuals with H_2O_2 only (alone) than with *H_2O_2 with plasma*. More polymers may be compatible with H_2O_2 with plasma than with H_2O_2 with no plasma.	

H_2O_2 with plasma is more limited than EO, because it is less permeable than EO gas, however, EO has longer processing times, more parameters, higher residuals and less

process control than H_2O_2 with plasma, which makes it (H_2O_2 with plasma) more attractive on a smaller production volume scale. Because of the oxidative nature of H_2O_2 some materials may not be recommended for use. When designing for devices, it is best to avoid decomposers—such as silver, copper, and copper alloys—and absorbers, such as PU, PA, ethylene vinyl acetate, and cellulosics. Durability is dependent upon specific moulding conditions, for example, a component with high residual stress may be less durable than a component that has been stress relieved. It may be important to recognise that material compatibility information for H_2O_2 vapour may not apply to H_2O_2 gas plasma sterilisation.

Plasma and oxidising agents are generally applied only to small niche and minimal sized loads or devices. Biocompatibility and functionality of polymers need to be further evaluated, depending upon their end use. Its use is predominantly in the general hospitals, and less in the medical device manufacturing industry.

4.4.4 Ozone Process

O_3 sterilisation has recently been introduced to healthcare facilities. It has, however, been recognised as a sterilant for over 100 years. It is a safe process because the O_3 is introduced *in situ* into a sterilising chamber. There are many polymers and materials that it may process adequately and others that it does not – **Table 4.7** shows some of these. O_3 may be more compatible to more polyacrylics, including elastomers, than the other sterilisation methods compared.

There are no toxic residues as such (except for the oxidised by-products that are created), and it may be more penetrable than H_2O_2 vapour (with plasma), but not as penetrable as EO, propylene oxide (PPO), steam, dry heat or irradiation.

In gaseous low temperature O_3 sterilisation, the typical process variations or parameters required, are vacuum, time, temperature, O_3 concentration, humidity, and pressure (rate, level, or both). The O_3 concentration is typically ~85 mg/l for 15 min at a temperature of 30-36 °C. The process temperatures are generally low (<36 °C), making it suitable to use for temperature sensitive materials.

Table 4.7 Polymer and material compatibility with O_3 sterilisation (Rating 1 – best to 5 – worse 1-5 and N for not recommended). For further details see TIR 17 [20]

Materials	Compatibility
Fluoropolymers	
PTFE	1
PFA	1
PCTFE	1
PVF	2-3
PVDF	1
ETFE	1
FEP	1
Thermoplastics	
ABS	1
Polyacetals	2
Polyacrylates (e.g., PMMA)	2
PA (e.g., Nylon)	2
PC	1
Polyesters, saturated	1
PE, various densities	2
Polyimides (e.g., PEI)	1
Polyketones (e.g., PEEK)	1-2
PP	1
PP – natural	1
PP- stabilised	1
PS	4
PSF	2
PU	4-5
PVA	2-4
PVC	1
PVC, plasticised	2
SAN	Unknown
Thermosets	
Epoxies	1-2
Phenolics	1
Polyester, unsaturated	1
Polyimides	Unknown
PU	4-5
PU – Aliphatic	4-5
PU – Aromatic	4-5

Adhesives	
Acrylic	2
Epoxy	1-2
Fluoroepoxy	2
Silicone	2
Elastomers (O_3 has poor elastomer compatibility, except with silicone)	
Butyl	4-5
EPDM	3-4
Natural rubber – latex	4-5N
Nitrile	Unknown
Polyacrylic	2
Polychloroprene (Neoprene)	4-5
Silicone	1
Styrene-butadiene	4-5
Urethane	4-5
Metals	
Aluminum	1
Brass	2-4
Copper	2-4
Gold	1
Magnesium	Unknown
Nickel	4-5
Silver	4-5
Stainless steel	1
Titanium	1
Ceramics/glasses	
Aluminum oxides	1
Silica	1
Zirconium oxides	1
Liquids (O_3 added to liquids separately)	
Other Materials	
Bioabsorbables	
PGA	Unknown
PLA	Unknown
Cellulosics	
Cellulose esters	2-5
Cellulose acetate propionate	2-5
Cellulose acetate butyrate	2-5
Cellulose, paper, cardboard	2-5
Liquid crystal polymers	1-2

For gaseous O_3, adequate materials and devices for its low-temperature sterilisation should be resistant to oxidation and moisture. Some materials and devices remain unknown in terms of compatibility. Woven materials such as PS, polyurethane butyl, natural rubber, polychloroprene, nickel and silver are not likely to be compatible. However, some cellulosics may be compatible. The shape of the device and material as well as the design of a device may be closely related to its stability and resistance of the device to sterilisation. Device and polymeric parts with wide surface to-mass ratios (e.g., fibrous material) may undergo faster oxidative degradation. While such devices and materials may be adequate for single use or used in the manufacture of a device that has limited re-use, such a condition might not be satisfactorily used for a device with a longer expiration period.

O_3 and oxidising agents are generally applied only to small niche and small sized loads of devices. Their use is predominantly in hospitals and currently limited in industry.

While O_3 and H_2O_2 are both oxidising agents, they may cause different types of damage.

O_3 may sterilise cellulosics better than H_2O_2, while, H_2O_2 may sterilise butyl rubber and natural rubber better than O_3. Silicone is sterilised better by O_3 than by H_2O_2.

4.4.5 Radiation Sterilisation Process

Polymers that are particularly sensitive to radiation are unstabilised PP, acetals, Teflon, PGA sutures, polymethylpentene and PVDF. Material degradation may be reduced by effective device design and material selection, i.e., the use of materials with appropriate additives and modifications in the polymer chains. Active electronics are also typically not compatible, but an increasing number are compatible with irradiation. Premature aging of plastics may occur due to ongoing oxidative effects in some materials. It is, therefore, always prudent to evaluate accelerated and real time aging of plastics to ensure that this is not a problem under real life conditions. Another means to overcome material compatibility issues in some cases is through the reduction of sterilisation dose required to achieve the desired SAL. Also, it is important to note that the compatibility of materials is a strong function of the application, and the related material stresses. For example, in some cases it is possible to utilise Teflon with radiation sterilisation despite it not being generally acceptable. Additional information about radiation sterilisation material compatibility is provided in AAMI TIR 17 [20], however, the biocompatibility and functionality need to be further considered and evaluated depending upon the end use of the polymer and under what conditions it will be used.

Radiation is increasingly used for sterilisation of many plastics in numerous medical devices today by means of additives and modifications in the polymer chains.

Understanding basic radiation chemistry may help to assess why a particular plastic is affected in a certain way. When a plastic is exposed to gamma radiation, in the case of ^{60}Co with energy levels of 1.33 and 1.17 MeV, molecular bonds are broken. The polymer may either recombine into its original configuration or, if cross-scission occurs, the molecular weight of the molecules is reduced and the polymer is weakened. Conversely, where crosslinking occurs, a large three-dimensional matrix is formed and the polymer is strengthened.

The effects of radiation on polymers are determined by:

- The chemical composition and formulation of the polymer.
- The morphology of the polymer (percentage of crystallinity, molecular weight, and density).
- Radiation dose and dose rate that is applied.

It is important to know that higher molecular bond energies produce more stable molecules and those polymers with a benzene ring are generally very stable. Oxidation, caused by the presence of O_2 in the gamma radiation process, may decrease crosslinking and increase degradation, or produce a tendency for chain scission to occur. Oxidation also causes peroxide, carbonyl, and hydroxyl groups to be formed. Post-irradiation effects on polymers may be attributed to trapped free radicals, the presence of peroxides, and possibly trapped gases. These post-irradiation effects help explain why a PP component may appear acceptable today, but will shatter in two years' time.

4.4.5.1 Some Materials Adversely Affected by Irradiation

A few plastics that may be adversely affected by sterilisation radiation doses, for example, 25 kGy may be sterilised by lower doses (>11 kGy). Some plastics which are sensitive to radiation are: unstabilised PP, acetal, Teflon, polyglycolic acid sutures, polymethylpentene, and PVDF.

All plastics are affected by irradiation to some extent. Some effects are favourable or negligible, while others are not:

- PE is predominantly crosslinked, but radiation treatment is acceptable. Slight odours may be associated with it, but these may be reduced through modification of the formulation.

- PP is both crosslinked and scissored. Embrittlement, breakage, and discoloration may occur.
- Polymethylpentene is affected in a similar way to PP.
- Radiation stabilised PP polymers, however, are available, using high molecular weights, co-polymerised and alloyed with PE with additional stabilisers. Use of electron beams at high irradiation dose rate may further reduce the oxidative degradation of PP.
- PS is very stable to radiation because of its benzene ring. It may begin to yellow at irradiation above 50 kGy.
- ABS is much less resistant to radiation than PS, but it may be suitable for a single dose of irradiation. High impact grades are less radiation resistant than standard grades.
- PVC may be compatible to radiation, but squelching of HCl, prevention of discoloration and plasticiser leaching, must be considered. Addition of antioxidants and heat stabilisers help.
- Resterilisation is not likely. Single use predominates. However, plasticised PVC may be resterilised.
- Acetal or polyformaldehyde copolymers are sensitive to radiation and their chains are easily scissored (embrittlement), and the material often changed from solid to dust, with a colour change from yellow to green.

PA are sensitive to radiation and this results in crosslinking, but many are suitable for a single dose; some for a multiple dose. PA10, PA11, PA12, and PA66 are more stable than PA 6. PA films and fibres are less resistant to irradiation.

There are some general considerations to be made in selecting plastics for irradiation:

- Aromatic polymers, for example, benzene rings, are more stable than aliphatic ones.
- Look at the ratio of scissoring and crosslinking.
- Polymers with low radical yields (G-values) after irradiation are more stable.
- Phenolic antioxidants contained in most polymers are responsible for discoloration. The use of non-phenolic additives will usually eliminate the problem.
- Most natural PP and PTFE (Teflon) are unstable with irradiation.

- PVC and PP should be specially stabilised to improve radiation compatibility.
- Polyacrylics, acrylics, PMMA. PMMA discolors with irradiation, to the extent that contact lenses made of PMMA are not suitable for use after irradiation. However, other uses of acrylics may be acceptable with colorant additives and so on. Polyacrylonitrile is more heat resistant than PMMA, and can be copolymerised to be more radiation resistant.
- High levels of antioxidants help radiation stability. In general, one may need to increase the level if the product is to be radiation sterilised.
- Within a given polymer class, the lower the density the greater the radiation stability.
- The elastic modulus is not greatly affected with one sterilising dose of radiation.
- Fillers and reinforcing materials usually improve the radiation stability of adhesives, coatings and potting compounds.

Polymers used in adhesives, films, fibres, coatings and encapsulates react much the same way to irradiation as the materials from which they are derived:

- Take extra care with nucleated polymers – nucleation increases embrittlement.
- If co-polymerisation with ethylene is possible – try it.

Some examples of radiation-compatible plastics are:

- Elastomers – silicones (peroxides and platinum cured), thermoplastic elastomer, polystyrene-ethylene-butylene styrene, TPPO, natural rubber (Isoprene), EPDM, urethane, nitrile, butyl and styrene-butadiene.
- Fluoroplastics (other than PTFE and FEP) – PVDF, PCTFE and polyethyltetrafluoroethylene.
- 'High-end' engineering resins – polyether ketone, PEEK and PEI.
- PA (Nylon), especially aromatics, PA12, PA11, PA6/12 and PA6/10.
- PE, LDPE< linear low-density polyethylene, HDPE and ultra-high molecular weight polyethylene (UHMWPE).
- PE and polyester terephthalate glycol.
- PC and its alloys.
- PSF.

- PVC flexible and semi-rigid, colour, plasticiser and hydrogen chloride corrected
- Polyurethane (8 chemical varieties).
- PP (stabilised) and polypropylene copolymers (PPCO) and polymethyl pentene – radiation stabilised.
- PS and co-polymers, ABS, PS and SAN.
- Thermosets – epoxies, phenolics, polyimides, polyurethanes and polyesters.
- Electronic circuit boards, typically are not always compatible, but some are compatible with irradiation. Premature aging of plastics may occur due to the oxidative effects of irradiation, consequently it is always prudent to evaluate accelerated aging of plastics to assure that this is not a problem under real life conditions.
- Some Teflons despite their high heat resistance are degraded by radiation, and generally not acceptable, although some thin films/coating and certain types of Teflons have been demonstrated to be radiation compatible at low doses.
- ABS and PC is generally considered to be acceptable to one dose of radiation.
- Acrylic polymers are sensitive to radiation. The effect of scissoring of the ester chain is the main effect of radiation. Polymethacrylate has been used for dosimeters, because it changes colour proportionally due to its sensitive to irradiation within sterilising dose ranges. However, radiation compatible acrylics are available.

Radiation tolerance of polymers must be balanced against resistance of product contamination, for example, the presence of *Acinetobacter* may be a concern. As part of an epidemiological investigation of hospital infections caused by *Acinetobacter spp*., the radiation resistance of 15 clinical isolates and four reference strains were assessed. The radiation resistance (equivalent to 10^6, decimal reduction values or 6 D-values), which is the dose necessary for reducing the initial number of colony forming units by a factor of 10^6 was, in general, higher in the isolates of *Acinetobacter radioresistens* than in the isolates of the *Acinetobacter calcoaceticus* – *Acinetobacter baumannii* complex and of *Acinetobacter lwoffi*. However, the least resistant isolates *of A. radioresistens* had a D-6 value equal to or lower than the most resistant isolates of the other groups. The lowest D-6 values found were for two of the reference strains. The highest D-6 value was 35 kGy, which is higher than many conventional radiation sterilisation processes. Three isolates of *Acinetobacter johnsonii* could not survive long enough in a dried preparation to make an assessment of the D-6 values possible. However, the continuation of the presence of clinical *Acinetobacter* in hospitals should raise a concern regarding adequate sterilisation or sterility assessments.

4.4.6 Moist Heat (Steam) Sterilisation

Different factors should be considered when selecting a material for compatibility to moist heat. Among them, it is important to recognise the influence that angles, load, mass, or stress might have on a material during moist heat sterilisation. Typically, the lower the temperature of the moist heat sterilisation process, the more materials are compatible, stable, and tolerable.

The glass transition temperature (T_g), as well as melting temperatures of many polymers is typically a good indication of material rigidity and compatibility to heat. For example, processing below a T_g typically maintains the optimal rigid compatibility of the polymer to moist heat. As the temperature of the polymer drops below the T_g, the polymer typically becomes more hardened or brittle. As the temperature rises above the T_g, the polymer becomes more rubber-like and capable of elastic or plastic deformation without fracture. Elastomers, in general, have a T_g considerably below room temperature but are moist heat compatible above their T_g. Reaching the melting temperature of the polymer must be avoided. Knowledge of the maximum operating temperature, upper service temperature, or heat deflection temperature of the material under consideration is key to ensure that the parameters for moist heat sterilisation cycle are suitable. Polymer heat stability will also depend on molecular orientation. Materials with a high degree of crystallinity typically will enhance thermal stability.

The number of polymers capable of tolerating moderate temperature and moisture are more numerous than often considered (see **Table 4.8**).

Unlike most other methods, steam is compatible with many liquids (including many drugs) or many filters that sterilise drugs. Steam may sterilise most metals (e.g., instruments), glass (bottles), and a large number of heat resistant plastic and polymer materials and devices.

Table 4.8 Moist heat material compatibility (rating 1 – best to 5 – worse 1-5 and N for not recommended). See AAMI TIR 17 [20] for more details	
Materials	Compatibility rating
Fluoropolymers	
PTFE	1-3
PFA	1-2
PCTFE	2
PVF	2-4
PVDF	2
ETFE	1
FEP	1
Thermoplastics	
ABS	1
Polyacetals	2-3
Polyacrylates (e.g., PMMA)	4-5
PA (e.g., Nylon)	1-4
PC	2-4
Polyesters, saturated	2-5
PE, various densities	2-5
Polyimides (e.g., PEI)	1-2
Polyketones (e.g., PEEK)	1
PP	1-4
PP – natural	3-4
PP – stabilised	1-3
PS	N
PS – syndiotactic (heat resistant)	1-2
PSF	4
PU	4-5
PVA	4-5
PVC	4
PVC plasticised	3
SAN	4-5
Thermoplastic polyurethane, rigid	1
Thermosets	
Epoxies	1-3
Phenolics	2-3

Polyester, unsaturated	1-2
Polyimides	1
PU	4-5
PU - Aliphatic	4-5
PU - Aromatic	4-5
Adhesives	
Acrylic	4-5
Epoxy	1-3
Fluoroepoxy	1-2
Silicone	1-3
Elastomers	
Butyl	2-3
Copolyester ether elastomer	5
EPDM	1-2
Natural rubber – latex	4
Nitrile	2-4
Polyacrylic	5
Polychloroprene (Neoprene)	4-5
Polymethylsiloxane	3
Silicone	1-4
Styrene-butadiene	3
PU thermoplastic elastomer	N
Urethane	4-5
Thermoplastic elastomers	1-2
TPO	1-2
Metals	
Aluminum	2-3
Brass	1
Copper	2-3
Gold	1
Magnesium	2-3
Nickel	1
Silver	1
Stainless steel	1
Titanium	1
Ceramics/glasses	1-2

Aluminum oxides	2
Silica	1
Zirconium oxides	2-3
Liquids (aqueous – no, oils- varies)	
Other materials	
Bioabsorbables	
PLGA	4-5
PLA	4-5
Cellulosics	3-4
Esters	3-5
Cellulose acetate propionate	3-5
Cellulose acetate butyrate	3-N
Cellulose, paper, cardboard	2-4
Liquid crystal polymers	1-2
Note: the lower the steam temperature below 121 °C (e.g., 105-120 °C) more polymers will become compatible, useable and reuseable in moist heat sterilisation.	

Drugs that are moist heat sterilised come in various forms such as:

- Parenteral or irrigating solutions.
- Many antibiotics, antiseptics, medicated gauzes and dressings.
- Various individual drugs - see Pharmacopoeias.

Besides aqueous drugs, there are also biologics such as:

- Amino acids, fat, vitamins, peptides, proteins, bags for blood and biological collections.
- Biological warfare agents, prions, may require 134 °C for greater than 1 h.
- Infectious wastes.
- Blood bourne diseases.

Plastics transfer heat more slowly than metals and may take longer to reach sterilising temperatures in the autoclave. Because of the differences in heat transfer characteristics between plastics and inorganic materials, the contents of plastic containers may take longer to reach sterilisation temperature (for example 121 °C). Therefore, longer autoclaving cycles are necessary for liquids in large-volume plastic containers.

Adequate cycles may be determined only by experience with specific liquids and containers.

4.4.6.1 Miscellaneous Concerns

Some miscellaneous concerns in steam sterilisation are:

- Some chemical additives in steam will attack transparent plastics and cause a permanently glazed surface after autoclaving.
- Some transparent plastics (e.g., PVC) may absorb minute amounts of water vapor and appear cloudy after autoclaving. The clouding will disappear as the plastic dries. Clearing may be accelerated in a drying oven at 110 °C. For PVC tubing, clearing is obtained at or above 75 °C for 2 h.
- PPCO bottles should be used instead of PSF bottles if there is Tween in the autoclave, because Tween will harm PSF at elevated temperatures, but not PPCO.
- Steam sterilisation of PU may result in the formation of toxic, leacheable MDA.
- Mixing of polysilicone and PU may result in acceptable biocompatibility.

Improvements in computer controls, monitoring devices, biological and chemical indicators and processing have paved the way for renewed applications of moist heat technology, and the growing need for more compatible materials. Sterilising some polymers in heated water can lead to less distortion than sterilising in just steam. Improvements in polymers for steam sterilisation, as in the case of radiation sterilisation, are being made with addition of heat stabilisers and co-polymerisation, improved polymerisation with metallocenes, pelletisation and moulding temperatures, and creation of syndiotactic polystyrene.

4.5 Enhancing the Properties of Polymers Using Sterilisation

4.5.1 Ethylene Oxide Sterilisation

A potential way to lower EO cycle times, as well as reduce toxic residual levels, is to increase the sterilising temperatures from traditional levels of 45-60 °C to 70-80 °C, as has been used with steam/formaldehyde. The higher temperature drives the EO and ethylene chlorohydrin residuals toward ethylene glycol, which is not as toxic according to the recent ISO 10993-7 [71]. Ethylene glycol is not regarded as a significant

residual, as EO and ethylene chlorohydrin are, except in ocular contact. Since the higher temperature and moisture at 70-80 °C would create more ethylene glycol, it should not be a significant problem as previously discussed before. Improvements of plastics with heat stabilisers and co-polymerisation would enhance the number of plastics that could be sterilised at these slightly increased temperatures.

Lower temperature and lower humidity are parameters, which at times, enhance the properties of some polymers, biomaterials, enzymes and electronics. For example, sterilisation of a monitoring device with active electronics and a polymer-enzyme complex was improved by reducing the relative humidity, temperature and EO concentration for sterilisation.

Pre-conditioning of some polymers (for example, cuprophane) prior to EO sterilisation allows for EO sterilisation of this moisture sensitive dialysing material, without in-vessel humidification. EO sterilisation requires that aeration and ventilation of toxic residues occur to minimium acceptable levels before medical devices or biomaterials are releaseable.

4.5.2 Moist Heat (Steam) Sterilisation

While polymers may be selected based upon melting temperatures to exceed sterilisation processing temperature, lower steam sterilisation temperatures may be implemented so that polymers with lower melting temperatures may be used. Possible future considerations include alternative or combination approaches to steam sterilisation. For example, dialysers may be steam SIP on carousels and released in a just-in-time fashion through process controls and parametric release. These dialysers may also be sterilised with water at high temperatures. Many pharmaceutical/healthcare plastic containers, such as HDPE, PVC and polyallomer (a copolymer of propylene and PE) filled with liquids may be steam sterilised at lower temperatures than 120 °C. Some of these containers with biomaterials are attached to devices and are classified as medical devices.

Steam sterilisation with other agents may further reduce sterilisation temperatures and acceptable lower melting temperatures of polymers. Currently there is a steam formaldehyde sterilisation method that runs between 65-85 °C. This approach could be applied to PPO resulting in a preservative as a by-product such as propylene glycol that may be beneficial to incorporation of some biomaterials.

While PPO may not penetrate as well as EO, PPO will diffuse and penetrate better than chlorine dioxide, H_2O_2, or H_2O_2 with plasma and O_3. PPO may be more stable and less reactive to materials at temperatures >36 or 55 °C for O_3 and H_2O_2, respectively.

Furthermore, PPO may not require as deep a vacuum (for example, 133 Pa) as O_3 and H_2O_2 for sterilisation and penetration.

High-density materials are typically more resistant than are low-density materials (e.g., PE). Sterilising some heat sensitive materials in heated water rather than steam vapor and pressure, will lead to less distortion and damage. Sterilising other materials in steam air pressure will lead to less pressure distortion of some materials and products.

4.5.3 Radiation Sterilisation

Irradiation sterilisation utilises high energy radiation to break or disrupt molecular bonds which ionise and/or excite material bonds. The level of ionising energy, dose, received by the product to achieve sterilisation is measured in kGy and may be delivered *via* gamma or X-ray photons or directly *via* high-energy electrons.

Reducing the irradiation dose (e.g., 25-15 kGy often results in enhanced properties of the polymers. Also the use of nitrogen in place of air helps to reduce the effect of oxidation of some polymers. Reducing temperatures down to 10 °C or lower (dry ice) also allows for sterilisation of very sensitive biomaterials.

The application of electron beams in place of -irradiation also enhances the properties of a number of polymers, by reducing the oxidative effects, but may result in slightly higher temperatures. Gamma irradiation may have a lower dose uniformity rate than the electron beam process, which makes it difficult to validate the product at its maximum dose delivered. Less oxidative effects may be found with X-rays as well as electron beams. Use of X-rays will also result in lower temperatures generated from the impact of electrons on the materials. It may have an excellent dose radiation uniformity ratio.

Aromatic materials are more radiation resistant that are aliphatic materials (e.g., PU). Aliphatic PU may breakdown to create toxic compounds.

4.5.4 Hydrogen Peroxide and Oxidising Agents

A reduction in concentrations of H_2O_2 and/or O_3 will improve the properties of some polymers, but may increase processing times. Increasing the temperature may reduce the processing time.

The use of the highest molecular weight materials (with the narrowest molecular weight distribution) is optimal for most applications.

4.6 Some Properties of Sterilised Polymers Following Implantation

There is a great possibility that damaged polymers could be implanted in the human body. It is a requirement that polymers be sterile, safe and non-toxic for implantation after sterilisation. One of the immediate considerations of properties of sterilised polymers following implantation, is that they must be sterile inside/within the polymers and not just on the surfaces or in the lumens. It is vital that polymers and biomaterials to be used as implants be sterilised in their entirety or as they come in to contact with tissues and so on during the period in which they are implanted. If they are going to be implanted long-term they need to be completely sterilised. Microbes (spores) entrapped within polymers will typically be more resistant to sterilisation than those on the surface, and over time may activate, germinate, and grow out from their entrapped site, and infect the human host upon exposure or outgrowth.

This means that the sterilised product must be typically totally evaluated, not just its surfaces, but areas within the polymers. Also, one must apply only sterilants that are capable of penetration. H_2O_2, steam, and O_3 are not penetrable sterilants unless the materials are highly diffusible. Dry heat, EO and irradiation are. Electron beams have less penetrable properties than gamma- or X-ray irradiation.

Typically PE is not permeable to steam or humidity, however, EO will drive humidity and moisture through PE films. Nylon is permeable to moisture, but not EO alone, however, moisture will take EO through Nylon films. Penetration of EO is important.

UHMWPE has been used in orthopaedic implants, particularly for surfaces, which are subject to high stress such the cavity of replacements hips or shin plates in knee replacements. However, 'lower molecular weight PE' could not withstand the high stress. Radiation performs better with high molecular weight PE than low molecular weight PE but EO is gentle on PE, but must be aerated to remove toxic residues, or they may be irritation of tissues, carcinogenicity, haemolysis, sensitisation and so on, from the presence of EO. Oxidation of UHMWPE by gamma irradiation results in some degradation. EO gas is a viable alternative to γ-irradiation that avoids the oxidation and fatigue strength degradation known to accompany irradiation of PE polymer bearing surfaces in total joint implants.

Acrylics have been used in implantable ocular lenses, bone cement for fixation of joint prosthesis, and in dentures, and maxillofacial prostheses.

Acrylics are sensitive to irradiation, and their use in implantation would not last long. EO is a more gentle sterilant and would improve the possibilities of implantation. PMMA has better properties than just polymethacrylate, and may have a higher melt temperature of 125 °C; consequently it may be sterilised with a lower temperature dry heat.

Commercially available biodegradeable polymers are employed in sutures, orthopaedic fixation devices, dental implants, ligature clips, tissue staples and skin covering devices. One example of the most widely used polymers are polyhydroxyl acids such as PLA and its co-polymer, PLGA - these implants are only required to serve for a certain time period ranging from weeks to months. Functional behaviour of these implants is generally determined by their glass transition temperature that may be as little as 10 °C. Additionally residual stresses may remain in the moulded parts after manufacturing which may lead to deformation on heating above the transition temperature. PLA, PGA and their copolymer PLGA are hydrolytically unstable. Consequently these polymers are affected by sterilisation. Moist heat and dry heat may lead to hydrolysis or melting of the implants and their deformation at higher temperatures. EO as a chemical may act as a plasticiser for the polymer. Additionally, EO sterilisation at 50-60 °C is well above the critical temperature for the polymer. Complete removal of residual traces from the gas is also difficult to achieve. H_2O_2 with plasma is a surface sterilant, and the bioresorbable implants need to be sterilised in their entirety to preclude patient infection during their degradation. Irradiation at 35 kGy may induce degradation of the polymer chain and result in reduced molecular weight and influence the mechanical properties and degradation profile of the polymer. However, radiation sterilisation at lower temperatures (e.g., 10 °C, dry ice) may be effective at 16 kGy or higher.

Silicone is another material used in implantation. Silicone is used for breast implants and other prosthesis. The sterilisation of implants with EO for example is strongly dependent upon the quantity of viable bioburden and non-viable materials, including particles, oils, proteinaceous films and extraneous production debris at the time of sterilisation.

Accumulation of particles, oils and hydrophobic substances may agglomerate microbes and protect them from the sterilant. Breast implants are filled with silicone gel and oils are particularly inappropriate substances for this method as well as for irradiation, which may crosslink silicone and make it stiff. Furthermore, EO is highly absorbed in these silicone gels, requiring extremely long times for off-gassing, that may not be reduced to safe limits. Breast implants and other silicone prosthesis often have multiple cavities and imperfections, which may harbour bioburden. Some types of silicone polymers are worse than others. Steam sterilisation is not a viable alternative to these multiple impenetrable cavities and the non-hydroscopic silicone.

Broad microbial contamination of silicone prosthesis and multi-lumen implants could result from the application of steam or EO sterilisation. Harbouring of viable microbes constitutes a significant risk of infection.

Most implants undergo one or more thermal treatments during their production, incidental to extrusion, moulding, vulcanisation and so on, which would impart some sterilisation or decontamination of the heated components. If applying good clean room conditions, the subsequent dry heat sterilisation process is expected to impart sterility on various silicone implants and prostheses. Silicone is highly heat resistant. The dry heat sterilisation process should be well established for this application, if bioburden quantities are kept low and under control. If it is sufficiently developed and validated it will yield reliable silicone products with an excellent level of sterility assurance of the probability of a survivor. For practical purposes sterilisation never leads to an absolutely sterile product, unless performed at extremely high temperatures that would carbonise organic matter.

Any polymer applied in any medical device or biomaterial must be demonstrated to be non-toxic, biocompatible and safe to the FDA and other regulatory agencies before use as an implant. Materials selected must meet the stringent requirements of ISO 109993-1-17 [75, 76] and more.

The materials are tested after exposure or special treatment to the sterilisation process (typically double sterilisation). The biological testing of the polymer is dependent on the intended contact duration and body contact. Contact durations are classified as limited (<24 h); prolonged (24 h to 30 days), and permanent (>30 days). Body contact polymers are then categorised as surface contact, external communicating, or implant. Implant polymers have the most stringent requirements (see Table 1 and Table 2 in ISO 10993-1 [19]).

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5 Current Alternatives, Non-Traditional and Novel Sterilisation Methods

A new generation of sterilisation advances and magic is emerging ...

5.1 A Few Alternative Sterilisation Methods

The number of methods capable of sterilising product or material without adversely affecting their product quality or material compatibility is only a few. The small numbers of methods that are reported in American Association of Medical Instrumentation (AAMI) Technocal Information Report (TIR) 17 [1] are: dry heat, ethylene oxide (EO), hydrogen peroxide (H_2O_2), moist heat, radiation and ozone (O_3).

5.1.1 Traditional Methods

Traditional methods are dry heat, EO, moist heat, and radiation, and possibly liquid chemical sterilants such as glutaraldehyde for sterilising single-use devices incorporating materials of animal origin, while alternative methods include $H_2O_2$with and without plasma, O_3, chlorine dioxide (ClO_2), EO within diffusible packages, peracetic acid (PAA) and so on (see **Table 5.1**).

Corporations may be looking for a silver bullet or an environmental friendly (green) sterilisation process that meets their particular needs, however, in sterilisation, the number of agents or processes capable of 'just' sterilising (achieving a sterility assurance level (SAL) of 10^{-6}) without adversely affecting material or product quality are few and limited (**Table 5.1**). A number of these non-traditional methods include oxidising agents such as ClO_2, H_2O_2 with and without plasma, PAA, O_3 and possibly oxides of nitrogen.

Alternative (oxidising agents) do not have the penetrating capabilities of traditional sterilisation methods such as dry heat, EO, moist heat, and irradiation. They are typically surface sterilants. Consequently, devices and products to be sterilised by alternative sterilants must be 'effectively cleaned' prior to sterilisation.

Table 5.1 Identification of some current alternative sterilisation methods	
ClO_2	This is not widely used possibly because of limited information on its material compatibilities, and its residuals, however, it has been EPA registered, and previously applied to sterilise oxygenators, contact lenses, and is currently used in isolators as well, and decontamination of the Hart Building during the threat of *Bacillus anthracis* contamination. Generally, ClO_2 still remains a novel process among some regulators. It has possibilities because of penetration.
O_3	This is a simple process for some heat sensitive materials, and O_3 is created on site, and has been approved for sterilisation of some materials and devices in hospitals in place of EO. It is considered more than a novel process and it is approved for use in hospitals.
H_2O_2	The vapours are used for heat sensitive materials in larger volume chambers than with H_2O_2 with plasma. The latter is used in smaller volume chambers. H_2O_2 without plasma has greater residuals than H_2O_2 with plasma. It still has not been cleared by the FDA for use in sterilisation in healthcare (hospital) facilities.
H_2O_2 with plasma	This is widely used and approved for use in hospitals in place of EO for many applications. H_2O_2 vapour with plasma is accepted as an alternative process, but H_2O_2 vapour without plasma is still deemed to be a novel process. It cannot penetrate as well as EO sterilisation. Other possible future (vapour) peroxides are oxides of nitrogen, PAA, nitrogen oxide, and performic acid. PAA with plasma is still considered a novel sterilisation method.
EO in-a-bag	This is an alternative sterilising agent for use within and by diffusion through bags. It differs from traditional EO because it sterilises within a bag, and diffuses out of the bag. It measures EO by weight (grams), rather than mg/l (concentration).
X-rays	These may be an alternative to γ-rays and electron beams in industrial sterilisation, and may be more adaptable to healthcare facilities that already use X-ray technology.
Liquid sterilant chemicals	These are limited - some alternatives may be liquid ClO_2, PAA, H_2O_2 (6%), superoxidised water (chlorine), OPA and glutaraldehyde.
EPA: Environmental Protection Agency FDA: US Food & Drugs Administration OPA: *Ortho*-phthaldehyde	

In general, traditional heat can distort and damage many polymeric materials, radiation can damage some polymeric materials and many materials are not typically

reusable, chemicals (for example, EO) can be extremely dangerous, toxic, heavily regulated, and leave residuals and so on. Consequently non-traditional methods are needed particularly in hospitals and for reusables.

5.2 Alternative Methods

Alternative sterilisation methods may be non-traditional as acceptable methods or as novel methods that may yet require approval by regulatory bodies (for example, the FDA).

Unfortunately most of the non-traditional alternative methods, except for X-rays and EO sterilisation within diffusible bags, do not have great penetration capacity to inactivate microbes within sealed, long lumens, mated surfaces and other non-surface areas; many are limited in the length and size of the lumens that they can reach. Most of the oxidising agents are good to excellent surface sterilising processes.

For example H_2O_2 with plasma and O_3 are newer and more recent methods that are deemed as acceptable and more readily approved for medical applications, where surfaces and some lumens are achievable, and some materials are compatible, but they are not as compatible (e.g., cellulosic, absorbers and so on) to as many materials as X-rays and EO sterilisation within diffusible bags.

Other non-traditional methods (**Table 5.2**), however, are deemed to be novel methods and include methods such as ClO_2, H_2O_2 vapour, PAA and EO processing within bags, that have an EO concentration of (7.2 g) instead of 500-600 mg/l may use an EO cartridge or capsule, with humidity producing chips and a long exposure dwell time of greater than 8 h.

Alternatives can be classified between non-traditional methods, which may be readily accepted, and non-traditional methods that are regarded as more novel methods, requiring future acceptance and approval by regulatory bodies such as the FDA.

There are no safe sterilants, only safe ways of using them. Several factors can affect the efficacy and effectiveness of typical sterilisation processes and these are shown in **Table 5.3**.

Table 5.2 Some acceptable alternative and potential sterilisation methods - advantages and disadvantages [2, 3]

Chemical	Advantages	Disadvantages
H_2O_2 with plasma	• Very rapid cycle times. • It is thought not be carcinogenic with plasma. • Peroxide residuals can be easily eliminated. • No aeration is required. • It is environmentally friendly to many products. • It has proven efficacy. • It is an approved non-traditional method. • It is suited for using with embedded electronics, except for embedded batteries.	• It is restricted on the length and diameter of lumens it can sterilise. • It cannot be used with cellulose (paper), cotton, linens, liquids or urethanes. • Some materials cannot be resterilised or reprocessed. • PA and acetals have a limited life. • It uses small chambers. • It cannot penetrate or sterilise items with a small diameter or long lumens. • It requires use of special packaging. • It has limited penetration, it is more of a surface sterilant. • It requires deep vacuums. • Higher cost than simple H_2O_2 vapour (without plasma). • It may adversely affect embedded batteries and semiconductors.
H_2O_2 – no plasma	• Uses a larger chamber than plasma peroxide. • Requires less expensive equipment than with plasma. • Rapid cycle time. • Limited toxic residues compared to EO residuals. • Less expensive than H_2O_2 with plasma.	• Without plasma it may require aeration to remove residuals from some materials and loads. • It is a novel process, and is not an authorised method for hospitals. • Its deep vacuum may affect embedded batteries.
O_3	• It is a very strong oxidising agent It breaks down quickly. • It can be produced on site where it is needed. • It is ultimately reduced to O_2, with no adverse residuals. • It has fast cycle times. • It is an approved, non-traditional method. • It penetrates longer lumens than H_2O_2 vapour.	• A number of materials cannot be sterilised or resterilised with this method. • It has limited penetration, compared to EO. • Products with a large surface-to-mass ratio (e.g., fibres) will undergo fast oxidative degradation. • It is not for devices with a long life span. • Materials and medical devices should be resistant to deep vacuum (135 Pa) as it causes surface oxidation.

		• Materials need to be resistant to moisture, but it should not be applied to textiles, PU, butyl or natural rubber, zinc, silver, nickel or copper and its alloys. • It may adversely affect embedded batteries with deep vacuum, or copper in electronics.
Chlorine dioxide	• It is not a carcinogen, it does not react the same as the other oxidising agents on materials. • It has a short exposure time. • It can sterilise many materials, for example, ABS, PA, PMMA, PE, PP, PS, Teflon, Vitron and stainless steel. • Penetration is good for longer lumens and dead ends, but is not as good as EO. It retains its activity in organic matter unlike other oxidants • It has short cycles. • It has been used to decontaminate rooms and buildings, for anthrax. • It has greater diffusability and penetration potential than other oxidising gaseous agents. • ClO_2 can be produced on site. • It can sterilise at very low temperatures (e.g., 25-30 °C. • It can sterilise electronics, batteries, and semiconductors that other sterilant agents may not be able to without some damage or adverse effects.	• Uncoated aluminum and copper, PC and PU may be adversely affected depending upon the formulation, however, it may leave or create toxic by-products. • It is highly toxic. • With a 30 min dwell time, a ClO_2 gas concentration of approximately 30 mg/l in 80-85% RH at 30 °C is necessary. • Corrosion may occur. • It may have a D-value of 4.1 min. It is, however, only regarded as a novel process, and is not cleared by the FDA. • Further information is necessary for it to be accepted in healthcare applications, however, it has been accepted as a *B. anthracis* decontamination process. But, in the latter situation, ClO_2 has emerged as the preferred biocide against anthrax-contaminated sites, having been employed in the treatment of numerous government buildings (e.g., the Hart Building) over the past decade. • Its chief drawback is the need to produce *in situ* processes to have the reactant on demand and material unknowns.
PAA (vapour)	• It was originally used as a decontamination agent and then used with plasma. • PAA with plasma breaks down into acetic acid, water and O_2, all of which have a low toxicity. • It is not absorbed into cellulosic materials as H_2O_2 typically is.	• It is a strong oxidiser. • Residual acetic acid may be corrosive to certain materials (for example, copper, metal acetates). • It cannot be used on liquids or products likely to be damaged by a deep vaccum. • It is still a novel process.

	• It is safe for employees and the environment. • It lasts longer allowing for deeper penetration.	
PAA (Immersible)	• With system control it removes salts, protein and microbes. • By-products are environmentally friendly. • It has a rapid cycle time.	• It has had some regulatory issues. • It sterilises only a small number of instruments at a time. • Some materials in small sterilisers are incompatible.
Performic acid	• No toxic residues. • Slightly higher effectiveness than H_2O_2 and PAA. • It causes less irritation than PAA. • It is more volatile than H_2O_2 or PAA. • It is likely that it will effectively inactivate prions within brain tissue.	• It is explosive above 80 °C. • It has a lower boiling point than PAA. • It has limited penetration. • It is unstable, and must be used within 12 h. • It is less stable than PAA. • This is no published information on material compatibility, and little data on its effectiveness.
Liquid sterilants	• ClO_2, PAA, can be liquid systems and in this form do not produce the adverse residuals that are formed with glutaraldehyde. They may not be as effective against spores as H_2O_2, glutaraldehyde, or formaldehyde [4]. • Potential (future) performic liquid technology may be what is needed in a 'total' endoscopic cleaning and sterilising system of 30 min, with only 1,800 ppm at 44 °C, but may not be available. Its breakdown products may be CO_2 and O_2.	• Adverse effects on materials by ClO_2 and performic acid are still unknown. • Residuals of glutaraldehyde and formaldehyde. • No routine BI.
X-rays	• Excellent penetration. • Excellent sterilisation. • Fast throughput. • Dosimetric release, no further testing required. • May be more applicable to hospitals that already have X-ray technology.	• It may not be able to resterilise a lot of radiation sensitive materials. • It is more costly than gamma or electron beam irradiation.

	• Simple to use • Maybe useful for decontamination and sanitising to reduce the bioburden to safe levels. • Maybe be synergistic with other methods and would thereby require a smaller irradiation dose.	
Aseptic processing	• It has the capacity to achieve device and material compatibility when other sterilising agents will adversely affect the finished product due to extreme oxidation, moisture, heat or irradiation.	• It will not normally provide a 10^{-6} SAL, but it should never be considered or applied without a high level expert sterilisation evaluation and study.
ABS: Acrylonitrile-butadiene-styrene BI: Biological Indicators CO^2: Carbon dioxide D-value: Death value O_2: Oxygen PA: Polyamide PC: Polycarbonate PE: Polyethylene PMMA: Polymethyl methacrylate PP: Polypropylene PS: Polystyrene PU: Polyurethane RH: Relative humidity		

5.3 Non-traditional and Accepted Sterilisation Techniques

5.3.1 Hydrogen Peroxide with Plasma (without Plasma)

H_2O_2 with plasma and O_3 are considered to be non-traditional sterilisation methods yet are readily acceptable by regulatory agencies such as the FDA, because they do not actually have any toxic residuals. H_2O_2 (without plasma) can exist in the vapour and liquid form. But the discussion here is about the vapour state not the liquid. In the vapour phase it generally follows the ideal gas law.

H_2O_2 has excellent antimicrobial properties against a wide range of micro-organisms including bacterial endospores. Under carefully controlled process conditions, H_2O_2 is also safe for use with many materials. H_2O_2 may be decomposed into water and O_2 rendering it environmentally safe, eventually.

Table 5.3 Factors affecting the sterilisation processes	
Factors	**Effects/results**
Environment	A controlled environment will improve or limit the number, types, environment and physiology of microbes on product and factors that make it difficult to sterilise product or materials: • For example desiccation or drying is sometimes difficult to overcome. • Salts, protein (organic matter, dirt) can impede the diffusion of a sterilant to a microbe. • *Propionibacterium* species as well as *Staphylococcus and Micrococcus* species coming off of the skin can produce a very high bioburden. • Personnel not wearing gloves and not cleaning their hands thoroughly can deliver Gram-negative microbes as well as *Clostridium*, however, to determine their presence may require blood in the recovery media and an anaerobic as well an aerobic recovery environment. • Controlling microbial contamination of air, surfaces, materials and personnel reduces potential of resistant bioburden. • Some sterilants require humidity, strong oxidants, irradiation and/or high temperature to sterilise, which may be incompatible to certain materials and polymers.
Cleaning	Failure to clean re-used instruments and equipment, can result in a higher bioburden, protein (organic matter) and salt loads. How the clean product is handled after cleaning can also influence what is on it. Using water that is contaminated and drying can increase microbial resistance. Unclean alcohol or processes, which are contaminated with surviving spores can result in failures. Clean, disinfect areas and personnel, keep sick and untrained persons out of decontamination/cleaning areas.
Bioburden and contamination	Bioburden needs to be low as required for FDA or other regulatory and agencies. Some devices will have lower numbers, while others will have higher. Types of microbes will vary in resistance. Typically spores are the most resistant, however, microbes contaminated and/or occluded in salts, protein, biofilms and desiccated can be very resistant. The bioburden needs to be reduced, and the product cleaned prior to use for oxidising agents.
Sterilant accessibility	Shielding of microbes from sterilants, such as in closed areas, matching surfaces, shadows, packaging, long, small and restricted lumens, hinged instruments or dead ends, reduces or prevents accessibility. Crevices, unavailable and inaccessible surfaces to the sterilant. Types of material and design, density of load, can vary sterilant accessibility and permeability to the microbe. Residual air, water and so on may limit sterilant accessibility.
Sterilant exposure and parameters	Sterilant concentration/intensity, exposure, and parameters [such as %RH, temperature, plasma, the number of repetitive steps, vacuum/pressure (s)] may be required.
Aeration and flushing	Removal of residuals if needed.
Post sterilisation handling	Without packaging, there is source of immediate contamination. Packages that are old, weak or have environmental leaks can contaminate.

H_2O_2 is a strong oxidising agent. H_2O_2 may primarily react with cysteine containing proteins, creating disulfide crosslinks between proteins. This oxidation of amino acid moieties within a given protein may cause secondary damage as the radicals modify other amino acids within that protein or other proteins. Another inactivation mechanism of damage may be through intracellular reactions. For example reactions may occur when iron(II), present in haeme groups or in other forms, reacts with peroxide forming hydroxyl radicals. Such hydroxyl radicals are highly reactive and can oxidise most organic molecules within a cell.

While H_2O_2 with plasma may have additional excellent microbiocidal properties, it has poor penetration. And while highly toxic in form before sterilisation it may be environmentally acceptable under process control. H_2O_2 is typically used in the vapour phase for medical materials and devices. While it is compatible with many polymers (see **Table 5.2**), there are some materials that are damaged (for example, acrylics, cellulosics (includes paper), natural rubbers, and bioadsorbables such as polyglycolides and polyesters.

Due to the oxidative nature of H_2O_2 vapour, some materials are not recommended for instruments intended for this sterilisation method. **Table 5.4** lists the durability of commonly used material families/grades for medical device fabrication. It is important to note that the durability is dependent upon the specific molding conditions. For example, a component of medical devices with high residual stress could be less durable than a component that has been properly stress-relieved.

Implantable devices may require special processing and, therefore, the equipment manufacturer should be contacted for specific applications. It is important to discuss the specific concerns of sterilant residuals with the equipment manufacturer. As with EO sterilisation, H_2O_2 residual level depends on the material family, grade, load density in the chamber, loading weight, the specific cycle parameter, and packaging used.

H_2O_2 does not have the same penetration as pulsating or pressurised steam, dry heat, EO or irradiation. It is primarily a surface sterilant, with some lumen diffusion or penetration, and diffusion and permeation through some packaging materials (see **Table 5.5**).

H_2O_2 can sterilise some lumens, but not lengthy ones. It cannot sterilise some polymeric materials and devices in their entirety. While its outcome is typically safe, it begins with the source of a very hazardous high concentration of H_2O_2. Plasma is effective in breaking down the H_2O_2 into water and O_2.

Because H_2O_2 has very high vapour or boiling point, very deep vacuums are required that may adversely affect some packaging, batteries and materials.

Table 5.4 Reliability of some commonly used material families/grades for medical device fabrication	
Material	**Reliability with H_2O_2 gas plasma sterilisation method**
Liquids	Do not use, unless freeze dried
Solids, mated surfaces, long lumens, sealed enclosures	Cannot penetrate
Cellulosics, cotton	Do not use
Paper/cellulose/cellulose fibres, cellulopane/cupraphame	Do not use
Cellulose esters	Do not use
Cellulose acetate	Do not use
Cellulose acetate butyrate	Do not use
Powders	Do not use
PA	May be grade dependent
Rubbers	
Latex	1**
Neoprene®	1
Silicone	5
Natural rubber, butyl and latex	3
Nitrile and polyacrylics	3 grade dependent
Polychloroprene	5, but degradation after 100 cycles
Thermoplastics	
PE (PE, UHMWPE, LDPE, HDPE)	5
PP	5
PETG	5
PTFE	5
Styrene block copolymer (e.g., Kraton®)	4
Plasticised PVC	5 but unlikely to be resterilisable*
Non-plasticised PVC	Some colour change after 50 cycles*
Silicone elastomer	5
Ethyl vinyl acetate copolymer	5
PS	5
PC	5
PMMA, acrylic	2
Polyacetal (Delrin®/Celcon®)	3-5 grade dependent
PU	4
Polyetherimide (Ultem®)	5
Polysulfone	Grade dependent
Polysulfone (e.g., Udel®)	5
Polyether sulfone (e.g., Radel® A200)	3

Polyaryl sulfone (Radel® R5000)	2
ABS	4.5
PA	1
Liquid crystal polymer	5
Poly(ether ether ketone)	5
Thermosets	
Epoxy	5
Phenolics, PU, and polyimides	Grade dependent; 3-5
Adhesives	Variable and grade dependent, contact equipment manufacturer
Metals	
Stainless steel	5
Aluminum	5
Cobalt chrome	5
Titanium	5
Gold	5***
Copper	5***
Silver	1 to 3 Only small amounts
Magnesium and nickel	3 Limited to small quantity
Ceramics/glass	5
Aluminum oxide and zirconium oxides	Limited to small amount
Silica	No change after 100 cycles

HDPE: High-density polyethylene
LDPE: Low-density polyethylene
PETG: Polyethylene terephthalate glycol copolymer
PTFE: Polytetrafluoroethylene
PVC: Polyvinyl chloride
UHMWPE: Ultra-high molecular weight polyethylene
See AAMI TIR 17 [1] for further details.
* Plasticiser may bloom on the surface after 50 cycles - compatibility may be grade dependent.
** May degrade after three cycles.
*** Use should be limited to electrical contacts or small plated surfaces.
Ratings:
1 – Severe material degradation after 100 cycles.
2 – Significant material changes or crazing after 100 cycles.
3 – Significant colour changes or slight material changes after 100 cycles.
4 – Some colour change or loss of gloss after 100 cycles.
5 – No change after 100 cycles.
Note: The H_2O_2 gas plasma method uses the plasma phase to further eliminate residuals, therefore, the most commonly used materials for medical instrument fabrication do not retain enough sterilant residuals to affect biocompatibility and post-sterilisation aeration usually is not required, with plasma process, however, with straight H_2O_2 aeration may be required.

Table 5.5 Compatibility of (packaging) material with H_2O_2 sterilisation method	
Material	**H_2O_2 vapour sterilisation method**
Paper, cellulose materials	Poor, not recommended
Teak	Excellent
PET/PE laminate film	Excellent – needs to have an equivalent area made of permeable material such as a non-woven polyolefin.
PE film	Excellent – needs to have an equivalent area made of permeable material such as a non-woven polyolefin.
Metallic laminates	Excellent – needs to have an equivalent area made of permeable material such as a non-woven polyolefin.
PETG	Good - needs to have an equivalent area made of permeable material such as a non-woven polyolefin.
Acrylic (XT Polymer)	Medium - needs to have an equivalent area made of permeable material such as a non-woven polyolefin.
Acrylonitrile – methyl acrylate copolymer (Barex)	Medium - needs to have an equivalent area made of permeable material such as a non-woven polyolefin.
Silicone elastomer	Grade dependent – discuss with equipment manufacturer.
Plasticised PVC	Not recommended – high absorption.
Polyaryl sulfone (Radel)	Not recommended – high absorption and limited durability.
Polyetherimide (Ultem)	Not recommended – high absorption.
PU	Do not use – very high absorption.
PA	Do not use – very high absorption.
PET	Unknown
PET: Polyethylene terephthalate	

A myriad of devices, materials and packages without limitations may be sterilised with H_2O_2 with plasma (see AAMI TIR 17 [1]), because it is a very strong oxidant, yet vulnerable to declining in a vaporous state.

5.3.1.1 Advantages and Reasons for Using Hydrogen Peroxide Vapour with Plasma or without Plasma

Some of the advantages and technical reasons for using H_2O_2, with or without plasma are given next:

- Certain packaging material is provided by the manufacturer, otherwise peroxide will not penetrate it or continue to be a viable oxidising agent as a vapour.
- Items with lumens up to 12 inches long are recommended for the process without the use of an adapter.

- Sterilisers would be classified as Class II medical devices, requiring performance standards. Unlike EO gas sterilisers, which have performance standards developed through American National Standards Institute/AAMI, the H_2O_2 system does not currently have a specific performance standard.
- It is a good candidate for many low temperature materials that tend to be hydrophobic and heat sensitive.
- It is compatible with many materials and a large number of medical devices.
- Non-catalytic, non-absorbent materials such as PTFE and PE can be used with H_2O_2, however, stainless steel, or low copper-aluminum alloys are not recommended for H_2O_2 without plasma.
- The system is currently on the market as a replacement for EO sterilisers particularly in hospitals and healthcare facilities
- Current non-traditionally accepted methods are H_2O_2 with plasma and O_3. However, H_2O_2 without plasma is not 'immediately' accepted by the regulators.
- H_2O_2 and plasma have excellent microbiocidal properties, and they are environmentally acceptable under process control, with varying and numerous parameters (**Table 5.6**).
- H_2O_2 is typically used in the vapour phase for medical materials and devices.
- It is compatible with many polymers, and many polymers can be resterilised after using it, rather than there just being a single exposure to the sterilant.

Sterilisation by this technique (H_2O_2 with plasma) is typically achieved in small sterilisation vessels, not large chambers or facilities, such as are needed with dry heat, EO, radiation, or steam.

Table 5.6 H_2O_2 gas with plasma sterilisation cycle parameters

Type of cycle	H_2O_2 concentration per injection	Exposure time	Chamber temperature	Plasma power
Hospital use - H_2O_2 gas plasma	6-18 mg/l	15 to 30 min (two exposure cycle)	40-55 °C	400-600 W
Industrial use - H_2O_2 gas plasma	6-18 mg/l	Multi-exposure cycles available	40-60 °C	400-600 W

Note: Use of H_2O_2 processing with plasma frequently reduces the exposure time of the products and smaller sterilisers have shorter exposure times, (e.g., 38 min) than larger sterilisers with longer exposure times, (e.g., 52 min).

H_2O_2 with plasma is not considered to be a traditional method but is regarded as an acceptable non-traditional method, whereas H_2O_2 without plasma is more limited.

Some of the unique limiting and disadvantageous qualities of using H_2O_2 with plasma and without plasma are:

- There is a misconception that it will be able to sterilise all the products or items, which EO previously sterilised, however, it cannot be used to sterilise long lumens, paper or cellulosic items, o-rings, mated surfaces, some hinged instruments, some procedural trays with pads or set ups with connectors and so on.
- Performing validation on this process is not as simple as for processes with standard exposure times.
- The technique requires special packaging and container trays.
- The technique typically uses a cartridge, called a pillow, with ten unit dose cells that contain a 58% solution of H_2O_2.
- H_2O_2 gas plasma is generated in the unit under a very deep vacuum and with electrical energy obtained from radiofrequency field.
- Unlike EO, which is an alkalising agent and penetrates through packaging and most devices, H_2O_2 gas plasma is an oxidising agent and has only surface contact capability.
- Only a specific variety of devices and materials can be subjected to the process, unlike EO that can sterilise many materials.
- No textiles or cellulose material common to a healthcare facility central service department can be used with the system.
- Some materials that are typically damaged or affected are acrylics, PU, natural rubbers, bioadsorbables such as polyglycolides, cellulosics, polysulfide adhesive and polyesters.
- Avoid H_2O_2 absorbers, such as PA, PU and decomposers such as silver, copper and copper alloys.
- Adhesives that use large proportions of amines as curing or crosslinking agents tend to be incompatible.
- H_2O_2 has an Occupational Safety and Health Administration (OSHA) and National Institute for Occupational Safety and Health (NIOSH) exposure standard of 1 ppm 8-h time weighted average (TWA), is listed in the OSHA Standard on air contaminants, and thus requires to be monitored.

- The manufacturer does not recommend environmental monitoring.
- Reports from the field indicate there may have been some health problems associated with the system. H_2O_2 at 35% or more is a toxic substance - this system may use a 58% solution.
- According to the Agency for Toxic Substances and Disease Registry (*www.atsdr.cdc.gov*) and Sax's Dangerous Properties of Industrial Materials [5], there are adverse health effects with both acute and chronic exposures.
- The immediately dangerous to life or health (IDLH) level is 75 ppm. However, over time lower concentrations may have ageing effects on exposed humans.
- The reportable quantities (RQ) is one pound in concentration >52% under Situational awareness and response assistant (SARA) Section 302 Extremely Hazardous Substances (EHS).
- Sterilisation by this technique is typically achieved in small sterilisation vessels, not large chambers or facilities as are needed for use of dry heat, EO, radiation, or steam.
- H_2O_2 does not have the same penetration as pulsating or pressurised steam, dry heat, EO or irradiation. It is a surface or topical sterilant. EO can permeate through some polymers and mated surfaces that H_2O_2 cannot.
- It can sterilise some lumens, but not lengthy ones.
- It cannot sterilise some polymeric materials and devices in their entirety.
- While its outcome is typically safe, it begins with a source substance with a very hazardous, high concentration of H_2O_2. Plasma is effective in breaking down the H_2O_2 into water and O_2. Without plasma requires it 'significant' aeration.
- Because H_2O_2 has very high vapour or boiling point, very deep vacuums are required that may adversely affect some packaging and materials.
- It is recommended that items are cleaned before sterilising them, to remove salts and proteins.
- Failure to clean and make all areas (for example, connectors) accessible could result in potential failures.

Some of the phases of a H_2O_2 with plasma and without plasma cycle are shown in **Table 5.7.**

Table 5.7 Steps in the H_2O_2 plasma process	
Process	**Description**
Evacuation	The sterilisation chamber is evacuated to remove air from the steriliser. This phase may have an effect on materials.
Conditioning	The chamber is conditioned to achieve conditions for sterilisation.
Peroxide injection	An aqueous solution of H_2O_2 is transferred to the steriliser vapourisation system.
Exposure	The H_2O_2 is vapourised and allowed to diffuse into the load. This phase may have an effect on materials. There may be multiple pulsing injections. For gas plasma systems, typical H_2O_2 concentrations are 6–18 mg/l, cycle times range from 15 min to 4 h, and temperatures range from 40-60 °C. For H_2O_2 vapour systems, typical H_2O_2 concentrations are 0.5–9 mg/l, cycle times range from 45 min to 8 h and temperatures range from 25-55 °C.
Plasma	H_2O_2 gas plasma processes use a strong electrical field applied to the steriliser electrodes to create the gas plasma. The plasma breaks down the peroxide into a cloud of highly energised species that recombine, turning the H_2O_2 into water and O_2. This phase may have an effect on materials.
Evacuation	Chamber pressure is reduced to prepare for the plasma and removes a portion of the H_2O_2 from the system. The evacuation system with plasma does not require aeration.
Final vent	At the conclusion of the cycle, the steriliser is returned to atmospheric pressure with microbial filtered air.

Because of the deep vacuum required for sterilisation, the items to be sterilised, products and packaging must be able to withstand the deep pressure changes. Some devices require special venting caps to allow for pressure equalisation.

Physical damage may occur if a device is not capable of withstanding both deep vacuum and the rate of pressure change from a given cycle. Plasma will affect some materials by surface modification - sometimes the effect is temporary, but devices should be evaluated for surface modifications and effects on their functionality.

H_2O_2 with plasma is not considered to be a traditional method yet, but it is an accepted non-traditional method.

5.3.2 Ozone

O_3 is a very strong oxidising agent, making it an efficient sterilising agent. It is a relatively new (gaseous) technique for sterilising medical devices, making it a non-traditional and acceptable process, although it has been traditionally used to sterilise water; previously used for medicinal applications since late 1800s and so on.

O_3 sterilisation can be achieved in water as well as in gaseous form. This information relates to O_3in the gaseous state. The sterilant must be able to penetrate all portions of a load and product areas intended to be sterilised. Materials must be resistant to oxidation. Gaseous O_3 requires a high humidity to be effective.

O_3 is a molecule comprised of three O_2 atoms. The molecule is bent with a measured bond angle of 116.8 ± 0.5º. The O_3 molecule exists in different 'resonance' forms. **Figure 5.1** shows two of its resonance forms.

Figure 5.1 Molecular formula of O_3 showing two of its resonance forms

O_3 boils at -112 °C, melts at -192 °C, has a molecular weight of 48 and it is soluble in water at approximately 1.0 g/l at 0 °C.

O_3 is produced by mean of an electrical discharge passing through O_2. In its pure state, O_3 is a blue coloured gas that is dangerous to breathe. Otherwise, it is colourless at room temperature. Due to its thermodynamic properties, O_3 is a metastable product; it decomposes slowly (in minutes) at ambient temperatures and rapidly (in seconds) at higher temperatures. O_3 is a powerful oxidising agent, far stronger than O_2. It is also unstable at high concentrations, decaying to ordinary diatomic O_2 (with a half-life of about half-an-hour under ambient atmospheric conditions).

O_3 is a strong oxidiser, which makes it an efficient sterilising agent. Since O_3 is a metastable product, it cannot be stored and is therefore produced *in situ*. O_3 sterilisation processes are particularly suited for sterilising heat-sensitive materials because temperatures within the load currently do not exceed 36 °C.

The O_3 molecule in the presence of humidity oxidises electron rich chemical groups such as highly unsaturated organic compounds. Critical molecules in the cells, such as amino acids (proteins) are then deactivated leading to cell death. Since O_3 decomposes rapidly into highly reactive species (hydroxyl radicals, atomic O_2 and so on), these are also attributed to O_3 sterilisation killing mechanisms of living cells.

O_3 is typically generated within the steriliser's self-contained O_3 generator from United States Pharmacopeia (USP) grade O_2 [6]. Items to be sterilised are placed in the

sterilisation chamber. The chamber is closed and a vacuum is drawn. The items are conditioned to a pre-determined state and then O_3 is injected. $O_3$is generated within the steriliser so that the operator does not handle the sterilant in any form. During the sterilisation cycle, items are exposed to O_3 at typical concentrations of 85 mg/l for 15 min at a temperature of 30-36 °C. The O_3 is allowed to dwell for a fixed time and then converted to O_2 through a catalytic converter and removed (exhausted or ventilated) from the steriliser by a series of vacuums and air injections (**Table 5.7**). During the dwell period, the O_3 breaks down in the chamber into reactive species, including free radicals. The breakdown residues of O_3, O_2, and water vapour are exhausted through a catalytic converter, yielding safe, non-toxic byproducts.

5.3.2.1 Advantages of Using Ozone

In a gaseous or vapour form, O_3 can be used to sterilise medical products and other materials within a chamber. Because O_3 is a metastable product, it cannot be stored and is therefore produced *in situ*, making it an environmental and safe acceptable process, but requiring special equipment. Its penetration may be limited or slow with organic matter, but better than H_2O_2. At the end of the process the O_3 degrades to O_2. Because of the strong oxidising nature of O_3, materials must be resistant to oxidation.

Some of the principle reasons and advantages of O_3 sterilisation are:

- It can potentially process some cellulosics and paper, that H_2O_2 cannot.
- It can sterilise many low temperature materials, as well as higher temperature materials.
- It has excellent microbial sterilising capabilities and possibly deactivate prions.
- It has the potential for sterilising larger loads than H_2O_2 with plasma.
- It leads to environmentally safe breakdown products - O_2 and water vapour.
- It may penetrate lumens and more difficult to sterilise areas, better than H_2O_2.
- No transportation of toxic consumables is necessary.
- O_3 is easily prepared/produced on site, safely with no cylinders of gas required.
- O_3 sterilisation is inexpensive or low cost.
- It can sterilise at low temperatures (e.g., 30-36 °C).

5.3.2.2 Disadvantages of using Ozone

Gaseous O_3 has been recognised as a sterilant for a long time in the treatment of drinking water, but only recently has it become available for medical devices, although it has been used to sterilise pharmaceutical (medical) water and so on. O_3 is a very strong oxidiser, making it an efficient sterilising agent. In a gaseous or vapour form, O_3 can be used to sterilise medical products and other materials within a chamber. Because O_3 is a metastable product, it is difficult to store it for long periods and it is therefore produced *in situ*, making it an environmental and safe process. Its penetration may be limited or slow with organic matter, and it does not penetrate polymer films like EO does. While the O_3 is degraded to O_2, at the end of the process, it requires materials to be resistant to its high oxidising power during processing. The potential disadvantages of O_3 include its oxidising reactivity with certain polymers. It may have some penetration limitations (for example, through organic matter and non-diffusible polymers).

Some of the limitations of O_3 sterilisation are:

- It has adverse chemical effects on some materials (for example, rubbers).
- It may cause adverse changes in steel, brass, latex, and other polymers over time.
- It is not recommended for all polymers or plastic devices.
- O_3 has to be generated on site, requiring special equipment and an O_2 source (e.g., cylinders).
- It is an oxidising and bleaching agent, however, unlike H_2O_2 it may sterilise some cellulosics or paper that H_2O_2 cannot.
- As an oxidising agent, it does not have a penetration capability such as EO but it may sterilise many lumens that H_2O_2 with plasma may not.
- OSHA and NIOSH exposure levels are 0.1 ppm 8-h TWA.
- It has an IDLH of 5 ppm.
- It produces surface oxidation.
- Materials must be resistant to (strong) oxidation.
- The shape of the materials as well as the design of a device is closely related to the longevity and resistance of the device to O_3 sterilisation.
- Polymeric components with large surface to-mass ratios (for example, fibrous material) may undergo fast oxidative degradation.

- It may not penetrate organic matter as well as EO.
- The RQ in case of a release is one pound SARA Section 302 EHS.
- It may adversely affect embedded batteries.

In the currently available system, O_3 is generated within the steriliser's self-contained O_3 generator from USP grade O_2. Items to be sterilised are placed in the sterilisation chamber. The chamber is closed and a vacuum is drawn. The items are conditioned to a pre-determined state and then O_3 is injected. O_3 is generated within the steriliser so the operator does not handle the sterilant in any form. During the sterilisation cycle (see **Table 5.8**), items are exposed to O_3 at a concentration of 85 mg/l for 15 min at a temperature of 30.8-36 °C. The O_3 is allowed to dwell for a fixed time and then removed. During the dwell period, the O_3 breaks down in the chamber into reactive species, including free radicals. The breakdown residues of O_3: O_2 and water vapour are exhausted through a catalytic converter, yielding safe, non-toxic by-products.

Table 5.8 The steps in a typical gaseous O_3 process

Phase	Action
1 - Vacuum/conditioning	A vacuum is drawn to approximately 133 Pa to remove air from the steriliser chamber and the load. The load is then conditioned to attain the sterilisation conditions.
2 - Humidification	Water vapour is pulled inside the chamber for effective sterilisation, consequently the pressure increases.
3 - O_3 injection/exposure	O_3 is generated immediately before its gradual injection inside the chamber. Once the O_3 concentration has reached its pre-determined dose, the load is exposed for a fixed period of time for a successful sterilisation.
4-6 Repetitive phases	Phases 4-6 replicate the same conditions as in phases 1-3.
7 - Evacuation/ventilation	O_3 is drawn through a catalytic converter where it reverts back to O_2 and water vapour. The steriliser chamber is evacuated and returned to atmospheric pressure.

Table 5.9 gives a list of some materials, which are compatible or tolerable with currently available O_3 sterilisation processes. This list is not exhaustive and manufacturers' should use it as general guideline for the selection of materials. Further information can be obtained from the manufacturers of commercially available O_3 steriliser.

Table 5.9. List of some compatible materials with the O_3 sterilisation process	
Type of material	**Examples of materials**
Thermosets	• Epoxy • Phenolic resins • Silicone • Unsaturated polyester
Thermoplastics	• Ethylene chlorotetrafluoroethylene • ETFE • FEP • Polyacetals or polyoxymethylene • PA, for example Nylon • PC • PCTFE, PVDF • PEK • PEI • PE (HDPE, LDPE, UHMWPE) • PMMA • Polyoxymethylene or acetal • Polysulfone • PP • PTFE, filled and unfilled • Rigid PVC
Elastomers	• Polydimethylsiloxane (silicone)
Metals/alloys	• Anodised aluminum • Stainless steel • Titanium
Ceramic/glasses	• Aluminum oxides • Silica • Zirconium oxides
Liquids	• Not recommended
Others	• Some cellulosics
ETFE: Ethylene tetrafluoroethylene FEP: Fluorinated ethylene propylene PCTFE: Polychlorotrifluoroethylene PEI: Polyetherimide PEK: Polyethylketone PVDF: Polyvinylidene fluoride	

Some of the properties and parameters of the O_3 process cycle that affect materials and lethality are:

- O_3: O_3 is a strong oxidising agent. Materials must be resistant to oxidation. Lethality is a direct function of the O_3 concentration injected into the sterilisation chamber. As with other sterilisation processes, sterility of the product cannot be ensured unless the devices are cleaned and dried prior to the sterilisation cycle. The sterilant must be able to penetrate into all portions of the load to be sterilised.

Thus, device construction and packaging may be more important to achieving sterility than are the materials used to construct the medical device. Materials and devices must be resistant to oxidation.

- *Temperature*: Materials and medical devices should be resistant to temperature ranges of 30-36 °C for short periods of time.
- *Humidity*: Materials and medical devices should be resistant to high RH level (>80%). A RH level >80% is required for O_3 to be effective as a sterilant. Lower RH (for example, 50%) may be possible. Even with a relatively low humidity level, it is possible for humidity to condense into liquid water on the surfaces of a device. This is most likely to occur during the load conditioning portion of the cycle, when a cold load is placed in the chamber.
- *Pressure excursions*: Materials, medical devices and packages should be resistant to vacuum (133 Pa). As such, the process is not recommended for the sterilisation of glass ampoules or liquids.

The question arises of how well do the previously acceptable, non-traditional, sterilisation methods compare to traditional sterilisation methods when it comes to material and device compatibility. This is shown in **Table 5.10**.

5.3.3 Some Factors to Consider in the Determination of Alternative Non-traditional Sterilisation Methods versus *Traditional Methods*

Although the material and medical device compatibilities of non-traditional and traditional sterilisation methods (see **Table 5.10**) are important, many other factors need to be considered too. Some of these factors were discussed in ***Healthcare Sterilisation: Introduction and Standard Practices, Volume 1*, Sections 8.3.3.1-8.3.3.4.**

5.3.3.1 Availability

Is the sterilisation process and method 'readily' available in a hospital or industrial setting, where the need or requirement is? Steam sterilisation is readily available in hospitals, pharmaceutical manufactures and to a lesser extent in medical device manufacturing factories. Dry heat is more available in dental offices, pharmaceutical areas, laboratories, some in hospitals, but typically less in device manufacturer's factories. EO has been available in hospitals, industries and through contractors. It is becoming less available in some hospitals. Irradiation is available to many large device manufacturers and contractors, but not actually in hospitals. H_2O_2 with plasma is being used in many hospitals and by a few device manufacturers. O_3 sterilisers are in a few hospitals at this time. Good Manufacturing Practice's (GMP) and non-GMP chlorine dioxide sterilisers are available.

Table 5.10 Comparison of compatible materials and devices to traditional sterilisation methods (dry heat, EO, moist heat, and radiation) compared to the acceptable non-traditional sterilisation methods (H_2O_2 with plasma, and O_3) and novel methods (for example, H_2O_2)

Material	Radiation	EO	Moist heat	Dry heat	H_2O_2 with plasma	O_3	Device applications
Polymers							
PE	Good to excellent, however, may off gas. Low and moderate density are more resistant, are likely to be able to be re-sterilised.	Excellent	Poor to good - high density more resistant.	Poor to fair, but a lower temperature improves it for high density products.	Excellent	Excellent	Medical containers, packaging, pouches, orthopedics, replacements in joints, ankles, elbows, shoulders and toes, tubing.
PP	Poor to good, stabilised, but single use only[a].	Excellent	Good, and excellent with heat stabilised grades and can be resterilised[a].	Good and excellent at low temperatures (up to 135 °C) with heat stabilised grades[a].	Excellent	Excellent	Catheters, clamps in intravenous (IV) sets, Irrigation containers, membranes in filters and dialysers, packaging, sutures, syringes.
Polymethyl pentene	Fair to good	Excellent	Good/excellent	Good/excellent up to 170 °C.	Unknown	Unknown	Container, covers for medical instruments, polymethyl-pentene (TPX®, Mitsui) film.
Co-polymers, for example, PE/PP, polyallomer, styrene polymers	Poor to good, stabilised, but single use only.	Excellent	Good, and excellent with heat stabilised grades and can be resterilised.	Good and excellent at low temperatures (up to 135 °C) with heat stabilised grades.	Excellent	Excellent	Parenteral solution containers, containers for packaging applications.

PS	Excellent compatibility because of its benzene ring.	Poor to good, but millions of parts have been acceptably sterilised and some formulations can be resterilised 2 to 5 times.	Poor to excellent with high impact or syndiotactic styrene.	Poor to excellent with high impact or syndiotactic styrene.	Excellent	Fair	Container, parts in IV sets, petri dishes, sputum cups.
Styrene acrylonitrile copolymers	Good to excellent	Poor to good, but many parts are acceptably sterilised.	Poor to fair	Poor to fair	Excellent	Poor-fair	Dialysis devices, IV connectors.
Acrylic polymers							
PMMA	Fair to good	Good	Poor to fair at low temperatures but not likely to be able to be resterilised[b].	Poor to fair at low temperature[b].	Fair	Good	Bone cement, contact lenses, corneal prosthesis, grout for artificial joints, orthopedics, ophthalmology lenses, membrane oxygenators.
Vinyl polymers							
Polyvinyl acetate	Good	Poor	Poor to fair	Poor to fair	Excellent	Unknown	Films
PVC	Good[c]	Excellent[c]	Poor to fair up to 120 °C[c].	Varies but is good.	Excellent	Good	Blood bags, catheters, containers, endotracheal tubes, films, hearing aid components, IV tubing, IV drip chambers, packaging, shrink tubing, storage bags, in ventilation systems.

Vinyl chloride copolymers	Good	Excellent	Moist heat (poor to good up to 120 °C.	Poor to good up to 120 °C.	Unknown	Unknown	Films, packaging.
Polyvinylidene chloride	Good	Excellent	Poor to fair up to 120 °C.	Poor to fair up to 120 °C.	Unknown	Unknown	Microporous hydrophobic membranes.
Fluorinated polymers (fluoropolymers)							
PTFE, Perfluoroalkoxy, PCTFE, PVDF, ETFE, FEP	Mixed- some poor, e.g., FEP and PCTFE.	Excellent	Fair to excellent	Fair to excellent -maybe up to 170 °C.	Excellent	Excellent	Artificial bone joints and vasculature, fibre optics, surface treatments, stop cocks, tubing.
PA (Nylon)	Poor/fair to good	Excellent	Poor to excellent	Poor to excellent	Good, but only has one use[e].	Good	Bags, catheters, films, kidney dialysis, laparoscopy devices, special packaging, PA spikes.
Polyester	Fair to good	Variable to excellent	Poor to excellent	Poor to fair	Excellent	Excellent	Covers, films, IV infusion fluid containers.
PET copolymers							
PETG	Good to excellent	Excellent[d]	Fair to poor	Good to excellent up to 134 °C.	Unknown	Unknown	Packaging
PET	Good to excellent	Excellent	Fair to poor	Good to excellent	Unknown	Unknown	Angioplasty balloons, blood collection tubes, specialty syringes, sutures, woven vascular prostheses, wound drainage systems.
Cellulosics, cellulose ester, cellulose acetate propionate, cellulose acetate butyrate, cellulose (paper, cardboard)	Fair to good (*Note:* esters degrade less than other cellulosics).	Variable to excellent	Poor to good at low temperatures and depending upon the cycle, some may be good.	Poor to good but higher temperatures will char.	Poor (with plasma) fair to good (without plasma).	Poor to good	Films, filters, haemodialysis membranes, IV burette,clamps packaging, thermosets.
Epoxy	Excellent	Good to excellent	Fair to excellent	Fair to excellent	Excellent	Fair to excellent	Adhesive, potting

Phenolic	Excellent	Good	Fair to good	Fair to good	Good	Excellent	Coatings on metals.
Polyimide	Excellent	Excellent	Excellent	Good to excellent	Excellent	Unknown	Tubing
PU	Good to excellent -aromatic better than aliphatic.	Poor to good	Poor to fair, may create toxic residues.	Poor to fair/ good, at low temperature, likely to have no toxic residues.	Good	Poor -varies with the type of PU.	Blood pumps, catheters, connectors, containers, enteral feeding tubes, lipid resistant stopcocks, needle-less syringes, vials.
Miscellaneous plastics							
Acetal	Poor, embrittlement.	Excellent	Fair to good up to 120 °C.	Good to excellent up to 120 °C.	Excellent. With plasma - good to excellent.	Good	Engineering plastics, structural keels for prosthetic devices, stop cock.
PC	Good to excellent	Excellent	Fair to good	Fair- good/ excellent up to 134 °C.	Excellent	Excellent	Blood sets, cases, covers, cardiotomy trocars, injection sites, drug delivery devices, IV connectors, reservoirs, surgical instruments, safety syringes, valve occluders.
ABS	Good	Excellent	Poor to fair	Poor to fair	Excellent	Fair	Administration IV sets, luers, roller clamps, spikes, Y connectors, and dialysis units.
Elastomers (rubbers)							
Butyl	Poor	Excellent	Fair to excellent	Poor to good	Good, but with only one cycle.	Poor	Tubing
Ethylene-propylene diene terpolymer	Good to excellent	Excellent	Good to excellent	Fair to good	Fair to good	Fair	Tubing
Nitrile	Good to excellent	Good to excellent	Fair to good	Poor to fair	Fair	Unknown	Gloves
Polyacrylic	Fair to good	Fair, but only for one cycle.	Poor	Poor	Fair	Good	Dental
Polychloroprene	Good	Good	Fair to good	Poor to fair	Excellent	Poor	Tubing

Silicone	Fair to good - can crosslink.	Excellent	Fair to excellent	Fair to excellent - up to 200 °C, many prosthesis processed by dry heat.	Excellent	Excellent	Catheters, membranes, prostheses (prosthetics), tubing.
Textiles and fibres	Poor to excellent	Fair to excellent	Fair to good	Fair to good, muslin up to 160 °C.	Unknown	Unknown	Covers, uniforms, packaging, wrappers.
Adhesives and cements							
Acrylic	Fair to good	Fair	Poor to fair	Poor to fair	Fair	Good	Adhesive
Epoxy	Excellent	Good to excellent	Fair to excellent	Fair to excellent	Excellent	Good to excellent	Adhesive
Fluoroepoxy	Excellent	Unknown	Good to excellent	Fair to good	Fair	Good	Moisture resistant adhesive
Silicone	Good to fair	Excellent	Fair to good	Fair to excellent	Fair – (peroxide only) H_2O_2 with plasma (good).	Good	Adhesive
Ceramics or glasses (medical applications – diverse)							
Aluminum oxides	Excellent	Excellent	Good to excellent	Good, up to 190 °C.	Excellent	Excellent.	In superconducting devices.
Silica	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent.	Desiccant
Zirconium oxides	Excellent	Excellent	Fair to good	Fair to good	Excellent	Excellent.	Dental prosthetic devices
Metals (medical applications – diverse)							
Aluminum	Excellent	Excellent	Good with one cycle.	Excellent	Excellent	Excellent	Needle component caps.
Brass	Excellent	Excellent	Excellent	Excellent	Good-excellent	Good	Oral instruments and brass collets.
Copper	Excellent	Good, watch out for acetylides.	Good	Excellent	Good	Good	Unlikely

Gold	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Gold-line wrist, and ankle weights, surgical instruments, in electronic devices.
Magnesium	Excellent	Unknown	Good	Fair	Good	Unknown	Implantable devices
Nickel	Excellent	Excellent	Excellent	Excellent	Good.	Poor	Oral instruments
Silver	Excellent	Excellent	Excellent	Excellent	Poor to good	Poor	Alert bracelets, wound dressing.
Stainless steel	Excellent	Excellent	Excellent	Excellent	With plasma - excellent), without plasma – poor.	Excellent	Cardiovascular instruments, prosthetics.
Titanium	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Hips, limbs, prosthetics, cardiovascular instruments.

[a]: Will vary depending upon formulation and presence or not of stabilisers.
[b]: Will vary depending upon the heat stabilisers added to formulation, and if they are copolymers.
[c]: May vary with discoloration, plasticiser and leaching, flexibility and/or residuals.
[d]: EO residuals may be a problem.
[e]: Nylon formulations will vary in response to high temperature.
The above plastics, elastomers and polymers will vary depending upon formulation, process parameters, and trade names.

5.3.3.2 Costs

Costs vary considerably. One must consider the cost of equipment, labour, consumables, and storage needs. H_2O_2 consumables are more costly than steam, dry heat or O_3. EO in-house *versus* contract costs can vary considerably depending upon labour and transportation costs, availability, regulations, and speed of delivery. More manufacturers are using contract facilities because of the numerous controls and regulations on the use of EO.

Ionising irradiation is performed mostly through contract facilities, due to the high initial costs of equipment, facilities, safety criteria and the professional level of personnel needed, however, many larger manufacturers have their own facilities.

Moist heat is less expensive than either the use of EO or irradiation. Dry heat is the least expensive method.

O_3 is relatively new. O_3 consumables are not expensive. Chambers can be larger than H_2O_2 vessels. Both Chambers require steel or non-corrosive metal.

Peroxide/plasma has the added expense of consumables.

5.3.3.3 Compatibility

This had been discussed previously in **Chapter 4**, EO is the most compatible and widely used method (including hospitals and industrial). It and steam sterilisation can sterilise re-usables. Disposability is critical for single use medical devices. Irradiation sterilises mostly single use devices. Dry heat can be more compatible by using or applying lower temperatures acceptable to materials. For example, PVC can tolerate use of up to 120 °C without any load on it. HDPE can tolerate 120 °C. Cellulose acetate with no load can go up to 120 °C. However, if higher temperatures are applied there is charring, melting, distortion and damage. Metal instruments can be more safely sterilised by dry heat than with moist heat, particularly at lower temperatures, with less corrosion.

5.3.3.4 Ease of Control and Monitoring

Steam, dry heat and irradiation are the easiest to control and monitor. O_3 and H_2O_2 are easier to control and monitor than ethylene oxide:

- *Environment*: Steam, dry heat, electron-beam and O_3 are the most environmentally friendly methods. H_2O_2 with plasma leaves no toxic residues or waste. EO and

gamma irradiation are the more environmentally hazardous, requiring elimination of hazardous wastes.

- *In-House* versus *Contract*: Most traditional methods (e.g., steam, EO, irradiation) are in-house or contract. Dry heat is primarily in-house. Contract is typically used for large sterilisers with more complicated processes and/or heavy controls. EO and irradiation are available *via* contract. There are a few contract steam sterilisers or autoclaves. O_3 and H_2O_2, are typically in-house processes. In-house EO and irradiation typically exist for larger industrial users.
- *Lethality*: All terminal sterilants are lethal, however, radiation and EO have the greater penetration capabilities. Dry heat can penetrate but it may be very time dependent. Heat sterilisation (moist and dry heat) can inactivate prions or pyrogens but the other methods are not recommended for these. The *Pyronema* cotton mould is easily inactivated by steam without damage to the cotton, but not easily destroyed by EO and/or irradiation.
- *Packaging*: Items sterilised by O_3, H_2O_2 and steam must have porous packaging. EO and irradiation need porous packages for reasons of humidity diffusion or degassing of off gases (odours).
- *Regulatory*: Dry heat is the least regulated with some safety precautions. EO is highly regulated because of toxic, carcinogenic and explosive gases with a large number of standards, regulations and criteria. Vessels are typically Association for the Study of Medical Education (ASME) coded.
- Ionising irradiation-radiation (isotopes) and electron-beams have irradiation regulations as well as sterilisation regulations (safety) and other regulations.
- Moist heat has some safety regulations as well as steriliser and other regulations. The vessel must be ASME coded.
- O_3 has few regulations, new process regulations, but some vessel regulations.
- Peroxide/plasma has user safety regulations, new process regulations, H_2O_2 consumable regulations.
- Regulations can vary depending upon the process and the type of vessel used from local, state to national ordinances, rules and permissions. There are always safety requirements and measurements and environmental constraints.
- *Reusability*: Re-sterilising devices and materials is part of environmental good stewardship, as well as reducing costs and waste. Steam, EO, H_2O_2 and O_3 are processes that can be used to re-sterilise many medical devices and materials. Irradiation and dry heat are typically one use, to sterilise materials and devices.

5.4 Novel Sterilisation Methods - The Magic Goes On

In addition to the previously mentioned non-traditional sterilisation methods, there may be unique or novel sterilants that have not yet been fully investigated or employed, for which there is no related audit history, or for which there is little or no published literature discussing its safety and effectiveness for its intended use. Such methods may include, but are not limited to, the use of PAA, ClO_2, other O_3 processing, microwave radiation, pulsed light, gas plasma and sound waves.

Non-traditional methods such as oxidising agents (H_2O_2/plasma) cannot sterilise all materials without adverse effects (e.g., cellulosics). O_3 is not commonly used in industry except for specialty applications (e.g., ozonation of pharmaceutical water), consequently there is a need for novel sterilisation methods.

While H_2O_2 with plasma and H_2O_2 without plasma are similar, H_2O_2 without plasma is deemed to be a novel sterilisation process, not readily acceptable and therefore subsequently discussed further.

5.4.1 Hydrogen Peroxide Vapour (without Plasma)

Like O_3, H_2O_2 can be used in a liquid or vapour state. The information described here relates to H_2O_2 as a vapour and not as a liquid or gas plasma. Gas plasma could not be used in a liquid state. Residual peroxide can have some microbiocidal activity as well as material effects. Good material candidates for use with H_2O_2 are hydrophobic, chemically stable, resist oxidation and moisture. Peroxide decomposers such as silver, copper and cooper alloys should be avoided.

H_2O_2 vapour without plasma typically uses a lower H_2O_2 concentration than H_2O_2 with plasma because it requires aeration to get rid of the peroxide residue and has a longer adverse contact on materials than H_2O_2 with plasma, which requires no aeration. Residual H_2O_2 will depend upon material family, grade, load density, loading weight, specific cycle parameters (**Table 5.11**), packaging and if plasma is used. Typically H_2O_2 sterilisation cannot sterilise such 'large' loads of products or materials as radiation, steam, dry heat or EO methods can.

There are many materials that may be sterilisable once but are not (likely) be resterilisable. Examples of these are:

- Acrylics;
- Butyl and natural rubber;

- Polyacrylates;
- Polyesters (unsaturated);
- Polyglycolides; and
- Polylactides.

The main advantages of using H_2O_2 without plasma are:

- H_2O_2 sterilisation can be used in larger sterilisers and in other applications easily such as isolators in aseptic processing.
- H_2O_2 sterilisation is less expensive than H_2O_2 with plasma.
- It can sterilise many low temperature materials, as well as higher temperature materials.
- It does not have to be manufactured on site.
- It has excellent microbial sterilising capabilities.
- Use of H_2O_2 ultimately leads to environmentally safe breakdown products - O_2 and water vapour.

The principle disadvantages of using H_2O_2 (without plasma) are:

- It has similar limitations to using H_2O_2 with plasma (e.g., it adversely affects cellulosics).
- It tends to produce more surface oxidation.
- Materials must be more resistant to oxidation.
- Attacks metals more than H_2O_2 with plasma.
- The shape of the materials as well as the design of a device is closely related to the longevity and resistance of the device to peroxide sterilisation.
- Polymeric components with large surface to-mass ratios (e.g., fibrous material) will undergo fast oxidative degradation.
- H_2O_2 may not penetrate organic matter well.
- H_2O_2 without plasma may require aeration to remove residuals from certain loads and materials.
- H_2O_2 has limited penetration of lumens.

Table 5.11 Cycle steps and parameters when using H_2O_2 vapour without plasma

Parameter	Notes
Evacuation and conditioning	The sterilisation chamber is evacuated to remove air from the chamber and packaging. The chamber is conditioned to achieve conditions for sterilisation.
H_2O_2 exposure	A solution of H_2O_2 and water is vapourised and allowed to surround and interact with the devices to be sterilised.
H_2O_2 vapour systems	Typical H_2O_2 concentrations are 0.5–9 mg/l, cycle times range from 45 min to 8 h and temperatures range from 25-55 °C.
Evacuation system of H_2O_2 without plasma	This system requires aeration.
Final vent	The chamber is returned to atmospheric pressure.

5.4.2 Chlorine Dioxide

ClO_2 was investigated in the mid- to late 1980s because it has excellent sporicidal properties, without the effects of chlorine. Research has shown that ClO_2 in gaseous and aqueous phases is an effective sanitising agent with both broad and high microbiocidal effectiveness. Aqueous ClO_2 has been reported to effectively inactivate pathogens such as bacteria, spores, viruses and algae. One of the interesting qualities of ClO_2 is its high water solubility, especially in cold water. ClO_2 does not hydrolyse when it enters water, it remains as a dissolved gas in solution. ClO_2 is about 10 times more soluble in water than chlorine. It can be removed by aeration or carbon dioxide.

However, gaseous ClO_2, has been shown to be more effective than its liquid form when applied in equal concentrations and for the same time. In the gaseous form, ClO_2 has been used at temperatures of 27-30 °C or 20-35 °C, gas concentrations of 10-50 mg/l, exposure times of 30 min – 2 h and RH of 80-85%. One of its advantages compared to H_2O_2, is that it has been shown to penetrate into hard to reach areas and dead ends in a vessel. ClO_2 has been used and applied in the decontamination of the Hart Building when there was a threat of *Bacillus anthracis* contamination. ClO_2 has previously been used to sterilise contact lenses, oxygenators, isolators. ClO_2 is able to sterilise complex equipment, such as in a filling line where it is difficult for non-gaseous decontaminating agents to reach all the surfaces and crevices effectively.

For sterilisers and isolators that are densely packed and have impeded circulation, ClO_2 exhibits better distribution than other sterilants such as H_2O_2. The good distribution of ClO_2 gas is apparent.

Chlorine dioxide alters the proteins involved in the structure of micro-organisms, the enzymic function is broken, causing very rapid bacterial kills. The potency of ClO_2 is attributable to the simultaneous, oxidative attack on many proteins, thereby preventing the cells from mutating to a resistant form. Additionally, because of the lower reactivity of ClO_2, its antimicrobial action is retained longer in the presence of organic matter, than with other oxidising agents.

While it has still not been cleared by the FDA, it has been used to decontaminate areas of anthrax contamination, and has been approved by the EPA (registration). Consequently there are some merits to the process as well as demerits. Because of its strong oxidising potential it may corrode some materials, but it is acceptable for use with electronics, batteries, and microconductors.

The properties of ClO_2 are:

- Chemical formula: ClO_2;
- Molecular weight: 67.45 g/mol;
- Melting point: –59.8 °C, crystallises; and
- Boiling point: 11.8 °C.

It is a true gas at normal-use temperatures and is, thus, not affected by temperature gradients that can cause condensation with vapours, but it is unstable. At concentrations greater than 15% volume in air, ClO_2 explosively decomposes into chlorine and O_2. The decomposition is initiated by light.

ClO_2 is a yellow to reddish-yellow gas at room temperature. It has an unpleasant odour, similar to the odour of chlorine and reminiscent of nitric acid. It is a respiratory irritant. Pure ClO_2 is stable in the dark and unstable in light. Inhaled (airborne) ClO_2 acts primarily as a respiratory tract and ocular irritant. In air, ClO_2 readily dissociates both thermally and photochemically and may form chlorine, O_2, hydrogen chloride, $HClO_3$, $HClO_4$, ClO, chlorine peroxide, and/or chlorine trioxide, dependent on temperature and humidity.

ClO_2 is toxic, but it can be produced on site, rather than transporting it from the manufacturer, and it can be neutralised, however, potential residuals remain a concern. The major obstacles of ClO_2 are its unknown material compatibility and the fact that it may produce toxic by-products with impurities or residuals. Consequently ClO_2 may not be considered as a full green sterilisation process.

Breakdown to 'some' very toxic chemicals or producing toxic impurities may be one of its limitations, but it has other limitations:

- It cannot be shipped or stored and must be generated on site, which would increase the complexity of steriliser design.
- Is unstable and classified as a hazardous substance.
- It has deleterious effects on uncoated aluminum foil, uncoated copper, carbon steel, PU, PC, silk, unbleached paper, and passivated stainless steel, however, no corrosion has been observed when using pharmaceutical-type materials such as high-grade 316 and 304 stainless steel, Lexan, and various other plastics such as Delrin (acetal), Teflon and UHMWPE.
- OSHA has an extreme (exposure) limit of 0.1 ppm 8-h TWA and NIOSH has a limit of 0.1 ppm 8-h TWA plus a 0.3 ppm short-term exposure limit (STEL). ClO_2 is highly reactive and causes serious adverse effects in the lungs, including congestion and pulmonary oedema. These effects are presumed to be the cause of death and are likely to be caused by a direct chemical effect on the tissue in the lung. As this effect is not expected to vary greatly among individuals or between species, intraspecies and interspecies uncertainty factors of three have been applied.
- Thermal decomposition of ClO_2 is characterised by a slow induction period followed by a rapid autocatalytic phase that may be explosive if the initial concentration is above a partial pressure of 0.01 MPa. Unstable chlorine oxide may be formed as an intermediate, and the presence of water vapour is hypothesised to extend the duration of the induction period by reacting with the chlorine oxide intermediate. When water vapour concentrations are high, explosiveness is minimised and all decomposition occurs in the induction phase; the water vapour inhibits the autocatalytic phase. The products of thermal decomposition of gaseous ClO_2 include chlorine, O_2, hydrogen chloride, $HClO_3$ and $HClO_4$.
- It has an IDLH of 5 ppm.
- Investigation is ongoing for industrial applications but not for healthcare applications.

The steps in the ClO_2 sterilisation process are:

- *Pre-conditioning*: A pre-conditioning room or in chamber is brought to the proper RH set point (typically 70-85%). Humidity can be generated using a variety of methods such as steam, fine particle-size atomisers, hot plates, or foggers. Steam offers the quickest, cleanest, and most efficient way to raise the humidity.
- *Conditioning*: Once the humidity is at the proper level (e.g., 70-85% RH), the cycle can begin its conditioning time (typically 30 min), during which the RH is monitored continuously. If the RH drops by any significant amount (5%), moisture

must be added to the chamber. This step conditions the spores and prepares them for the charging step.

- *Charging*: During charging, ClO_2 gas is generated and introduced into the chamber through a small tube to achieve a set gas concentration. The target concentration is dependent upon various factors including cycle time, the stability of the sterilant, amount of reagent gas, ambient pressure cycle, and vacuum chamber cycle. If the cycle time is extremely important, a higher concentration is sometimes selected to achieve a faster kill (15–50 mg/l). At higher concentrations, the D-values are achieved much more quickly, thereby shortening the overall cycle. If a site has limited consumables or reagent gas, a lower concentration can be used to preserve consumables (1–15 mg/l), but the exposure time must be extended accordingly. Because ClO_2 is easily measured in real time, the target concentration can be achieved each and every time in a straightforward manner, thus ensuring a repeatable and reproducible decontamination cycle. When gas concentration reaches the target concentration, the cycle proceeds to the next step.

- *Exposure*: During exposure, the ClO_2 gas concentration is monitored and maintained at the selected concentration for the entire exposure time (typically 30 min to 2 h). In addition, if the gas concentration drops during the cycle because of ClO_2 absorbance by cellulose materials, ClO_2 gas is added to ensure the required ClO_2 concentration is maintained during the entire decontamination exposure step.

- *Aeration*: This involves removing ClO_2 to the outside by an exhaust system and the time required to bring the ClO_2 concentration in the chamber to a safe level (0.1 ppm). During aeration, ClO_2 gas is removed from the chamber by allowing clean air into the chamber and evacuation of the ClO_2.

5.4.3 Peracetic Acid

Peracetic acid (PAA) has been historically used as fumigant, widely used in the food industry and demonstrated to have a high order of sterilisation capacity, however, it can damage or degrade some materials (e.g., copper). A typical wet PAA concentration may be 35% or greater, but lower (e.g., 0.2%) have been used.

PAA is a strongly oxidising solution, typically in an equilibrium mixture of acetic acid and H_2O_2, and PAA. It is typically a wet process, used as a liquid for reprocessing of some healthcare products. While it has been used as a vapour, it was first recognised as a vapourised liquid sterilant. It is extremely reactive and consequently a very hazardous chemical. It has had limited use - basically for sterilising endoscopes and other approved items (e.g., articles or devices). Unapproved items when used have

caused damage to humans (e.g., eyes). The equipment is a close designed system. It requires rinsing with a neutralising agent. Liquid PAA solutions of 0.8 and of 0.2% have been approved by regulatory bodies and the EPA for processing endoscopic equipment. The buffered solution at 50-55 °C is circulated through and around the devices for about 12 min. This is followed by an automated rinse to remove the sterilant from the products. Specifically designed trays or containers are used for positioning the instrumentation. As with any liquid process, monitoring is a problem. The thermophile *Bacillus stearothermophilus* BI is used with PAA. Acetic acid is the by-product and has an OSHA and NIOSH exposure limit of 10 ppm for an 8-h TWA. The IDLH for acetic acid is 50 ppm. The RQ for PAA in case of a release is one pound under SARA Section 302 as an EHS. PAA may denature proteins, disrupts cell wall permeability, and oxidises sulfhydral and sulfur bonds in proteins, enzymes, and other metabolites in microbes.

Advantages of using the PAA liquid system:

- Active ingredients (e.g., 22% H_2O_2 and 4.5% peroxyacetic acid).
- Compatible with a wide variety of materials and instruments.
- Environmentally friendly by-products (acetic acid, O_2 and water).
- Excellent microbial killing at low concentrations.
- Fully automated.
- Low temperatures 50-56 °C.
- No adverse health effects to the operators.
- Rapid cycle time – only 12 min.
- Suitable for devices such as flexible/rigid scopes.

Disadvantages of using the PAA liquid system:

- A buffered solution at 50-55 °C is circulated through and around the devices for about 12 min. This is followed by an automated rinse to remove the sterilant from the products.
- A by-product, acetic acid, has an OSHA and NIOSH exposure limit of 10 ppm 8-h TWA.
- A liquid PAA solution of 0.2% has been approved by FDA and EPA for processing endoscopic equipment.

- As with any liquid process, monitoring is a problem. *Geobacillus stearothermophilus* BI are used with the process.
- BI are not suitable for routine monitoring.
- Extremely reactive and consequently a very hazardous chemical.
- Limited basically to scopes, and some other healthcare products.
- No packaging, need to use immediately after treatment.
- Only a small number of instruments can be processed in a cycle.
- Requires rinsing with a neutralising agent.
- Specifically designed trays or containers are used for positioning the instrumentation.
- The equipment designed as a closed system.
- The IDLH for acetic acid is 50 ppm.
- The RQ for PAA in case of a release is one pound under SARA Section 302 (EHS).

5.4.3.1 Peracetic Acid Hydrogen Peroxide Vapour System

The PAA - H_2O_2 vapour system with plasma has a questionable use in the market at times, due to a previous withdrawal by the FDA. It has been previously recognised as a vapourised process, without plasma, using an alkalising agent to decompose it. Chemically, PAA - H_2O_2 is the equilibrium mixture of H_2O_2, PAA, and acetic acid. In the concentrated form (>30% solution), it is corrosive to equipment and irritating to human tissue.

In the plasma process (phase one of the process), a 5% solution of PAA is introduced into the sterilisation chamber under a deep vacuum; in phase two, a non-flammable mixture of hydrogen gas, O_2 and a carrier gas is subjected to microwave electromagnetic energy to create the plasma. The PAA vapour breaks down to H_2O_2 and acetic acid vapour. The manufacturer recommends exhausting of both phases *via* a dedicated or common outside air duct.

Unlike EO, which is an alkalising agent and penetrates through packaging and most devices, PAA/H_2O_2 vapour are an oxidising agent and have surface contact capability only. With this system, however, up to six deep vacuums can be drawn to enhance penetration.

5.4.4 Glutaraldehyde

Glutaraldehyde may not be classified as either a non-traditional or novel process because it has been used in devices consisting of animal origins or enzymes [7].

However in healthcare it is typically used in aqueous solutions, but generally in a closed system, to keep residuals and toxic air levels down. It may be a high-level disinfectant but becomes a sterilant (if left in solution for required time). Glutaraldehyde in aqueous solution has received considerable attention, aimed at increasing its potency and rapidity as a sporicidal agent, but it remains a long process. Some examples of oxidising agents in solution are ClO_2, H_2O_2, hypochlorite, iodine, ozonised water, peroxyacetic acid, peroxyformic acid, superoxidised water, but they are typically too oxidising and damage tissues and enzymes. Liquid sterilants need to be able to penetrate into crevices and beneath films of organic matter. The final sporicidal composition should be able to rapidly permeate, flood and kill microbes in mass. Using these agents with ultrasonics may improve their effectiveness, but equipment and handling is needed. Many surfactants have been found to enhance this quality.

Some mixtures such as formaldehyde and glutaraldehyde continue to be used as the only means to sterilise certain biological matter, enzymes, and tissues.

Some typical sterilising glutaraldehyde concentrations are 2 or 2.65%. Some authorised mixtures (regulatory approval) of glutaraldehyde are: glutaraldehyde and phenol/phenate, and glutaraldehyde and isopropyl alcohol. Some advantages of using glutaraldehyde are:

- Kills vegetative bacteria in minutes.
- Sterilises in 6-10 h.
- Non-corrosive to metals.
- Compatible with most rubber and plastic materials.
- Can sterilise some enzymes.
- Can sterilise tissues.

Some of the limitations of using glutaraldehyde are:

- It is essentially a liquid or wet system.
- It is unstable, 14-28 day products. Testing should be done periodically (e.g., every 2, 4, 6 and 8 days, to check its effectiveness). Typically if the concentration goes below 1% do not use.

- It typically requires activation at pH 7.5-8.5.
- Used typically for unwrapped items only.
- Has hazardous residuals, which must be rinsed off.
- Vapourises.
- Produces noxious odours and residuals.
- Has no cleaning ability so the products must be cleaned first.
- Causes contact allergies, it is a mucus membrane irritant, and it is malodorous.
- There is a new American Conference of Governmental Industrial Hygienists ceiling limit of 0.05 ppm threshold limit value - ceiling limit as of May 1997.
- It requires exposure monitoring.

5.4.5 Ortho-phthaldehyde

OPA may be used as a liquid substitute for glutaraldehyde but it is regarded more as a high-level disinfectant than as a sterilising system. However, 0.55% OPA may be mixed with 2% glutaraldehyde for sterilisation.

The OPA molecule is a dialdehyde, consisting of two formyl (CHO) groups attached to adjacent carbon centres on a benzene ring.

OPA has a melting point of 56 °C, consequently it is less volatile and less irritant than glutaraldehyde. It is soluble in water. It has a very low vapour pressure at room temperature of only 0.69 Pa.

OPA (0.5%) was not sporicidal within 270 min of exposure, however, increasing the pH from its unadjusted level (about 6.5) to pH 8 improved sporicidal activity. It may sterilise after 32 h.

OPA has several potential advantages compared to glutaraldehyde:

- It does not require exposure monitoring.
- It has a barely perceptible odour.
- It has excellent stability over a wide range of pH (3-9).
- It is not a known irritant to the eyes and nasal passages.

- It received clearance for use as a disinfectant from the FDA in 1999.
- It requires no activation.
- OPA has excellent material compatibility.

Disadvantages of using OPA are:

- It is not typically regarded as a sterilising agent, but it can be mixed with glutaraldehyde for use as a sterilant.
- OPA stains proteins gray (including unprotected skin) and, thus must be handled with caution (i.e., use of gloves, eye protection, fluid-resistant gowns when handling contaminated instruments, contaminated equipment, and chemicals).

Some recommendations for all alternative non-traditional and typical novel processes are:

- Cleaning is necessary to remove salts and proteins (organic matter) before sterilisation.
- Failure to ensure exposure of micro-organisms to the sterilant in hidden areas, long lumens, connectors, and matted surfaces could result in failure of the process.
- Unlike most traditional methods they do not penetrate well.

5.4.6 The Chemiclave and Low Temperature Steam Formaldehyde

The Chemiclave is a machine (steriliser) that sterilises using a small quantity of formaldehyde, alcohol, ketones, and some water vapour (steam). The Chemiclave is also a formaldehyde/alcohol vapour chamber process used primarily in small 'table-top' dental sterilisers. It is a vapour at elevated temperatures (132 °C or higher), with a minimal pressure of 0.14 MPa. The sterilant is a mixture of formaldehyde, alcohol, ketone, and low-level steam with a 7-8% RH.

It has the following advantages:

- Faster than the conventional steam process with air washes.
- No drying cycle is required.
- No rust or corrosion of instruments.

It may operate at 132 °C and 0.14-0.27 MPa, and with a reasonable cycle time of 20 min. Some older vessels operate at 30 min exposure times. It is mainly used for

unwrapped dental instruments, the chemiclave is appropriate for heat and/or moisture-sensitive medical devices.

It has the some disadvantages, in that it is toxic and may not penetrate as well as steam.

The OSHA worker exposure levels - for formaldehyde may be 0.75 ppm for an 8-h TWA and 2 ppm for a 15-min STEL. The IDLH for formaldehyde is 20 ppm and it is considered a potential carcinogen. The RQ in case of a release is 45 kg under the Comprehensive Environmental Response, Compensation, and Liability Act. Alcohol also has OSHA exposure levels. Its advantage is that is prevents dulling of sharp, cutting, working instruments due to moist heat and oxidation of moisture. Its largest application is in dental surgeries.

Steam sterilisation is no panacea. It has long been known that steam sterilisation penetrates better than the chemical Vapo-Steril (ethanol and formaldehyde) of the Chemiclave. But 100% humidity of steam causes fibre optic degradation, rusting, and removal of water is difficult. Steam sterilisation is not necessarily the optimal method that preserves hand piece function best for all dental hand-piece designs.

The Chemiclave alcohol/acetone/formaldehyde vapour must penetrate thin packs and condense on dry instruments to kill spores. It requires approximately 131 °C and 0.14 MPa pressure, about 30 min total time and must be operated according to manufacturer's directions:

- Do not skimp on time if timing can be varied.
- The cleaned instruments must be dried well before sterilising.
- Only the wrap prescribed by manufacturer must be used, not cloth.
- Only the manufacturer's steriliser fluid must be used.
- Avoid breathing the vapour.
- When possible, the steriliser must be cool before opening door to reduce fumes.
- It is not suitable for towel packs.
- It may not penetrate as well as saturated steam.

5.4.6.1 Low Temperature Steam-formaldehyde

Low temperature steam-formaldehyde is a process used in Europe, India, and Asia. It is applied to polymers and materials that cannot withstand the high temperatures of

steam sterilisation (e.g., 121-134 °C), since its temperature typically stays below 85 °C.

The process consists of four steps:

- Pre-treatment with a series of evacuations and steam to remove air from the steriliser and provide heat (e.g., 65-85 °C).
- Injection of formaldehyde in the form of heated formalin solution that is evaporated (8-16 mg/l formaldehyde) and 75-100% RH.
- Exposure to maintain temperature, formaldehyde concentration, and humidity.
- Post-treatment is to remove the formaldehyde with steam and evacuation pulses that will flush out the formaldehyde residuals.

Formaldehyde is toxic, and carcinogenic. However, the process is faster than EO and less costly. Its irritating odour even at extremely low levels prevents its increasing use. Ammonia, however, has been used to neutralise and cut its odour.

5.4.7 Chlorine and Superoxidised Water with Chlorine

Chlorine has long been used as the disinfectant in water treatment and hypochlorites are widely used in healthcare facilities in a variety of settings, but it may also be used as a high-level disinfectant or sterilant for endoscopes.

Low concentrations of free available chlorine (e.g., HOCl, OCl^-, and elemental chlorine (Cl_2) have a biocidal effect on mycoplasma (25 ppm) and vegetative bacteria (<5 ppm) in seconds in the absence of an organic load. Acidified bleach and regular bleach (5,000 ppm chlorine) can inactivate 10^6 *Clostridium difficile* spores in <10 min.

The microbicidal activity of a new high-level disinfectant, 'superoxidised water with chlorine', has been evaluated. In October 2002, the FDA cleared this superoxidised water as a high-level disinfectant.

The idea of electrolysing saline to create a disinfectant or antiseptics is intriguing because the basic materials are only saline and electricity, both of which are inexpensive and the end product (i.e., water) does not damage the environment. The principle products of this oxidised water are hypochlorous acid (e.g., at a concentration of about 144 mg/l) and chlorine.

As with any germicide, the antimicrobial activity of superoxidised water is strongly affected by the concentration of the active ingredient (available free Cl_2). One manufacturer generates the disinfectant at the point of use by passing a saline solution

over coated titanium electrodes at 9 A. The product generated has a pH of 5.0–6.5 and an oxidation-reduction potential (redox) of >950 mV. Although superoxidised water is intended to be generated fresh at the point of use, when tested under clean conditions the disinfectant was effective within 5 min when 48 h old. Unfortunately, the equipment required to produce the product may be expensive because parameters such as pH, current, and redox potential must be closely monitored. The solution appears to be non-toxic to biological tissues, non-corrosive and non-damaging to endoscopes and processing equipment. With such a germicide formulation, the user should check with the device manufacturer for compatibility with the germicide. Additional studies will still be needed to determine whether this solution could be used as an alternative to other high-level disinfectants, germicides or antiseptics for hand washing, skin antisepsis, room cleaning, or equipment disinfection (e.g., other endoscopes, dialysers and so on).

5.4.8 Super Critical Carbon Dioxide

Super critical carbon dioxide ($scCO_2$) is an effective alternative for terminal sterilisation of biological materials and medical devices. Moreover, this process is gentle, as the morphology, ultrastructure, and protein profiles of inactivated microbes are maintained. This gentle sterilisation process has been commercialised for the sterilisation of advanced biomaterials. The gentle nature of this process makes it a very valuable tool for human and xenogenic allograft sterilisation. This has been a previously unmet medical need. Because current good tissue practices are centered on donor screening and aseptic processes, they leave the recipient of transplant tissue vulnerable to post-transplant infections. Radiation and EO have effects on the tissue or recipient, which restrict effectiveness. Other new technologies such as H_2O_2 with plasma simply do not penetrate the tissue and achieve sterilisation in the deep layers of the tissue. In addition, H_2O_2 plasma technology must produce large quantities of free radicals to achieve sterilisation, these free radicals can react adversely with the materials being sterilised. The tissue banking industry has made great strides in reducing risk, but the addition of a sterilisation technology with minimal impact on tissue can further improve the safety of the tissue supply. $scCO_2$ is able to achieve a SAL of 10^{-6} without any degradation of the valuable finished product. Additional established applications of $scCO_2$ include, but are not limited to, polylactic-*co*-glycolic acid - polyglycolic acid, PEK, absorbable sutures, active pharmaceutical ingredients, some drug delivery devices, fabrics, various polymers and plastics, and surgical metals.

Innovative $scCO_2$ processing may sterilise products of animal origin, which eliminates the weakening effects of radiation. It also performs at a near body-temperature (32 °C) process to minimise protein denaturation and yet exceeds a SAL of 1 part in 10^6.

In $scCO_2$ sterilisation, the CO_2 is compressed slightly beyond its critical point, becoming a fluid with the high permeability of a gas and solvent features of a liquid. Its pressure is less than 10.1 MPa (critical pressure = 7.6 MPa) and the critical temperature may be less than body temperature (critical temperature = 31.1 °C); and minor changes in temperature and pressure above the critical point can yield steep variations in diffusive and solvent properties. It has no residual solvent (CO_2 outgases at room temperature); and it provides green processing with no exposure to hazardous materials such as ethylene or radiation, and may result in a reduced inflammatory reaction.

5.5 Future Possibilities

5.5.1 Liquid Performic Acid (HCO_2OH)

A relatively new and novel sterilisation (process) is performic acid (PFAc). Aqueous solutions (up to 90%) can be prepared by mixing 70–98 wt% of formic acid (in water) with 35–50 wt% of H_2O_2. The reaction is reversible:

$$HCOOH + H_2O_2 \leftrightarrow HCO_2OH + H_2O$$

Advantages of using PFAc are:

- Standard urea H_2O_2 mixed with formic acid or its ester is useful for creating PFAc.
- The D-value may be as low as 5 min at a low concentration of only 1,800 ppm Association of Official Analytical Chemists (AOAC) sporicidal challenge) at 44 °C.
- The solution may be diluted with water before use.
- It is more stable than O_3.
- O_3 decomposes slowly (in minutes; ½ h life) at ambient temperatures and rapidly (in seconds) at higher temperatures, while PFAc is stable up to 12 h.
- It is more volatile than PAA - it boils at 50 °C ((13.3 kPa); 90% pure acid), while PAA boils at 25 °C (1.6 kPa).

More than one solution may be mixed before use. One component of it, may be an ester of formic acid that could be mixed with an oxidiser (e.g., urea H_2O_2) and water).

The uniqueness and advantages of this technology are that it has been used as a 'total' endoscopic cleaning and sterilising process system in 30 min, with only 1,800 ppm at 44 °C to inactivate >6 log AOAC sporicidal test. At a higher PFAc concentration and a lower temperature it can be made more rapid and faster.

PFAc is a stronger oxidiser than PAA, but is lower than O_3. PFAc breaks down into CO_2 and water, while PAA produces acetic acid and water. *Note:* the corrosiveness of PAA, metal acetates, and by-products mean it is not to be used with some medical devices.

PFAc is a colorless liquid, soluble in water, alcohols, ether, benzene, chloroform and other organic solvents. Its strong oxidising properties are used for cleaving disulfide bonds in protein mapping, as well as for epoxidation (converting ethylene to EO), hydroxylation and oxidation reactions in organic synthesis.

The disinfecting action of PFAc is also faster than that of the related compounds PAA and H_2O_2.

PFAc is non-toxic - it does irritate the skin, but less so than PAA, however, the concentrated acid (above 50%) is highly reactive. Its acidity can have a pKa of only 7.1.

There are no reports of tumorigenic properties.

Use of different esters, an alcohol for creating higher concentrations of PFAc; may also be 'possible' if using a processing aid with the mix, for greater effectiveness.

Of the major drawbacks of PFAc are its handling dangers related to its high reactivity, as well as its instability, especially upon heating (explosive at 80-85 °C), which means that the acid must be used within about 12 h of it being synthesised. PFAc is fast acting with no residual effects.

As an aqueous mixture with formic acid and H_2O_2, when accurately mixed, they form a highly efficient biocide with PFAc as the active substance. PFAc decomposes to form hydroxyl radicals that kill the bacteria.

For disinfection of water, after 10 min the water is disinfected and within an hour after dosing, the active substance can no longer be detected. It breaks down into carbon dioxide and water. No toxic disinfection by-products are detected.

It has a distinct advantage because of low energy consumption, competitive operational costs, minimal maintenance requirements, and a much lower investment cost compared to ultraviolet (UV)-light or O_3 disinfection.

Further future sterilisation possibilities exist for sterilants, particularly PFAc that not only sterilises, but also leave a preservative environment and more.

5.5.2 Propylene Oxide and Steam

The combination of steam and propylene oxide as a means of sterilisation could leave their by-product - the preservative propylene glycol on the product or item sterilised.

Another example, a synergistic and magical pesticide has recently been ‘proposed’ for (sterilising) safe buildings (e.g., contaminated with anthrax). One of the agents has already been accepted as a pesticide *in lieu* of O_3 depleting methyl bromide for strawberries and so on. It may create a preservative upon exposure to sterilising UV light. This approach may be not only potentially very fast and effective but potentially capable of preserving the item it treats with an antiseptic substance with antimicrobial protection properties.

5.5.3 Ozone (Cold Sterilisation)

O_3 has continually been re-investigated as an ambient sterilant. Another option, not necessarily applied yet, for example, is O_3 under cool/cold temperatures, because O_3 has been used effectively for cold storage of fruits and vegetables. Under cooler or cold temperatures, O_3 is more stable and humidity or moisture content increases relative to ambient or higher temperatures. Also less oxidation and reactivity should occur at lower temperatures (cooler) than at ambient or higher temperatures.

While H_2O_2 works well at a humidity of 10-15% RH, O_3 requires higher humidities ($<80\%$ RH) in order to demonstrate low temperature sterilising conditions of low water activity in microbes (dry or dessication) and effective oxidation from the O_3.

5.5.4 Low Temperature Dry Heat

Dry heat under extremely low water activity has been demonstrated to be an effective sterilant down to 45 °C. With assistance from other chemical as well as physical agents, its temperature and exposure time may be drastically reduced. For example, sterilising by heating under vacuum with infrared rays is a possibility. Such low dry heat conditions may be favourable for many heat and moist heat sensitive products.

5.5.5 X-ray Sterilisation - Technology of the Future

Sterilisation is a critical aspect of modern medical care when we think of all the patients who require syringes, surgical instruments, surgical tools, oxygenators, dialysers, IV sets, endoscopes, sutures and bandages. The first breakthrough in irradiation sterilisation technology was with electron beam and gamma emitting sources in 'industrial sterilisation,' which kills microbes with electrons and gamma irradiation. They have been used in industrial applications for more than half a century. Today hospitals with re-useable heat sensitive medical devices, equipment, and products may be sterilised with EO, H_2O_2 or O_3 sterilisation but not typically with gamma or electron beam sterilisation, however, there is another method with numerous advantages: bathing the products and its packaging with a focused beam of X-rays from an X-ray machine.

X-rays are a good, practical alternative to traditional irradiation methods for medical device sterilisation.

Commercial use of X-ray sterilisation for medical care began approximately 15 years ago but full commercial adoption has been slow because of the low output power of early accelerators. The invention of a high-power, high-energy accelerator (e.g., a Rhodotron) is quickly changing its magic.

Modern industrial accelerators have increased the throughput rates in X-ray processing facilities so that this irradiation method is now competitive and more economical than medium and large ^{60}cobalt facilities. Today, there are sterilisation facilities with X-ray capability in Europe, Japan and North America.

High-energy X-rays are suitable for sterilisation processes in which the materials and products are too thick to be penetrated by electron beams. The radiation processing of materials and commercial products with high-energy X-rays can produce beneficial changes that are similar to those obtained by irradiation with gamma rays emitted by ^{60}cobalt sources. The chemical and biological effects of X-ray and gamma-ray processing are similar because both types of energy can produce ions and free radicals in irradiated materials. The practical differences are attributable to physical characteristics such as angular distribution, penetration, dose uniformity and dose rate.

Low energy, soft X-rays have been used in hospitals for many years. With the advent of threats from bioterrorism and the potential presence of *B. anthracis*, X-ray sterilisation has been revisited. First with the use of the Rhodotron hard X-ray irradiation of mail, and also the possibility of other soft X-ray sources for irradiation of facilities and medical applications [8].

Hospitals already have some knowledge of X-rays from soft X-ray machines, but they could more easily adapt to hard wave X-rays than going to electron beam and gamma emitting sources. X-rays have long been recognised as a method of sterilisation since it was demonstrated as early as 1896 that they inactivated micro-organisms. X-ray sterilisation of medical products has been studied theoretically and experimentally by the US National Bureau of Standards, universities and accelerator manufacturers since it was first investigated more than 40 years ago.

But its practical application followed EO, and irradiation with the continuous improvement of plastic materials and medical devices. Now is the time for going back to using X-rays.

Unfortunately, germicidal UV radiation will not penetrate most common materials such as paper, plastics, fibres or metals. In contrast, electron beam will, but will not penetrate deep enough and high energy gamma rays will penetrate many objects, but require very large doses due to the small probability of interaction with the biological pathogens of interest, thus requiring massive shielding for safe use. X-ray radiation can found to be a suitable decontaminant, is penetrating, and can be controlled simply and safely.

The main points of X-ray sterilisation are:

- It has long been recognised as a sterilant (since 1896).
- Much of the early work with irradiation was done on the use of UV light, including repair mechanisms with UV light.
- UV light worked if there were no penetration problems.
- Much research was performed on other forms of ionising radiation, particularly electron beam and later gamma rays.
- X-ray is a ‘hybrid’ between electron beam and gamma irradiation. Its radiation, like electron beam is generated from electron accelerators, but X-rays are converted from electrons that are transformed into photons. X-ray photons are nearly identical to photons from gamma sources. Both have high penetration capabilities.
- It is recognised that radiation may damage a few materials, but by varying the dose and irradiation wavelength or energy, more materials may be irradiated and resterilised with X-rays in the future.
- Soft-hard X-rays at different wavelengths may vary in their sterilising capabilities, soft X-rays may not have as much penetration as hard X-rays.

- It was quickly learned that infrared radiation was due mainly to heat not ionising irradiation.
- Electron beam and gamma radiation were not used in hospitals, but in industry, while X-rays have been used in the hospitals before being used as a sterilant or in industrial applications.

There has long been a need for a sterilisation method which would be easy to prepare products for sterilisation, highly penetrable, and compatible with heat sensitive materials.

In the 1950s and 1960s (electron beam and gamma) radiation sterilisation became a near panacea for industrial sterilisation because of its excellent penetration capabilities, its fast release of treated products and simplicity of routine operation as compared to EO.

The principal advantages of X-ray and ionising irradiation are:

- UV light is like a magic wand but it cannot penetrate as well as X-rays can. Its bactericidal effect occurs in radiation range of 240-280 nm. Its prime use is in treatment of water, and the interiors of biological safety cabinets.
- X-rays have no toxic residuals, no toxic wastes, and do not generate as much O_3 as gamma sterilisation.
- X-rays may have deep penetration, unlike UV and electron beams.
- X-rays do not induce radioactivity in the treated products, just as an X-ray of the human body does not make a person radioactive.
- X-rays have a quick dosimetric release.
- X-rays have short exposure times, and they are faster than gamma irradiation.
- X-rays have a simple routine operation as compared to gamma two-dimensional direction and electron beam, two-sided irradiation. However, sometimes efficient X-ray utilisation of some product loads would need to be irradiated from both sides.
- X-rays are also spread over a wider angle than an electron beam.
- X-rays could sterilise all of the heat and moisture sensitive materials that gamma and electron beams currently do and possibly more (without O_3 or heat generation by the electron beam).
- X-rays have no environmental waste concerns as with gamma rays.

- X-ray dosimetry would be closer to gamma requirements than electron beam.
- X-rays could handle more dense product and larger pass through than electron beam irradiation and are faster than gamma irradiation.
- X-rays do not have the temperature rise concerns of electron beam.
- X-ray product handling can be more robotic controlled and less labour requiring than gamma and electron beam facilities.
- X-rays will have a faster turnaround time than gamma radiation.

Some of the disadvantages of using X-rays are:

- High initial capital and operating costs.
- Inefficient cost conversion of electron beam total energy into X-rays.
- Incompatibility with some plastic materials (e.g., acetal, natural PP, some Teflons) as with other current irradiation methods.
- It requires elaborate safety precautions, but X-rays may be concentrated along one-dimension, while gamma ray emission from isotopic ^{60}Co pencils are usually laid out in a rack with a two-dimensional extension.
- There may possibly be an extended length of time for qualifying of irradiated materials, except for materials already qualified for electron beam and gamma irradiation.
- There is no disposal of radioactive waste with X-rays as with gamma emitting isotopes.

X-rays require only the dose to be delivered. The method is simple, however workers must be trained for safety. Focused facility designs and controls are needed to minimise and eliminate the risk of irradiation to workers or the surrounding environment. Like electron beam radiation, X-rays are generated by a machine rather than by gamma emitting cobalt. And while X-ray sterilisation isn't quite as fast as electron beam processes, they would be faster than gamma processing. X-rays penetrate much more deeply than particle-based electron beams, similar to gamma radiation.

The system may cost a bit more than gamma processes, however, X-ray sterilisation will be much faster than gamma, which will shorten turnaround times. X-rays may also penetrate deeper than gamma radiation, and may be less harmful to some products because of the shorter exposure times, and focused irradiation dose that will reduce the maximum dose of gamma irradiating sources. The longer (and the higher doses) some plastics and pharmaceutical (drugs) stay in front of the irradiation

source, the more likely they will be damaged from gamma radiation, but with X-rays the exposure is shorter.

X-ray sterilisation is still in the experimental phase for combination products (medical devices and drugs), but the technology is likely to evolve within the market within the next few years. It is also expected that the operation will be more robotic controlled and less labour intensive as gamma and electron beam facilities are now.

For convenience and safety, manufacturers may deliver combination devices such as syringes with the pharmaceutical drugs already inside, as well as sterile surgical tools in sterile packages, ready to open in the sterile environment of the operating room. They need to kill every single micro-organism on both the product and packaging without adversely affecting the product (e.g., drug) quality.

Use of electron beam tends to overexpose the material with excess electrons and raise the product temperature and gamma irradiation will deliver both a low (minimum) and high (maximum) dose with a high minimum/maximum dose ratio. The high (maximum) dose may be incompatible for some materials, while the minimum dose may not adversely affect it.

Electron beam sterilisation is sometimes out of the question, because it cannot penetrate the total product contents, while gamma-ray sterilisation may damage the active ingredients in a drug with use of a high maximum dose. Techniques involving radiation emitting material raise concerns about worker safety and proper waste disposal.

While use of X-rays may sidestep problems, with faster speeds than gamma, and the penetrating problems of electron beam, X-ray energies may also be varied in the future - change of wavelength may also improve material compatibilities and the number of resterilisations for hospital sterilisations.

X-ray sterilisation can treat the same product range as gamma rays and it is the only irradiation technology able to reach dose uniformity ratio values of between 1.2-1.3 when sterilising pallets of product. It is believed that irradiation sterilisation will slowly migrate towards X-rays. Drivers for this migration will be the market demand for better sterilisation quality and microbiocidal effectiveness. There will be stronger hurdles related for ^{60}cobalt, such as continual price increases, more regulations linked to the transport of radioisotopes, hazardous wastes, and limited availability of ^{60}cobalt in the future.

5.5.6 Combination of High Intensity Ultraviolet and X-ray Radiation

The combination of a high-intensity UV emitter and an X-ray radiation source within a single package would offer a clean, non-toxic, and non-corrosive alternative for the surface and sub-surface multi-biological warfare agent decontamination or sterilisation of buildings and equipment. An electron beam pumped semiconductor UV source technology that combined with an X-ray source, could result in a UV/X-ray bio-decontamination unit.

5.5.7 Alternative Aseptic Processing Techniques

With all of the new alternative sterilisation techniques such as H_2O_2, ClO_2, O_3 and PAA, aseptic processing and assembly areas may be thoroughly decontaminated and virtually sterilised.

While these alternative aseptic processing techniques have many advantages such as its capacity to achieve device and material compatibility when other terminal sterilising agents will adversely affect the finished product due to extreme oxidation, moisture, heat or irradiation; it has the severe disadvantage of not being able to provide a 10^{-6} sterility assurance. These new alternative decontamination methods should never be considered or applied, without a high level expert sterilisation evaluation and study.

5.5.8 Microwave Radiation

A microwave works by vibrating water molecules and the heat results from that friction. Sterilisation by microwave is created by a lower temperature than by pure steam heat alone, but typically you cannot sterilise metal without difficulty. Microwave radiation is not the same as ionising irradiation (for example electron beam, gamma rays, UV or X-rays).

Microwave sterilisation may continue to be an option in the future. Steaming with microwaves may potentially inactivate anthrax spores in letters, if followed by drying. The sterilisation action is essentially heating by two mechanisms: dielectric and ionic. The dielectric heat is principally due to the forced resonant oscillation of the dipolar nature of water. Ionic heat may be mechanically caused by the oscillating electromagnetic field, producing heat. Efficacy of the process is influenced by the presence of water, and microwave power.

The principal advantage of microwave heating is simply because it heats faster than conventionally heating. Particularly for thick cross-section solids, where conventional

methods would rely on the slow process of conduction of heat from the exterior to the interior.

The other advantage, is that sterilisation may be achieved at lower temperatures from microwave

Heating, than from conventional heating. This may improve material compatibility.

Its principal disadvantage is its non-uniform heating. To achieve reliable sterilisation, heating needs to be nearly uniform throughout the material. The control of uniformity of heat is difficult to achieve, resulting in microwave facilities that are very large, complex and costly. When compared to dry heat, microwaves affected the cutting capacity of carbide burs, while dry heat did not.

Microwave processing is also useful as a technique in food processing.

Microwaves can create low pressure, plasma sterilisation.

5.5.9 Pulsed Light or High Energy Pulsed Light

The mechanism of pulsed light or white pulsed light is a function of both the high peak power and the broad spectrum of the flash. Certain wavelengths such as UV may be more effective for lethality, but it can be filtered out if it were to cause undesirable photochemical effects on materials to be sterilised. Longer wavelengths (without UV or non-ionising radiation) may have less material compatibilities, however, it may cause some heating photothermal effects on some materials. Most of the energy of the light is concentrated in the 170-2,600 nm wavelength range (for example, 25% UV (200-280 nm); 45% visible (380-780 nm), and 30% infrared (780-1,100 nm). The spectrum is similar to natural sunlight at sea level, but its intensity may be as much as 20,000 times greater. The pulses for example, from high powered xenon lamps for example, are typically delivered at a rate of 1 to 20 pulses per second, where each pulse may last from 1 µs to 100 ms.

The flash light in the UV spectrum targets deoxyribonucleic acid (DNA) molecules but other wavelengths may cause destructive effects on cell membranes, proteins, enzymes, and cellular structures.

Its main advantages are that pulsed light may reduce or eliminate the need to use chemicals or irradiation on materials, and the process is measured in seconds rather than minutes or hours.

It has been used to sterilise packaging material surfaces, water, and other clear solutions and some medical equipment surfaces.

The inactivation of microbes on surfaces may be sterilised in a fraction of time (virtually seconds) compared to other sterilisation methods and with no undesirable residues.

It principal disadvantage is its very low penetration of materials, used principally for surfaces only, it cannot go around corners for example, but will penetrate through clear films and solutions) but not opaque materials, and may have some potential undesirable photochemical or thermal effects of some wavelengths that might need to be filtered out.

5.5.10 Gas Plasma

Low vacuum, and low temperature gas plasma continues to be an attractive substitute for EO processing which leaves adsorbed toxic residues, whether in combination with H_2O_2 or PAA. In O_2-based plasmas, the de-activated spores are assumed to be due to their slow combustion with the active species, which produces CO_2 and H_2O. In the absence of ion bombardment, the concentration of O_2 atoms is an important parameter to determine the plasma sterilisation efficiency. An initial improvement would be to generate the plasma in the sterilisation volume itself, rather than in a separate compartment. A number of parameters can be involved such as the pressure or the type of gas or chemicals applied, or the electrical energy supplied to create the plasma.

Plasma is basically ionised gas. When an electric field is applied to a gas, it gets ionised into electrons and ions, creating a fourth state of matter.

Plasma sterilisation operates differently from other sterilants because of UV photons or radicals created (atoms or assembly of atoms with unpaired electrons, therefore chemically reactive, e.g., O and OH).

An advantage of the plasma method is the possibility, under appropriate deep vacuum conditions, of achieving such a process at relatively low temperatures (<50 °C), allowing for the integrity of polymer-based instruments, which cannot be subjected to autoclaves and ovens or even irradiation. Also, plasma sterilisation can be safe, both for the operator and the patient, in contrast to EO.

One disadvantage of plasma is that it cannot penetrate as EO can. Additionally plasma sterilisation with its low penetrability, has the inability to effectively sterilise

complex geometries, and its efficacy tapers off with increasing bulk and or product to be sterilised (i.e., size and loading effect), and there may be certain materials that cannot be sterilised without adverse effects. EO is typically a gentle chemical medium. Because of these considerations plasma sterilisation cannot completely monopolise the sterilisation industry. Recently, however, industrial reactors based upon plasma have been installed in pharmaceutical packaging, where surface decontamination or sterilisation may occur.

5.5.11 Sound Waves

Sound waves have some microbial activity combined with other sterilising agents, but ultrasound is sound waves at a frequency of 20 kHz. Ultrasound is widely used for cleaning, disinfecting and other activities. Its sterilising activity is principally due to micromechanical damage of cellular structures *via* cavitation, but also to the heat that is generated. Like microwave its disadvantage is uniformity. Consequently it is often combined with other processes such as heat and pressure.

It has held great promise in the past for combined cleaning and sterilisation for faster sterilisation.

5.6 Future

A recent market survey(s) indicated the following, in regard to sterilisation methods:

- Steam (moist heat) sterilisation will still be one of the most efficient and safe methods of sterilisation in central supplies.
- H_2O_2 with plasma is gaining popularity over EO sterilisers.
- The future ban on hydrochlorofluorocarbon and high sterilisation time are key factors for the decline of EO sterilisation.
- Most rigid endoscopes need to be sterilised, whereas most flexible endoscopes need high-level disinfection.
- H_2O_2 with plasma can reprocess both rigid and flexible endoscopes.
- Ultrasonics may be useful to process some endoscopes, without damage.
- O_3 sterilisation is expected to increase in use.

- Rapid readout BI will continue to gain, and will improve the time to release EO and other sterilised products.
- Glutaraldehyde is still a liquid disinfectant or sterilant of choice in many healthcare facilities, including sterilisation of tissues.
- The safety concerns of some liquid disinfectants and sterilants will force the development of alternative sterilisation technique for reprocessing endoscopes.
- Liquid disinfectants and sterilants with fast turnaround time are being researched.

5.6.1 Sterilisation by Nitrogen Dioxide and Peroxynitrites

Nitrogen dioxide (NO_2) is a 'recent' room temperature alternative to traditional sterilisation methods and it particularly compatible with some plastics. It is a 'relatively' new process. It requires vacuum, injection of sterilant and injection of humidity. If, in a sterilisation chamber where NO is mixed with air, (instead of NO_2) most of the NO reacts to form NO_2. The only other oxide of nitrogen that forms under these circumstances and is stable at concentrations higher than 1 ppm is N_2O_4, which exists in equilibrium with NO_2 and the concentration of which is determined by the NO_2 vapour pressure. If the air is humidified, NO_2 can be converted into nitric acid ($HONO_2$) at trace levels.

The sterilisation process uses low concentrations of NO_2 gas (<21 mg/l) in the presence of air and water vapour. The NO_2 is an effective sterilant at low concentrations, often between 8-10 mg/l and typically less than 21 mg/l, depending on its application. Therefore, relatively small containers of the NO_2 are required. NO_2 sterilant is supplied as a liquid, from which vapour is dosed into the chamber during the cycle. This is a space saving alternative to large gas cylinders. NO_2 is non-explosive and non-flammable, non-carcinogenic, but toxic.

The process is typically delivered at or near room temperature and consists of evacuation of air from the chamber, the introduction of the sterilant, and the addition of humidified air to a preset pressure, which is typically at or near ambient pressure. These steps may be repeated several times or the sequence may be be changed. At the NO_2 concentrations used, the operating temperature and pressure of the process, the NO_2 remains in the gas phase and acts as an ideal gas throughout the sterilisation cycle. NO_2 can flow, either under vacuum or by diffusion, into complicated geometries such as lumens and mated surfaces; while with H_2O_2 (at a boiling point (T_b) = 150 °C), its condensation can occur prior to reaching the innermost regions of a device as the sterilant concentration approaches the saturated vapour pressure. This condensation may result in localised sub-lethal conditions leading to non-sterile devices. With NO_2

(at a boiling point T_b = 21 °C), this is not an issue because of the high saturated vapour pressure at the sterilisation temperature. Diffusion into lumens can occur without any driving force required to overcome the condensation.

NO_2 sterilisation is a room or ambient 'temperature' process, which provides designers and developers with a good choice for the sterilisation of temperature-sensitive materials such as bioresorbable polymers. Some of the formulations of bioresorbable polymers, such as polylactides, have glass transition temperatures that approach, or reside below, the elevated processing temperatures for EO and H_2O_2 gas sterilisation.

With NO_2 lethality proceeds more rapidly with increasing humidity, but a SAL of 10^{-6} can still be achieved at a reduced %RH with increased cycle duration. For example, a cycle with 80%RH will typically require a sterilant exposure time of only 20-40 min, while an exposure with <25 %RH will be in the order of several hours. This provides balance, with short exposures and the high humidity against longer exposures with low humidity in order to maximise polymer device compatibility with the NO_2 sterilisation process. If %RH is not critical to device integrity, then the higher humidity cycles minimise the overall exposure to the NO_2 gas.

At the end of the cycle, the NO_2 can be scrubbed. The spent scrubber material is a non-hazardous solid waste product (considered landfill-safe in the United States) that can be disposed of in accordance with local regulations.

Most polymers are rather impermeable to NO_2, unlike EO, particularly over the relatively short exposure required with NO_2 gas sterilant, there is virtually no permeability nor residues. This makes aeration with NO_2 a faster process.

NO_2 has been developed, and used to sterilise special catheters, and combination products. It can sterilise PEI, silicone rubber, and cyclic olefin co-polymers (COC). Devices and materials sterilised by this sterilant retain their biocompatibility. For example, PEI (Ultem, Sabic) has characteristics which include chemical and temperature resistance, high strength and durability.

Pure silicone rubber: polydimethylsiloxane exhibits a challenge to NO_2 sterilisation that is similar to that of Ultem or stainless steel. It is relatively inert in terms of reactivity with the sterilant. However, additives are often used in silicone to impart colour or improved mechanical properties. These additives can increase the challenge to sterilisation that is presented by the silicone rubber.

Sterilisation of COC proceeds rapidly, similar to stainless steel, due to the inert nature of the polymer compared to the NO_2 sterilant. COC components remain clear with no colour change after exposure to the NO_2 sterilisation process. Additionally, the NO_2 gas does not permeate the COC material during the sterilisation cycle, which allows

for rapid aeration of the sterilant. This means that residuals from the sterilisation cycle are low. When COC syringe barrels that have been exposed to the NO_2 sterilisation process are filled with American Society for Testing and Materials Class I water, the water remains within the standard limits of 'water for injection' when analysed for sterilant residuals.

An NO_2 sterilisation cycle can be accomplished without the aid of a vacuum to introduce or remove the sterilant. This allows for the room temperature sterilisation of the exterior surfaces of pre-filled syringes and vials without altering the contents of the syringe or vial. This is important as drug manufacturers move towards single dose syringes, many of which require sterilisation as they are used in the sterile field of the operating room or an outpatient facility.

The NO_2 process has some polymer limitations, it cannot sterilise acetal, PA or PU. These materials may limit, and inhibit sterilisaton with NO_2 and they may undergo scissoring. These materials could be substituted with other polymers that would tolerate NO_2 treatment.

NO_2 sterilisation, like H_2O_2 also is not compatible with cellulosic materials like paper and cardboard.

NO_2 sterilisation can use an industrial (contract) steriliser. Currently, the typical cycle time ranges from 60 to 90 min, and the load size is approximately ⅕ of a pallet, which allows a manufacturer to process a pallet per day through the steriliser. The steriliser, which has a self-contained, on-board scrubber system to remove the sterilant from the exhaust gas, can be placed in the manufacturing line making it truly part of the manufacturing process. For manufacturers for whom contract sterilisation makes more sense, a contractor offers services and may work with other contract manufacturers to set up sterilisation hubs in key cities. Switching from EO to NO_2 can help to reduce inventory carrying costs, due to the reduced cycle time and lack of aeration phase.

NO_2 sterilisation provides another alternative for medical device manufacturers. Designers of medical products can choose the NO_2 sterilisation efficiencies through material selection and design choices. The advantage of the NO_2 sterilisation is that because it is in-house sterilisation, it can save time and money by eliminating transportation and inventory carrying costs associated with contract sterilisation. Industrial manufacturers are often reluctant to bring EO or gamma sterilisation in-house because of the substantial capital investments and safety issues that are associated with the methods. Gamma units and facilities are capital intensive, and require cooling water or other facility modifications. Large EO units also require facility modifications, such as abators (scrubbers) and explosion-proof walls, and monitoring because of the explosive, toxic and carcinogenic nature of the EO gas.

NO_2 can be stored at room temperature, and would require similar steps to validate and routinely control it as do traditional methods. Some properties of NO_2 are shown in **Table 5.12**.

Table 5.12 Properties of NO_2	
Molecular weight	46.05 g/mol
Solid phase	
Melting point	-11.2 °C
Latent heat of fusion (101.3 MPa, at triple point)	159.41 kJ/kg
Liquid phase	
Liquid density (0.1 MPa at boiling point)	1443 kg/m^3
Liquid/gas equivalent (0.1 MPa and 21 °C)	424 vol/vol
Boiling point (0.1 MPa)	21.1 °C
Latent heat of vapourisation (0.1 MPa at boiling point)	430.4 kJ/kg
Vapour pressure (at 20 °C)	0.1 MPa
Critical point	
Critical temperature	157.8 °C
Critical pressure	10.1 MPa
Gaseous phase	
Gas density (0.1 MPa at boiling point)	3.4 kg/m^3
Compressibility Factor (Z) (0.1 MPa and 15 °C)	0.992
Specific gravity (air = 1) (0.1 MPa and 15 °C)	1.59
Specific volume (0.1 MPa and 21 °C)	0.512 m^3/kg
Heat capacity at constant pressure (Cp) (0.1 MPa and 24.3 °C)	0.036 kJ/mol.K
Viscosity (0.1 MPa and 20 °C)	0.000132 Poise
Thermal conductivity (0.1 MPa and 50 °C) :	167.47 mW/m.K

Another oxide of nitrogen, peroxynitrite (PON) sterilisation may be a process of the near future. It appears to have less oxidising damage than typical oxidising sterilants such as H_2O_2 and O_3. Spores not killed by PON due to DNA damage had no loss of dipicolinic acid (DPA) during the process. However, PON killed spores with loss of DPA. Although dead, the PON killed spores still germinated and initiated metabolism but never went through outgrowth.

5.6.2 Other Future Sterilants

Other techniques that may be used for sterilisation in the future may be:

- Oscillating magnetic field, but it is currently not a practical reality.
- Pulsed electric fields currently result in a significant log reduction of microbes but not practical sterility.
- High voltage arc discharge may yield several log reduction of microbes under some circumstances.

These processes may be considered as potential alternatives to pasteurisation at the moment, but not yet as alternatives to sterilisation.

5.6.2.1 Vapour Phase Performic Acid (HCOOOH)

PFAc has been considered as a potential liquid sterilant (see ***Healthcare Sterilisation: Introduction and Standard Practices, Volume 1*, Section 5.5.1**). Although no known vapour phase PFAc sterilisation appears to be under development, PFAc should be easier to vapourise than PAA (see ***Healthcare Sterilisation: Introduction and Standard Practices, Volume 1*, Section 5.4.3.1**), so it may be used more readily as a vapour sterilant system. At effective sterilising concentrations, it may have less toxic residues, than PAA (e.g., acetic acid), and it may be synergistic with other sterilants. PFAc breaks down into CO_2 and water. At effective sterilising concentrations, PFAc may be less irritant than PAA; may be less corrosive than formic acid, as a sterilant. There are no reports of tumorigenic properties. However, it explodes above 80-85 °C. PFAc may be more effective microbiologically than PAA, H_2O_2, and O_3.

5.6.2.2 Hydrogen Peroxide and Ozone

A new process utilising H_2O_2 vapour and O_3 may have synergistic and potentiation effects. By first introducing H_2O_2 vapour into the chamber and then O_3, a synergistic effect on microbes may occur which would facilitate penetration of sterilisation into long narrow lumens. Furthermore, it would enable use of significantly lower concentrations of O_3, lowering exposure time, and possibly enhancing further compatibility of polymers and plastics.

5.7 Summary

Healthcare products and facilities will continue to change and modify sterilisation technologies.

Technologies will expand in both in hospitals and manufacturers and in other areas such as: anti-terrorism, aseptic processing, foods, laboratories, military, spacecraft and so on. These will include design changes in products, materials, handling and components. Considerations of availability, cleanliness, cost, material compatibility, minimising regulatory restrictions, rapid speed of processing, fast release, reusability safety, toxicity and the environment will be highlighted. Throughout its history and in the present, a new magic of sterilisation is always beginning, however what will the future be? What will be, will be. The future is not ours to see, but to believe and determine.

So what is an ideal sterilant and what attributes should it have?

The following list gives a few ideas:

- Effective against all biological entities, including prions.
- Fast and just in time.
- Non-toxic or low toxicity.
- Detoxify (e.g., endotoxin).
- Safe and efficacious.
- Available and useable at all locations.
- Can sterilise all materials, products and items without adverse effects or damage.
- Can sterilise biological matter, enzymes, chemicals, drugs, waste and so on.
- No toxic or undesirable residuals.
- Low hazard and environmentally green.
- Meets regulatory requirements (local, national and international).
- Reusable and resterilisable.
- Can be used in liquid and gaseous states.
- Is penetrable, like heat, EO or irradiation.
- It is able to be validated and can be trusted.
- Is inexpensive - both for any capital equipment, energy, steps and/or consumable that is needed.

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6 Sterile Packaging and Sterilisation: A Packaging Perspective

Sterilise: protect the product within the package and keep contamination out.

Without special packaging of healthcare products, infectious disease would exist and likely persist everywhere in a hospital or healthcare facilities, and it is debatable that antibiotics would ever be able to cut or mitigate the impact of infections that occur everywhere. Besides infectious microbes, packages are needed to keep out pyrogens, chemicals, particulates, moisture and so on. Packaging also helps to keep the product in; this may include a liquid, powder, or a solid product. Packaging keeps the product safe and protected from damage and outside environmental liabilities. Packaging must also allow the sterilant to penetrate, to sterilise the enclosed product. Packaging must not be damaged, deformed, altered by its sterilisation method, intended use, storage or handling. Consequently packaging has tremendous value and is of great concern to medicine and healthcare institutions, for product delivery of safe to use, without failure.

The primary purpose of package is to accommodate the sterilisation method and maintain the sterility claims given on the label; to protect the product through all the shipping and storage requirements; and to enhance the usability of the product, without adventitious contamination. It is a packaging truism that 'at the moment of use' a sterile product must be sterile.

If a product is to be terminally sterilised, the sterilisation process as the final step in the manufacturing process, the packaging materials and design must be compatible with the sterilisation process. Ethylene oxide (EO) gas sterilisation, as well as hydrogen peroxide (H_2O_2) and ozone (O_3) methods, requires the sterilant to contact the micro-organisms to inactivate the microbial population and achieve sterility. The package material must be capable of allowing the gas, humidity and so on, to penetrate the package, without allowing any additional micro-organisms to enter the package. For steam, dry heat, radiation sterilisation, including gamma, electron beam and X-ray, the material must be capable of performing properly even after the potentially degrading impact of heat or radiation dose.

A sterilisation process is only as good as its packaging. If a sterilisation procedure should achieve a statistical probability that not more than one non-sterile article

can occur out of one million products, it is important that the package not lose this statistical probability. In order to achieve this and similar criteria many factors must be involved in the design and manufacture of the package. Among these factors are:

- Resistance of the packaging material to bacteria contamination. For example, wetting of packaging can lead to bacterial and other contamination, as well as loss of label identification (streaking, loss of colorants and so on). The use of moisture resistant and moisture impermeable films or materials is optional. Porosity size of packaging can minimise contamination. To maintain sterility of the product after sterilisation, microbial impermeability of the packaging must be demonstrated (see **Sections 6.4** and **6.9.4**).
- Strength of the package - burst, seal, and tear strength (see **Table 6.5** and **Section 6.9**).
- Type of package (see **Sections 6.1**, **Table 6.2** and **Table 6.10**).
- Testing of package –visual, seals, tensile strength, burst test, dye test and more (see **Table 6.4** and **Section 1.4.3**).
- Type of opening - ease of opening without contamination and so on (see **Section 6.12**).
- Suitability of the packaging material to the sterilisation method - bursting, melting, oxidation, discoloration, and more (see **Tables 6.1, 6.7-6.10** and **Section 6.6**).
- Stability and storage - expiration dating, proper environment and more (see **Section 6.5** and **Section 6.10**).

Some attributes for packaging for sterilisation by different modalities will vary (**Table 6.1**).

Once terminal sterilisation has been achieved, the package must be capable of maintaining sterility to the sterility assurance level (SAL) for a length of time equivalent to the labeled shelf-life of the device. The seals of the package must be of sufficient quality and strength to withstand the stresses to which they will be subjected over the labeled shelf-life. Also, the packaging material itself must be of sufficient quality and strength to withstand the abrasions, and stresses to which it will be subjected. This includes resisting impact punctures from sharp components of the product or inserts in the packaging and resisting abrasion as the product is shipped and handled.

Table 6.1 Types of attributes of packages to different Sterilisation modalities

Steam	Dry heat	EO Sterilisation
• Be highly permeable to moisture and air • Resistant • Heat and vacuum/pressure • Change resistant • Allow for rapid heat transfer • Conducts or transfers heat • Water resistant packaging	• Tolerant to high heat • Capable of transferring heat through package • Heat resistant material • High temperature • Conducts heat or diffuses heat	• Highly permeable to EO, gases, air, to moisture • Resistant to pressure changes remove air. • EO under vacuum • Good at low temperature (ambient) • Accept vacuum changes • Low absorption to EO • Desorbs EO gas
Irradiation	**H_2O_2**	**O_3 Sterilisation**
• Resistant to deleterious radicals and reactions of them • Allow for release of odours • Radiation can penetrate	• Resistant to oxidation • Resistant to deep vacuum • Requires permeable packaging • Requires high porosity. • Damages paper, cellulosics	• Resistant to high oxidation • Deep vacuum • Requires permeable packaging • Package resistant to oxidising

6.1 Types and Characteristics of Packaging

There are different packages, which vary in function and quality (see **Table 6.2**).

Table 6.2 General types of packages

Type of package	Background
Primary or product package	The immediate package that protects the product directly. Primary packages must be kept clean and in a reasonably dust free environment, preferably in an unhygienically controlled environment
Secondary or user package	A secondary package that protects the primary package - typically multiple primary packages. The internal transportation of the secondary package must provide protection against contamination of the primary package, as well as against mechanical damage and dust
Tertiary or transportation package	A third degree package - typically the transportation package that encompasses the primary packages and secondary packages. The transportation package must not be allowed in the hygienically controlled environment

During storage, handling and transportation of packages, the packages must be protected against at least five dangers to maintain the safety and their ultimate sterility. They are:

- Mechanical and physical damage. It is self-evident that a damaged product package will not keep its contents sterile. Rigid containers can provide this protection, but are expensive. They have high unit cost, and they are often difficult to fill automatically industrially, but can be manually filled in hospitals.
- Quick temperature changes. For example, this can potentially warp the package, and lead to damage such as bursting, stretching, shrinking, seal breakage, and/or creasing that can lead to breakage of the sterile barrier.
- Moisture, wetting, chemicals, visible or ultraviolet (UV) light.
- Excessive heat. This can cause warping, melting, seal breakage, creasing, plasticiser migration and bursting of the package.
- Dust and contamination. Dust and contamination may penetrate the package, or may indirectly be transferred to the user who is attempting to open the package aseptically.

The attributes of primary packages are many, based upon what their qualities and roles determine. Some attributes of primary packaging are:

- All primary packages must have labelling to describe the product or its use. If it is a drug it must state the name of the vehicle, preparation, its concentration(s), percentage of the amount of active ingredient, date of preparation, expiration date and so on. If the preparation is manufactured outside of the user (e.g., the hospital), the name of the manufacturer or distributor must be given.
- Typically labelling is arranged so that sufficient area of the container remains uncovered to see the product, and even to be able to inspect its contents.
- If it is a solution it should not allow loss of liquid or its ingredients. The package must, in some cases, allow the solution to be delivered sterile and at minimum required rate.
- The package should afford easy removal of the contents without contamination, provide an adequate barrier to micro-organisms, particulates, and fluids, and maintain the sterility of the package contents until opened.
- The package should not accumulate contaminates (e.g., moisture, EO or peroxide residuals or odours, particulates, extractables or by-products created by irradiation).

- The package should enclose and protect the product, but in a way that maintains its functionality. For example, a parenteral solution must not leak through its enclosure (e.g., stopper) and be labelled to maintain its stability or demonstrate its expiration date; and polyvinyl chloride (PVC) tubing must be coiled and packaged correctly to prevent kinking or deformation of tubing.
- The packaging must allow for aseptic presentation upon use and handling.
- The sterilant and related parameters must be capable of sterilising the product within the package throughout, allow sterilant penetration and direct contact with the item and surfaces, and the removal of the sterilant.

While it is a truism that 'at the moment of use' that a sterile product must be sterile, other conditions may be needed:

- Be free of contaminants, toxic ingredients, particulates and non-fast dyes.
- Provide complete and secure enclosure of the sterilised item(s).
- Protect and provide safety for package contents from physical damage (e.g., compression and stacking).
- Provide adequate seal integrity.
- Resist tears, punctures, abrasions, and prevent the transfer of micro-organisms.
- Provide a tamper-proof seal against accidental opening, which must be able to be sealed only once, unless it is a reusable package.
- Allow adequate toxic residuals.
- Be low-linting with no particulates. In some cases it should be static resistant.
- Demonstrate identification of contents and have labels.
- Be of adequate size to evenly distribute the contents and mass.
- Be user friendly when opening the package or container.
- Be odour free and appear clean.
- Be recyclable or reusable.

The sterilisation method must be compatible with the package and provide for protection of the product within the package. Consequently, the packaging systems should be appropriate to the method of sterilisation. The packaging system should be compatible with, and designed and approved for use with, the specific sterilising

technology applied, and be able to withstand physical and chemical conditions of the sterilisation process, particularly if reused and resterilised. While the packaging system should be compatible with the intended sterilisation process(es) and equipment, it should be verified and validated. Instructions for using and handling the packaging should be available and information given on the new package to be used for a product that is being resterilised.

The product should not be of such a weight, configuration, state, or sharpness as to weaken the package seal, or to pierce or penetrate the package.

Types of primary packages will vary and some types of primary packages are:

- Peel package – a rigid/semi-rigid container – paper or plastic film peel lid to rigid plastic container: One common type is the pre-formed tray that provides convenient packaging for many devices. An appropriate lid may be sealed directly to the tray. The single formed tray is typically disposable for clinical or home use, however in hospitals generic reusable trays are commonly used for procedural set ups.

- Peel-pouch-paper or plastic film peel lid to plastic film: Pouches are packages in a pouch form and sealed on three sides; so that the fourth side or site may be sealed at the manufacturing site or in the hospital after the product has been added or placed within it. The device may be fitted with a support structure, e.g., a nest, a tray, prior to being inserted into the pouch.

- Re-usable packages: They are typically a rigid sterilisation container system - such as metal or plastic trays with lids, or surrounded with wraps and so on.

- Rigid container/closures: Some examples are glass containers with stoppers and so on or blister packs which are used in the pharmaceutical industry and for some devices. The product is in liquid or solid form sealed within the blister pack. In hospitals rigid containers or pans with lids are frequently employed.

- Wrap packages typically consist of woven textile wraps made of muslin, jean cloth, barrier cloths, non-woven fabrics or spun bonded fabric, or polymer materials that are wrapped around the product in a singular form, wrap or multiple times.

Some types of secondary packages may be chipboard boxes, corrugated shippers, or plastic liners. Some types of secondary packages are:

- The chipboard and corrugated cardboard boxes are often used in addition and support to the primary (disposable) packaging listed previously for on the shelf, and shipping protection, respectively.

- Additionally plastic liner bags within corrugated boxes may be used for additional shipping and environmental or storage protection to primary package.
- Hospitals will frequently apply additional wrapping for processed and packaged re-usable as a secondary barrier, for storage purposes.

Tertiary packages may include corrugated cardboard, plastic or metal containers, and sometimes large bags. Examples of tertiary packages are:

- Corrugated cardboard - typically used in shipping.
- Totes with covers - totes may be used for long-term storage.
- Plastic or metal containers - typically used in hospitals for storage of sterile disposable packages.
- Large, strong and impervious bags - sometimes these are used to ship products in the mail.

6.1.1 Packaging Considerations will vary for Different Sterilisation Methods and Sterility Criteria

Packaging will vary depending upon the sterilisation method. Steam, EO, H_2O_2 and O_3 sterilisation require packages that are permeable to the gases and vapours. Steam and dry heat require heat resistant materials. H_2O_2 and O_3 require oxidation resistant materials. Radiation requires resistant materials but does not require permeability, except for the some damage and elution of odours that may occur. For example irradiated rubber gloves can give off tremendous malodours after irradiation. If they were irradiated with a sterilising dose in a non-breathing packaging, the off-gassing of polymers, rubber and so on, could be highly recognised immediately upon opening.

6.1.1.1 The Package Must Protect and Keep the Product Safe

Because people's lives are dependent on the proper functionality of the product it is essential that the package adequately maintains and protects the product during transportation of the product and throughout its entire shelf-life, for example, biomaterials, medical devices or pharmaceuticals. Barrier packaging may be required when a product is sensitive to light (particularly UV light). A device or other package validation programme must demonstrate that the package safeguards and maintains the biomaterial or the device's intended functionality. Testing and validation of packaging is critical for the maintenance of sterility and handling, and

for manipulation of the packaging after sterilisation. Transportation simulation is often employed as part of the validation programme.

6.1.1.2 The Type of Sterility Claim can Change the Type or Characteristic of the Product

Packaging may be as diverse as the products they protect or do not protect. For sterility, packaging may be divided into three main categories:

- One is the sterile fluid pathway or location, in which the container acts as a dust cover and general particulate protector until the time of use but sterility is maintained only in the fluid pathway or in the location under the protective barriers or covers. For example, a sterile fluid path product (not sterility of the entire product content, only the fluid path) will typically not require the strict integrity that a sterile content product will, because generally a sterile fluid path will have end covers to protect the contents from recontamination. Consequently, the sterile fluid package typically has a primary package that is a dust cover and may have some end caps that are tortuous paths, which prevent migration or ingression of microbes.
- A second category is a sterile pack or sterile product, which delivers the entire product to an operating field in a sterile condition. A sterile content packaged product will provide strict barriers to the product. An implantable sterile product will often have double packaging. A sterile pack transported from the central supply area to the operating field may consist of a package of several layers of fabric or woven textile materials, which may be a wrap around the product rather than a sealed container or pouch. Such packages have very short shelf-life, or expiration dates.
- The third category is the non-sterile product. Packaging is dictated by the label claims or functional needs. Most packages are designed to provide security for the product. For the sterile product package or critical product of use (e.g., drug, pharmaceutical, food and so on) it must be possible to demonstrate immediately if the package has been tampered with or opened.

Testing and validation of sterile packaging is critical for maintenance of sterility and handling, and manipulation of packaging after sterilisation. To perform testing, a protocol is typically required to define the rationale, test methodologies, test levels, and pass/fail criteria for the shelf-life periods. These protocols will describe the parameter to be used for the physical distribution of the environmental stress testing such as shock, vibration, compression hazards, package strength evaluation using seal strength methods, and sterility validation using physical leak detection methods.

The loss of sterility in a package is typically a dynamic, event related incident rather than a time related phenomenon. Damage to a package can be caused by a variety of factors including:

- Damage to the barrier materials due to shipping and handling.
- Loss of the seal integrity due to the effects of ageing or shipping and handling events.
- Improper manufacturing and production processes.
- Damage due to the design and configuration of the product, e.g., sharp piercing corners.
- Damage due to the sterilisation process, e.g., quick pressure changes in gas or steam during the post-vacuum phases.
- Damage to packaging material (H_2O_2 on cellulosics or paper), and high absorptive peroxide materials; damage due to high temperature dry heat (canvas, glassine, Cellophane, polyethylene (PE), PVC); damage due to autoclaving (low-density polyethylene (LDPE) and damage due to irradiation (polypropylene (PP), PVC, Teflon films).

Among packaging failures there are two general types of failures, for example, shrinking, bursting during the sterilisation cycle, and film tears and/or paper shear when opening a package. Bursting is the result of inadequate seal strength between the plastic laminate and the medical grade paper. Tearing and shearing is the result of a laminate that is weaker than the seal strength between the laminate and the paper. Consequently there is a paradox – a strong seal strength can lead to tearing of a weak laminate. Be careful that irradiation does not create a stronger seal. A seal weak enough to protect the laminate, is more likely to burst under the radical pressure changes imposed by modern pre-vacuum or post-vacuum sterilisers. The key to reducing failure may be a stronger laminate. Remember - the stronger the seal, the less likely it is to burst, but it is possibly more likely to tear. *Note:* in irradiation there is typically no vacuum and consequently no bursting of the packaging.

6.2 Some Considerations for Packaging Sterility

Sterilisation or sterility evaluation is not a singular case or discipline, but an interfacial area of study of product design development, manufacturing, and environmental control, particularly in the new era of device/drug combinations. It requires a multi-disciplinary effort. These areas of effort include biology, chemistry, environment control, material safety, biocompatibility, microbiology, engineering, material/drug

safety, mathematics, manufacturing, Research & Development, and quality of the product/package design.

The control of sterility ends with the design of the package. Packaging is frequently one of the last things that a company introducing a new product thinks about. Furthermore, many packaging engineers found their roles by accident, so that they may not know how a packaging system actually fits into a company or understand compliance, and industry standards.

Develop design inputs - Create documents that clearly define product protection requirements, including 'human' sensitivity, fragility, light, temperature, oxygen (O_2) and moisture levels, before selecting a package material.

Define the package functions - For example, implantable and parenteral packaging must maintain the sterility of the contents. Liquid products may have to have a leakage specification.

Key physical properties to consider in package design during the initial concept are:

- Weight
- Shape
- Barrier requirements

Key chemical considerations for the protection of the package are:

- Chemical characteristic of the device or product or sterilisation (e.g., residuals)
- Water vapour
- Other agents (e.g., plasma)
- Visible or UV light

The initial packaging concept may include:

- Marketing needs.
- User expectations and needs.
- Two-dimensional packaging (e.g., pouch, bag).
- Three-dimensional packaging (e.g., flexible, rigid).
- Irrespective of their dimensions, packaging may require bonding, printing, breathability, strength, transparency, shape forming, configuration and seal(s).

Drivers for material selection are:

- Sterilisation types, and their criteria - compatibility, porosity, coatings and so on.
- Labour intensive - requires materials which operate in a broad range of packaging/ sealing conditions.
- Capital intensive - requires the use of 'fast' material, which produces packages under mechanically stressful conditions and operations.
- See also **Sections 6.2.1** and **6.3**.

Evaluate competitive devices and understand how and why they package their products to learn from excellent and bad examples.

There must be a design quality assurance system in place to prevent problems. Some quality issues to consider are:

- When design input opportunities occurs.
- Written specifications or procedures.
- Personnel interfaces.
- Design verification is documented.
- Post-sterilisation, shipping and handling test inspections.
- Stability and/or expiration testing.

6.2.1 Package Selection

Selection of the appropriate packaging materials and accessories is a critical consideration. Materials have to be screened based on physical and chemical compatibility with the product as well as their ability to withstand sterilisation. To achieve product/package and process control, there must be procedures in place. Some packaging control opportunities are:

- Adequate procedures.
- Standards and documentation.
- Audit of suppliers.
- Receiving inspection.

- Post-sterilisation inspection (during qualification, and periodic stability evaluation).

A number of standards are used for evaluation, in particular American Association of Medical Instrumentation (AAMI) International Organization for Standardization (ISO) 11607-1 [1] and AAMI ISO 11607-2 [2]. To design a package, consider ways that a package design may fail [l], for example, just folding flexible packaging in different axes can lead to pinhole failures. Determine if the package will be aseptically used and if it will require a single or double barrier (e.g., be implantable). To reduce work, leverage previous qualifications to reduce tests and evaluations.

When it comes to labelling, take enough time to determine potential problems. How will you label the package and how will the label's print hold-up during sterilisation, and in the post-cleaning/handling and environment? Labelling is very critical because it is the primary way of identifying the product.

Develop a prototype of the package design. The 'physical' design provides a physical representation that gives an immediate package design feedback and is the first step for testing and evaluation of the package and design system. This may include how the design fits the sterile barrier efficacy, device safety and efficacy.

There are some critical considerations when sterilising healthcare product/packages – these include:

- Help to define and determine an acceptable product and package.
- Note changes beyond the material attributes.
- Note changes beyond mere sterility.
- Evaluate packaging integrity.
- Evaluate product/package stability.
- All packaging tests and standards are continually liable to change and updating, depending upon use, location, and regulations.
- Meet regulations.
- Select proper labelling.

Specific tests and standards may be required or substituted that will reflect the specific product and package to be marketed and released. These may include FDA regulations and international standards.

The choice of packaging material may depend on several important requirements (see **Table 6.3**).

Table 6.3 Types of sterilisation, requirements, and acceptable packaging materials

Sterilisation method	Packaging material requirement	Acceptable materials
Steam autoclave	Should allow removal of air and be penetrable to steam	• Paper (Kraft, Glassine, Parchment, Crepe and so on) • Plastic (Tyvek®) • Cloth (Muslin) • Paper peel packages, • Wrapped perforated cassettes • PP trays (breathable path) • Metal trays (breathable path)
Dry heat	• Should not insulate items from heat • Should not be destroyed by the temperature used	• Paper bags • Polyfilm plastic tubing • PA • PP • Wrapped perforated cassettes • Aluminum wrap • Metal containers, trays • Glass containers
Gas (e.g., EO, O_3, or H_2O_2	• Vapours should be able to penetrate, diffuse and contact contents • Vapours should not react with packaging material • Plastics should not contact the sides of the steriliser	• Wrapped perforated cassettes, Plastics (PE, PP, Tyvek®, PET/PE laminate, acrylic) and metallic laminates • Not recommended for O_2 or H_2O_2 absorbers such as: PVC, PU, Polyamide, paper or cellulosics
Irradiation	• Do not adversely affect material • Odour reduction through gas permeable films (e.g., Tyvek®)	• Many polymers such as Tyvek®, paper • Unacceptable materials are glass, PP
PA: Polyamide PET: Polyethylene terephthalate PU: Polyurethane		

Some considerations when choosing a packaging material are:

- The type of product, which has to be packed.
- Whether the sterilant has to pass through (porosity) or penetrate (dry heat or irradiation).

- The material should not release any harmful substances nor go through considerable change (chemically or physically) during sterilisation.
- The material has to pack easily, so it has to be soft, yet strong during packaging, sterilisation, transport, and storage.
- It must show damage and not create false safety (pinholes).
- The packaging material must prevent recontamination. Be impermeable to microbes and so on.
- Aseptic opening must be possible, to prevent introducing adventitious contamination.
- The material is possibly being used as a sterile field, meaning it may have to be repellent to low-tension liquids and have a low static charge.
- The material must be lint free - lint can be a potential hazard for the patient.
- The material must not be deformed or the porosity altered or damaged during processing (e.g., sterilisation) and handling.

6.3 Other Considerations of Packaging Selection

Non-breathable packaging is acceptable for irradiation penetration, however, irradiation often causes off-gassing of polymers so that breathable packaging may be needed to allow diffusion and reduction of obnoxious fumes.

Because H_2O_2 has a very high vapour or boiling point, very deep vacuums are required that may adversely affect some packaging (bursting) and materials (e.g., cellulosics).

Other packaging materials may be used to sterilise with EO within a package. For typical EO sterilisation, packaging must be strong enough for gassing, removal of air, input of humidity and EO gas, as well as post-evacuation pulses of gas and air for removing or diluting EO residuals. Packaging damage is typically found in EO sterilisation during the post-evacuation phase of the cycle where deep and multiple air washing removes EO residuals.

6.4 Sterility Assurance Level of Packaging

Trying to find the minimum permissible sterility necessary to provide the required assurance of packaging compatibility to sterilisation and maintenance of sterility is

elusive. It requires a minimum sterilisation to deliver to the package a 10^{-6} SAL but the package has to maintain this sterility level (10^{-6}) as well a minimum of 10^{-3} SAL for topical application use. While sterilisation must typically prove and demonstrate a 10^{-6} SAL, how can most medical device packaging demonstrate an equivalent SAL standard? Therein lies a paradox. The process must demonstrate a 10^{-6} SAL, while the terminally sterilised package does not and typically cannot. However, because of the clinical significance of sterility of some products (implantable or parenteral solutions or even drugs), some products are double packaged or placed in very tough hermetic sealed packaging to assure greater assurance of the maintenance of sterility of the package, because an equivalent SAL cannot 'readily or typically' be demonstrated for packaging.

However, drug containers are immersed and challenged at levels greater than 10^8 of microbes (after sterilisation, ageing, and/or stability, to demonstrate that no organisms can permeate or penetrate their dense configuration or surrounding barrier(s) material(s).

Compared to medical device packaging materials, that are much thinner, vapour/air permeable, porous and flexible and breathable, device packaging typically would fail if challenged with immersion in a liquid microbial challenge. However, double packaging of the same material and configuration may not fail as fast as single layer packaging. Achieving and maintaining sterility is the challenge.

The loss of sterility is typically a dynamic, event related incident rather than a time related phenomenon. Damage to a package may be caused by many factors. Factors, which can lead to failure of sterilisation, include:

- Unclean or uncontrolled cleanliness of packaging materials.
- Improper packaging type, method, or material(s).
- Wrong packaging material for the method of sterilisation.
- Excessive packaging material.
- Weak packaging material and/or seals.
- Improper loading or overloading of the steriliser.
- No separation between packages or containers even without overloading. This may prevent or prohibit thorough contact of the sterilising agent with all items in the chamber.
- Improper sterilisation parameters.

- Incorrect operation of the packaging equipment.
- Variation in monitored parameters in the sealing equipment.

Investigation of packaging failures usually finds some of the following problems:

- Seal integrity affects contamination. Seal breaches from broken seals allow for the possible passage of microbes on to patients, which may be fatal.
- Consider the purchasing department. If they purchase materials from another supplier to save money, the films may differ and may not meet the original engineering or validation requirements.
- When something goes wrong with the package, go back to the distributor who packaged the materials.
- Packaging may fail during testing just by the way they are opened. Opening must be consistent by tester and user.
- Wrinkles in foil or other packages may be caused by incorrect or uncontrolled tension during packaging as well as temperature, dwell time or pressure.
- Follow the manufacturer's instructions. For example, if a coating is applied to one side of a film structure, when the vendor recommends it is applied to the other side of the structure.

Packaging will vary depending upon the sterilisation method and sterilisation parameters. This can be determined through microbial challenge to pharmaceutical products that lie within moisture proof barrier containers such as glass and plastic, but it is not as easily maintained in medical devices held within breathable and moisture passing packaging. For international use nothing less than a SAL of 10^{-6} is allowed.

To evaluate the previous criteria, product, and packaging must be sterilised to at least the highest cycle to be delivered routinely, double sterilised (when applicable) and tested to the highest useful life of the product in the package.

If the product is a pharmaceutical product it often is immersed in a solution of growing small microbes with a concentration of greater than 10^6. To do this with most medical devices in breathable packages would result in failure, except for packaging, such as the PA type sterilisation pouch available for dry heat sterilisation. Immersion would not be a practical test for Tyvek or paper lid pouches or trays used in many devices because moisture with the microbes would diffuse and penetrate these materials. For radiation most packages must be breathable and porous to allow for degassing of odours. For chlorine dioxide (ClO_2), O_3, and H_2O_2, porous packages are needed for permeation of these sterilants. EO will penetrate some films without porous

packaging, however, these films may not allow for easy diffusion of humidity. With PE, EO will diffuse well and drive some humidity through this non-hygroscopic film. With Polyamide, EO will not naturally diffuse through this hydroscopic film, but moisture or high humidity will help take some EO molecules dissolved within the moisture, through this film.

Typically sterilisation of medical device packaging relates to thermal formed blister packs, sterilisation wraps, peel pouches, trays, tray accessories (silicone mats, medical device holding-devices), and reusable sterilisation containers.

6.5 Some Qualification and Stability Considerations of Packaging

A variety of factors must be considered for qualification and stability of packaging.

6.5.1 Qualification

Make a package process evaluation. Determine if the package will be used in a clinical trial or for a full scale production. With clinical trials you may make do with existing packaging equipment. Apply the same determinations for a contract packager's equipment. Plan for equipment purchases two or more years ahead of your launch if applicable and perform factory acceptance tests at a supplier's facilities before in-house qualification of the equipment.

Develop an installation qualification of packaging equipment. AAMI ISO 11607-2 [2] can be used. Focus on utilities for the equipment, making sure all parts of machinery and equipment perform as anticipated and calibrate critical operating functions such as the heat seal timer, temperature, controller, pressure gauge(s), software validation for any programmable logic controller, data collection and so on. Finally, a thorough inspection must be done.

6.5.1.1 Develop the Packaging Process and Perform an Operational Qualification of Equipment, Processing and Machinery

Determine the minimum and maximum process limits and the acceptable operating range of your packaging process, for example sealing. Use design of experiment of tools (either manual or software programmes which will provide assistance in defining process limits and identify any for example 'sweet spot(s)' for the packaging process. The so-called 'sweet spot' may be defined as the operating range where the process output is optimised (pre-determined requirements) with minimal output variability.

Perform multiple runs to make certain there is consistent seal integrity.

Note: temperature and dwell time at a certain value does not necessarily mean the package is sealed because there may be variation depending upon where the temperature is monitored and when the dwell time is started in the process. While it may appear that process inputs are controlled and monitored, they are in some cases where they can change the sealing process, but no one will know it until the package fails. Consequently, the key to a sealing process is to reduce the variations that may be introduced in to the process.

Next conduct process qualification evaluations.

6.5.1.2 Process Qualification Evaluation

Conduct three consecutive production runs at normal operating conditions and settings. Typically do these at different times (e.g., different days or shifts), with different people and with the machine operating at different temperatures. Finally conduct a system design validation.

6.5.1.3 Packaging System Design Validation

In this evaluation, use validated or validatable sterilisation methods and the appropriate materials and processes. By selecting packaged products which have been run through manufacturing, sterilisation, and then subjecting them to shipping, handling, strength integrity tests, and accelerated ageing, it can be assessed if the production process and package/product damage are adequate to maintain sterility, throughout the expected shelf-life of the packaged product and under extremes in handling and shipping. Some typical packaging standards or tests are described in European Norm (EN), American Society for Testing and Materials (ASTM), ISO, and AAMI, as shown in **Table 6.4**.

Note: Standard EN 868 is made up of standards, both current and historical that are general requirements for sterile packaging materials. Requirements for specific materials such as sterilisation wraps, papers, and Tyvek® are included in vertical standards Parts 2 to 10 of EN 868. Spitzley, one of the writers of the original and revised versions of ISO 11607 [1, 2], has said that the authors of the 1997 version of ISO 11607 thought EN 868-1 would eventually be replaced after ISO 11607 was published. However, European companies and regulatory bodies did not readily accept ISO 11607, and so the standard was not widely recognised or used in Europe. As a result, many US firms assumed they needed to comply with both ISO 11607 and

EN 868-1 as separate standards. However, revisions to ISO 11607, accomplishes what the original 1997 version failed to do. The new standard eliminates the need for the European packaging standard EN 868-1 by incorporating its requirements. The harmonised document covers materials, manufacturing, and package design requirements for terminal sterilised medical device packaging, such as tests for package burst strength, seal strength and so on (see **Table 6.5**) [5].

Table 6.4 List of various sterilisation standards	
AAMI ISO 11607-1 (2010) [1]	*Packaging for Terminally Sterilised Medical Devices - Part 1: Requirements for Materials, Sterile Barrier Systems and Packaging Systems.* **{This standard replaces EN 868-1 (1997)}**
EN 868-2 (2009)	*Packaging for Terminally Sterilised Medical Devices - Part 2: Sterilisation Wrap - Requirements and Test Methods*
EN 868-3 (2009)	*Packaging for Terminally Sterilised Medical Devices - Part 3: Paper for Use in the Manufacture of Paper Bags (Specified in EN 868-4) and in the Manufacture of Pouches and Reels (Specified in EN 868-5) - Requirements and Test Methods*
EN 868-4 (2009)	*Packaging for Terminally Sterilised Medical Devices - Part 4: Paper Bags - Requirements and Test Methods*
EN 868-5 (2009)	*Packaging for Terminally Sterilised Medical Devices - Part 5: Sealable Pouches and Reels of Porous Materials and Plastic Film Construction - Requirements and Test Methods*
EN 868-6 (2009)	*Packaging for Terminally Sterilised Medical Devices - Part 6: Paper for Low Temperature Sterilisation Processes - Requirements and Test Methods*
EN 868-7 (2009)	*Packaging for Terminally Sterilised Medical Devices - Part 7: Adhesive Coated Paper for Low Temperature Sterilisation Processes - Requirements and Test Methods*
EN 868-8 (2009)	*Packaging for Terminally Sterilised Medical Devices - Part 8: Re-Usable Sterilisation Containers for Steam Sterilisers Conforming to EN 285 - Requirements and Test Methods*
EN 868-9 (2009)	*Packaging for Terminally Sterilised Medical Devices - Part 9: Uncoated Non-woven Materials of Polyolefins - Requirements and Test Methods*
EN 868-10 (2009)	*Packaging for Terminally Sterilised Medical Devices - Part 10: Adhesive Coated Non-woven Materials of Polyolefins - Requirements and Test Methods*
ISO 15223-1 (2012)	*Medical Devices - Symbols to be used with Medical Device Labels, Labelling and Information to be Supplied - Part 1: General Requirements (ISO 15223-1:2012)* **{This standard replaces EN 980 (2003)}**
ASTM D882 (2012)	*Test Method for Tensile Properties of Thin Plastic Sheeting*
ASTM D3763 (2010)	*Test Method for High Speed Puncture Properties of Plastics Using Load and Displacement Sensors*

ASTM D4169 (2009)	*Practice for Performance Testing of Shipping Containers and Systems*
ASTM D5420 (2010)	*Test Method for Impact Resistance of Flat, Rigid Plastic Specimen by Means of Striker Impacted by a Falling Weight*
ASTM F88/ F88M (2009)	*Test Method for Seal Strength of Flexible Barrier Materials*
ASTM F1140 (2012)	*Test Methods for Internal Pressurisation Failure Resistance of Unrestrained Packages*
ASTM F1886/ F1886M (2009)	*Test Method for Determining Integrity of Seals for Flexible Packaging by Visual Inspection*
ASTM F1929 (2012)	*Test Method for Detecting Seal Leaks in Porous Medical Packaging by Dye Penetration*
ASTM F2054 (2012)	*Test Method for Burst Testing of Flexible Package Seals using Internal Air Pressurisation within Restraining Plates*
ASTM F88/88M [3]	*Standard Test Method for Seal Strength of Flexible Barrier Materials* **{This standard replaces ASTM F88}**
ASTM F1585	*Standard Guide for Integrity Testing of Porous Barrier Medical Packaging* **{This standard has been withdrawn, but included here for historical citation or reference}**
ASTM F1929 (2012)	*Test Method for Detecting Seal Leaks in Porous Medical Packaging by Dye Penetration*
ASTM F2638 (2007)	*Standard Test Method for Determining the Microbial Barrier of Porous Packaging Materials (Aerosol Filtration Method)*
ASTM D4169 (2009)	*Practice for Performance Testing of Shipping Containers and Systems*
ASTM D4332 (2006)	*Practice for Conditioning Containers Packages, or Packaging Components for Testing*
ISO 11607-1 (2006)	*Packaging for Terminally Sterilised Medical Devices - Part 1: Requirements for Materials, Sterile Barrier Systems and Packaging Systems*
ISO 11607-2 (2006)	*Packaging for Terminally Sterilised Medical Devices - Part 2: Validation Requirements for Forming, Sealing and Assembly Processes*
ISO/NP 11607-3	*Packaging for Terminally Sterilised Medical Devices - Part 3: Guidance on the Application of ISO 11607-1 and ISO 11607-2 TC 198* **{See also AAMI TIR 22 (2007) [4]}**
ISO 10993-5 (2009)	*Biological Evaluation of Medical Devices - Part 5: Test for In Vitro Cytotoxicity*
ISO 10993-1 (2010)	*Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing within a Risk Management Process*
AAMI TIR 22 (2007)	*Guidance for ANSI/AAMI/ISO 11607, Packaging for Terminally Sterilised Medical Devices – Part 1: and Part 2: 2006*
ANSI: American National Standards Institute TIR: Technical Information Report The information on packaging given in this table does not include all the standards available	

Table 6.5 Examples of physical tests for package burst strength, seal strength, and tear strength

Packaging test	Standard
Package burst strength	ASTM F2054 [6]
Package Seal Strength	ASTM F88 [3]
Tear strength	ASTM D1004 [7] and ISO 6383-1 [8]

Despite use of these standards, packaging should be visually inspected at point of use for any obvious physical damage, cosmetic appearance, including wetting, because Tyvek® and paper packages cannot withstand water (microbial) contamination, and water is an excellent source of microbial movement contamination. A composite foil is available for flat-pack and deep-drawing machines along with coated or uncoated Tyvek® and specialty composites. In order to minimise environmental impact, only water-based inks are used for printing and only solvent-free dispersion and hot-melt systems are used for seal coatings.

Double sterilise if applicable: See Annex E of ISO 11607-2 [2] for the applicable test methodologies. Use predetermined worse case manufacturing conditions for operational qualification to qualify and validate the packaging design. Look for physical integrity and package seal strength standards for qualification. Some suitable test methods may be:

- Dye penetration tests around the perimeter of the tray pack (for example for seal integrity. This tests only the seals of the package.
- Bubble testing or submersion leak testing in water for whole package integrity evaluation). This tests for gross failures of whole package.
- Immersion in a microbial soup as used in drug testing, or sterility testing if the assembly is aseptic. This tests whole package integrity and potential microbial penetration.
- Vacuum tests may be used for rigid jars and packages.

Any device or healthcare product delivered in a sterile state, must have been manufactured and sterilised by an appropriate method and maintained in a manner, that upon sterility testing will not demonstrate any viable micro-organisms, no matter how many samples are tested, unless there is proven adventitious contamination during the testing, or those which when used will not compromise the safety of the patients using the product.

Sterile packaging should be cost efficient, effective, and efficacious and safe. Some critical attributes of sterile packaging are:

1 The package has been properly designed, qualified, produced on a validated machine with a validated sterilisation process.

2 All customer and regulatory inputs and requirements have been considered.

3 The safety and assessment risk needs of the patient are met over an acceptable time period (e.g., stability evaluation, expiration date).

4 The primary concern is always to protect the product inside, and this becomes challenging when combined with the need to reduce packaging costs.

Validation or revalidation of a package of a medical device must be considered under the following conditions:

- Have there been changes to the packaging element including instructions or protective barriers?
- Have additional barriers been added or changed (e.g., a container case that may interfere with the sterilant or humidity penetration or removal of air?
- With gamma irradiation penetration may be attenuated with increase in density of the package and product. With electron beam treatment, electrons will not penetrate as easily as gamma rays.
- Has a decrease in the porosity of the packaging material occurred (e.g., weight, density, coating treatment, taping, additional or larger labels?
- Has the surface area of the venting material decreased or an underlying opening occurred?
- Has the packaging resulted in an increase of bioburden on the product?

6.5.1.4 Pharmaceutical Packaging Tests

The tests for pharmaceutical packages are similar to those used for medical devices, but may be more diverse. Some pharmaceutical packaging tests [9] are:

- Visual inspection is the easiest leak test technique, but also the least sensitive.
- There are now automated on-line inspection systems.
- The bubble test involves submerging the package in liquid, pressurising or pulling vacuum and then observing for bubbles.
- The pressure/vacuum decay test looks for a change in pressure or vacuum and is measured inside or outside in a sealed package chamber.

- Dye tests visually or instrumentally detect a movement across a seal.
- A chemical tracer test is where a solution containing a tracer chemical is applied to one side of a package seal, and pressure or vacuum is applied as a driving force and the chemical leakage is detected by the appropriate instrumentation.
- A microbial challenge test is performed by filling containers or packages with media and the seal and entire package content is either challenged directly with an external solution of a microbial soup or it is allowed to sit in an ambient storage environment. The presence of microbial growth, indicates a failure. Sometimes the entire package or container is submerged in a microbial soup and growth or no growth is observed. The microbial soup may consist of microbes with high motility (e.g., *Pseudomonas*) and or small size.
- The weight change technique is where a container is filled with liquid or desiccant, sealed, stored at various stress conditions and then re-weighed over time.
- Helium mass spectrometry is where helium is placed either inside or outside the container and a vacuum is applied to the seal interface and the migrating helium is detected by mass spectrometry.
- Gas detection tests involve a tracer gas placed on one of the container or package seals. Inert carrier gas is passed across the opposite seal side. Tracer gases are detected either by coulometric detector or by a photoelectric sensor. Instruments, which are designed to pierce containers and test package headspace for the gases are used to detect the gas.
- The vacuum ionisation test is where a high voltage, high frequency field is applied to the vials sealed under vacuum. The field causes the residual gas to glow. Glow intensity is a function of vacuum level.
- Electrical conductivity/capacitance tests – high frequency high voltage is applied to the sealed container or package. An increase in conductivity is correlated to the presence of liquid along the seal or a capacitance increase in dielectric constant across seal is detected due to presence of liquid.

Following qualification, stability of packaging must be considered.

6.6 Stability of Packaging

The major factors for evaluating the stability of packaging are:

- Distribution

- Storage
- Handling
- Dropping

A thorough analysis of distribution conditions for the product must be performed. For clinical trial packaging, low volumes of packaging are sent to many distant sites. An environmental monitoring study of cold/hot packaging, is conducted to understand how a package may be exposed to extreme conditions.

Real time aging is used to test a package's shelf-life and stability. Companies often conduct accelerated ageing tests because they want quick answers and do not want to wait for the data.

Determine the effect of time on seal integrity as provided in an Arrhenius reaction rate as given in ASTM F1980 [10]. Q_{10} is the expected or observed change in the rate of a reaction occasioned by a 10° C change in the thermal environment of the reaction. $Q_{10} = 2$, is a common and conservative estimate for most polymer systems. The resulting creation of an expiration date or shelf-life is based on the use of a conservative estimate of the aging factor (for example, Q_{10}) and is tentative until the results of real time aging studies are completed on the sterile barrier system

In evaluating packaging, the maximum rate of sterilisation must be delivered. If the product package can be resterilised, then it must be evaluated. Typically new packaging is used for resterilised products, but nonetheless they must be evaluated. The other parameters to be considered are zero time testing and heat ageing at 60-65 °C for 12 h to simulate worse case truck/transportation testing and real time testing for the lifetime use of the product. Abbreviated accelerated criteria to simulate real time testing may be 60-65 °C for 2 weeks.

The time of useful life of product should be determined (**Table 6.6**) and label claims may be imposed, e.g., expiry dating - internationally.

Note: For further details see ASTM F1980 (2011) [10].

Expiry dating may be assumed to be for five years. For field trials, expiry dating may be different, e.g., one year. To achieve expiry dating and material stability and compatibility for the product and packaging, it is possible to collect parallel five year test data through accelerated testing and scheduled stability testing.

The effects of materials, components, packaging, and/or product failure rates is dependent upon temperature and stresses, and is often assumed to follow the Arrhenius

law. In establishing accelerated ageing conditions, apply a worse case Q_{10} (a 10 °C rise of temperature) of 1.8 and a room temperature of 25 °C to the Arrhenius law.

Table 6.6 Some general ageing/stability case conditions

Study	Storage condition	Minimum time period covered by data at submission
*Long term	25 ± 2 °C/60 ± 5% RH or 30 ± 2 °C/65 ± 5% RH	12 months
Intermediate*	30 ± 2 °C/65 ± 5% RH	6 months
Accelerated	40 ± 2 °C/75 ± 5% RH	6 months

RH: Relative humidity
* Intermediate testing is performed for accelerated aging of a package to be stored long-term at 25 °C

Common temperatures used to evaluate effects from temperature and determinations of acceleration ageing are 40-50 °C and 60-65 °C. It is important to keep in mind that a temperature of 60-65 °C may have other effects on the product in combination with radiation, if applied; therefore, a lower temperature should also be used.

A stability schedule should be established to periodically test the product between zero time testing and the established three to five year life period.

If accelerated ageing at 60 °C is 6.6 weeks for one year, and for 50 °C, it is 12 weeks, and the real room temperature goes up to 5 years, then an accelerated aging plan may be tentatively constructed as follows:

- Zero time: may include conditioning at 60 °C for 12 h

Accelerated time testing:

- Samples are tested at 6.6 weeks (1.5 months) at room temperature (23 °C), 6 months at 50 °C and 1 year at 60 °C.
- Samples are tested for 11 weeks at 55 °C and ambient humidity to simulate 2 years of real time, or
- Samples are tested for 13.2 weeks (3 months) at room temperature, at 50 °C for longer than 1 year) and at 60 °C for 2 years.

- Samples are tested for 36 weeks (9 months) at room temperature, 50 °C for 3 years, and at 60 °C for 5 years.
- Samples are tested for 52 weeks (1 year) at room temperature and at 50 °C for more than 4 years.
- Samples are tested for 60 weeks (5 years) at 50 °C.

Real Time testing:

- Two years at room (ambient) temperature.
- Three years at room temperature.
- Four years at room temperature.

Other more precise and applicable approaches to looking at packaging stability may be obtained in AAMI TIR 17 [11].

6.7 Packaging for Different Sterilisation Techniques

In general, packaging for different sterilisation techniques will vary but before using sterilised packaging, packaging materials should be stored and processed properly to maintain the qualities required for sterilisation.

Before use, packaging materials should be stored properly, for example, in hospitals at 20-23 °C and at a RH of 30-60% for least 2 h before use. Maintaining room temperature and moisture content of packaging materials facilitates steam penetration and prevents superheating during the sterilisation process, where steam is used and for pre-humidification. Room temperature and humidity levels in the packaging area should be monitored. Follow packaging manufacturer's instructions for storage of the packaging materials.

Single-use packaging material should be used for one sterilisation cycle, only. Package contents should be assembled, handled, and wrapped in a manner that provides for low bioburden, and an eventual aseptic presentation of package contents.

Effective sterilisation of product is not only dependent upon the parameters of sterilisation, but also the penetration of the packaging to sterilise the product from within the package, maintenance of sterility and product function, and to allow for elution of any toxic residues, moisture residues in the case of dry product, and removal of malodorous odours produced by some sterilisation techniques. In some cases, no

packaging was used. This was typically referred to as flash sterilisation, and used in emergency situations. Today it is referred to as 'immediate to use sterilisation'.

Among the early reported types of packaging (see **Table 6.7**) used with traditional sterilisation method was that published in Perkins' book [12]. The type of packaging material is important because a sterile product will require slightly different handing to a product that is not sterile at time of use. Some products will be packaged on site, where it must be delivered sterile for use; while other products are packaged and made sterile off site possibly at a distance and consequently will have different handling and environmental conditions to those packaged on site.

Note: Reusable woven textile materials should be laundered between every use for rehydration. Re-sterilisation without re-laundering may lead to superheating and could be a deterrent to achieving sterilisation. Over-drying, heat-pressing, and storage in areas of low humidity also may lead to superheating and sterilisation failure. When woven textiles are not rehydrated after sterilisation, and/or if repeated sterilisation is attempted, the textiles may absorb the available moisture present in the steam, thereby creating a dry or superheated steam effect.

Some materials, (e.g., PP and PA) may be dry heat sterilised at slightly lower typical dry heat temperatures [1]. Paper is typically used for disposable hospital products. Coarse brown wrapping paper has been used for wrapping gloves and other small articles. Repeated use of paper wrappers is considered an unsafe practice. Any article to be re-used, either a product or a package is marked to be decontaminated and then sterilised prior to the use on the next patient.

Packaging has evolved significantly since the 1950s and early 1960s.

Table 6.7 Packaging materials, types and sterilisation					
Material	**Nature/type**	**Thickness or grade**	**Steam**	**Dry heat**	**EO**
Muslin (cotton)	Textile wrappers (reusable)	140 thread count	Yes	Yes, not to exceed 204 °C	Yes
Jean cloth	Textile wrappers (reusable)	160 thread count	Yes	No	No
Broadcloth	Textile wrappers (reusable)	200 thread count	Yes	No	Yes
Canvas	Textile wrappers (reusable)	Do not use, as a sterile barrier, but can be used as a dust cover			
Kraft - brown	Paper, wrap, bags	13-18 kg	Yes	No	Yes
Kraft - white	Paper, wrap, bags	13-18 kg			
Glassine	Coated 'envelopes'	13 kg	Yes	No	Yes
Parchment grade (Patapar 27-2T)	Paper wrappers	Patapar 27-2T	Yes	No	Yes
Crepe grade (Dennison Wrap)	Paper wrappers	Grade Dennison Wrap	Yes	No	Yes
Cellophane	Cellulose tubing, wrap, Weck sterilisable		Yes	No	Yes
PE	Plastic bags, wrap	25-75 μm	Yes & No*		Yes
PP	Plastic film	25-75 μm*	Yes & No*		Yes
PVC	Plastic film, tubing	25-75 μm	No	No	Yes
PA	Plastic film, wrap	25-50 μm*	No**	Yes	No
Aluminum	Foil wrappers (may be reusable)	25-50 μm	No	Yes	No
Trays and pans with lids	Metals (e.g., steel, plastic (e.g., PP) (may be reusable)	Varies	Yes Must be breathable for moisture and heat resistant	Yes Must be heat resistant, and breathable for release of pressure	Yes Must be breathable
* Can be used up to the polymer heat resistance temperature, e.g., 121-126 °C). **Not recommended - difficult to eliminate air from sealed films.					

6.7.1 Packaging for Moist Heat

Packaging systems for moist heat (steam) sterilisation should permit adequate drying, or otherwise contamination may occur after sterilisation with wet packages. The efficacy of steam sterilisation can be affected not only by moisture (wet or dry) and

altitude, but also by packaging material, package contents, load (dense or large), position of packages within the steriliser, size, and the parameters of the sterilisation cycle on the package. In hospitals, manufacturers' written instructions for each packaging system for steam sterilisation must be followed.

Wrapped items and more dense or larger load sizes may require longer times for adequate heating and sterilisation. Non-liquid products typically need permeable packaging such as Tyvek®, muslin, or paper. However, while PA is permeable to steam, air cannot penetrate Polyamide and air pockets can mitigate steam penetration. Liquid products typically require non-permeable materials such as glass, metal, PVC bags, PP/PE combinations, e.g., polyallomer. Also, some materials such as PVC will leach out plasticisers. Some packaged containers filled with liquids require hot water to sterilise rather than steam, in order to keep distortion or change in the packaging configuration.

Packaging which enters the sterilisation process must be capable of withstanding the high RH range, moisture (wetness) and saturated steam found in moist heat sterilisation. Some special moist heat processing may be used to keep some packages with entrained air or heat liquid from bursting or collapsing. Types of moist heat sterilisation methods are:

- Steam air mixture processing is used to mix air and steam together to keep packages with entrained air or heat liquid from bursting them. This often requires circulation fans. Air cooling is often used during the post-exposure portion of the cycle. It is less effective than saturated steam, but useful.
- Water immersion processing is sometimes applied to keep semi-rigid packaging from distorting under high heat.
- Air over pressure – is used to maintain effective pressure particularly during cool down or the evacuation phase of the cycle to prevent packages from exploding or bursting their seams and so on.

Immediate to use or flash sterilisation is a process where packaging may not be used – this is mostly used in a hospital or healthcare facility. Condition(s) where 'no' sterile package is used are:

- Unpackaged devices/products at high temperatures may given flash or emergency sterilisation of product in minutes.
- Flash sterilisation may increase the risk of infection to patients due to the additional pressure placed on staff and personnel to skip steps in the cleaning and sterilisation process. Therefore, flash sterilisation should always be kept to a minimum and

only applied when there is insufficient time to process by the preferred packaging or wrapping method.

- Flash sterilisation is never appropriate for implantable devices where the risk of surgical site infection may occur.
- If flash sterilisation is unavoidable due to a documented emergency, a rapid action biological indicator (BI) should be used along with the product. The implant should be quarantined on a back table until the rapid action BI provides a negative result.

6.7.1.1 Factors to Consider when Designing Tests to Determine Moist Heat Material Compatibility

The material effects from moist heat sterilisation are caused by exposure to the maximum specified heat and/or moisture for the maximum specified time and/or repeated exposures to the conditions of sterilisation.

Where appropriate, the effects of repeated sterilisation exposures should be evaluated and documented. Any treatment required before sterilisation should also be validated as part of the resterilisation procedure. *Note:* the initial packaging of a product is not typically reused, but the product is repacked in a new package.

For a given moist heat materials qualification, the degree of degradation may be temperature and time or number of iterations dependent. For example, material qualification performed at a low (moist heat and dry heat) temperature and single iteration may reveal less degradation, (e.g., softening, hydrolysis, distortion, melting) than a high temperature and multiple processing in moist heat sterilisation.

To help select a suitable steam sterilisation package it is important to consider moisture vapour transmission rates through the film or paper (see **Table 6.8**).

Films and papers with less than a high permeability rating should not be used as a packaging material with steam treatment. *Note:* some materials will not let air out, and would not be recommended for steam, unless another breathable film or breathable mechanism is provided or the air is removed before sealing.

6.7.1.2 Loading the Steriliser

Permeable packages must be carefully positioned to ensure adequate air removal, sterilant penetration and contact, removal and drying within the steriliser. Paper and plastic pouches should not be placed within wrapped sets or containment devices,

because they cannot be positioned to ensure adequate air removal, sterilant contact and drying. Small perforated, mesh bottom baskets may be used for absorbent, single layer flat wraps, medical grades of all paper bags or appropriate foam products, if they have been validated by the manufacture for this use.

Packages typically should not touch the walls or door of the steriliser.

Table 6.8 Moisture vapour permeability of some common films and papers, for steam, EO or O_3

Material	Nature	Type	Thickness (µm)	Permeability[1]
Cellophane	Cellulose	Sterilisable	75-500	High
Cellophane	Cellulose	Moisture proof	50-100	0.2-0.6
Kraft	Paper	Wrap, bags	50-230	High
Ethyl cellulose	Ethyl ether of cellulose	Film	25-50	High
Glassine	Plain	Bags, envelopes	25-50	High
Glassine	Lacquered bags	Envelopes	25-50	0.2-1.0
Glassine	Waxed bags	Envelopes	25-75	0.2-1.0
Fluorocarbon	Plastic (Aclar)	Film	75-125	0.15-0.55
PA 6	Plastic	Films	25-75	High
Pilofilm	Film	Film	25-50	0.5-1.0
PA	Plastic	Film	25-75	0.5-1.0
LDPE	Plastic (olefin)	Film	75	High
PE	Plastic	Film	25-100	0.25
PP	Plastic	Film	75	0.46
PVC	Plastic	Film	75	High
Vegetable	Parchment	Paper wrap	50-200	High
Vinyl coated	Paper	Film	75	High

[1] Permeability measured as grams of water transmitted per 645 cm^2 per 24 h at 38 °C and 95% RH.

6.7.1.3 Packaging for Dry Heat

Packaging as part of material and device construction compatibility for dry heat must be considered. Package materials must be heat resistant. Some examples of dry heat resistant packaging materials are:

- Tyvek®, high-density polyethylene and PP may tolerate heat-up to 125 °C for low, dry heat processing.

- Polyamide may tolerate heat-up to 180 °C for high dry heat processing.
- Glass and metal containers may be used in high dry heat processing, however, metals should not exceed their temper temperature.
- Aluminum may be used as a wrapping material.
- Paper may be treated at temperatures that do not cause charring. These may be coated to improve heat resistance and sealing.
- Glass containers and metal trays with lids may also be used as rigid type packages.

Wrapped items and load sizes require longer times for adequate heating (air or chemical penetration and heat diffusion) for dry heat sterilisation. Non-liquid products typically need permeable packaging such as Tyvek®, muslin, or paper. However, while PA is permeable to steam, air cannot penetrate PA and cannot be removed. Air pockets may reduce time to heat. Some packages may require an external air pressure to balance the internal pressure within packages to prevent them bursting with heating.

Newer packaging is available for low temperature dry heat sterilisation (e.g., 105 and 120 °C) from Tyvek®, paper, foils, and newer plastics such as polyolefins with metallocene and co-extrusions. Some packaging materials typically sterilised by steam that may be acceptable at low-temperature dry heat are: jean cloth, broadcloth, canvas, Kraft paper, glassine, parchment, crepe, Tyvek® paper/plastic, and PP. Polyamide (e.g., PA 6) and that are heat resistant are acceptable for sterilisation at high temperatures, as are metal trays, and glass containers. Some dry heat processes may sterilise instruments without wraps or packaging in the doctor's/dentist's office.

When designing tests to determine dry heat and packaging material compatibility some consideration should be made for how heat will be transferred through the package.

The material effects from dry heat sterilisation are caused by exposure to a maximum specified heat and/or dehydration for the maximum specified time and/or repeated exposures to the conditions of sterilisation.

Where appropriate, the effects of repeated sterilisation exposures should be evaluated and documented. Any treatment required before resterilisation should also be validated as part of the re-sterilisation procedure. Typically, resterilised product will be repackaged in a new package, and the initial package will be not be reused.

For a given dry heat material's qualification, the degree of degradation may be temperature and time or dependent on a number of iterations. For example, a material qualification performed at a low (moist heat and dry heat) temperature and single iteration may reveal less degradation (e.g., softening, dehydration, distortion, melting)

than a high temperature and multiple processing in dry heat sterilisation. Note, like irradiation, minimisation of oxidation on polymers or metals may be reduced by using nitrogen gas.

6.7.2 Packaging for Ethylene Oxide Sterilisation

In general packaging for EO should:

- Be permeable to EO, moisture, and air.
- Permit aeration.
- Be constructed of a material recommended by the steriliser and sterilant manufacturer, and maintain material compatibility (i.e., be non-degradable) with the sterilisation process.

Woven, non-woven, peel-pouch packages, and some rigid container materials are permeable to EO and do not impede rapid aeration of contents. Woven materials, however, may absorb a large amount of the RH that is needed for EO sterilisation. This may prevent adequate hydration of micro-organisms for penetration of EO gas to all surfaces of the package contents.

Typically an EO sterilised medical device must be sealed in a strongly designed, gas-permeable package that enables the EO gas to enter in and pass out. Furthermore, this package must meet a variety of engineering, regulatory, and marketing requirements, specified by the manufacturers. Sterile packaging is a cost driver at every step in a product's life cycle. Generally, smaller is better when it comes to sterile device package design.

EO sterilisation utilises multiple conditions in routine processing, (for example, heat, moisture, pressure changes, and exposure to EO and or its non-flammable diluents). Packaging must be designed to allow removal of air and penetration of steam and EO. Consideration shall be given to the potential physical and chemical effects of these conditions and the formation of residuals. During an EO sterilisation process, products can be subjected to environmental stresses such as vacuum and pressure changes, elevated temperatures and changes in humidity. The package may also react with EO and/or diluent gases used. The product design should ensure that functionality and safety are not compromised by exposure to the anticipated range of sterilisation conditions. Furthermore, high moisture content and changes in pressure may affect the strength of package seals with a consequent loss of integrity. Other packages require an air steam/EO mixture to balance internal pressure within the packages to prevent bursting. This is typically referred to as balance pressure EO process.

Designing packages for EO sterilisation may be a challenging process, because there are number of gas flow exchanges in and out of the package during the sterilisation process. The behaviour of these exchanges may be represented by the combined gas law as follows:

$$P_1V_1/T_1= P_2/V_2/T_2 \quad (6.1)$$

Where:

- P is pressure.
- V is volume.
- T is temperature.

The combined gas law can be used to calculate a specific factor(s) for a device package, which may be related to a specific EO sterilisation cycle. The combined gas law describes the behaviour of gases using the formula in **Equation 6.1**. The gas law takes into account four major variables in an EO sterilisable package design using Tyvek as follows:

- Package volume.
- Area of the package's Tyvek.
- Porosity of Tyvek.
- Sterilisation cycle specifications.

Note: While product packaging must be permeable to gas and humidity and so on for EO sterilisation, the packaging system for industrial sterilisation generally consists of layers of packaging (e.g., a number of barriers, beyond just Tyvek for example) and the material absorptivity, density will influence permeation and exchanges of volumes of gases.

Relative humidity may be another variable to consider, for example it has been observed that high levels of humidity may create a seal between two overlapping PE films, that are not normally sealed together but sealed together with a strip of Tyvek, to allow breathability in and out of the bag, as a cover lid, for example, in a Shuster bag, with film and a vertical strip of Tyvek.

Some considerations to be made when designing tests to determine packaging material compatibility for EO sterilisation are: EO sterilisation utilises multiple conditions in routine processing, i.e., heat, moisture, pressure changes, and gas concentration. Post-sterilisation residual testing should also be conducted at worse case (maximum) sterilisation processing parameters. During an EO sterilisation process, products can be subjected to environmental stresses such as vacuum and pressure changes, elevated temperature and changes in humidity. Extremes of all environmental conditions should be considered where feasible, as well as reusability.

6.7.2.1 Packaging Materials

When choosing materials suitable for packaging, initial consideration must be given to the intended method of sterilisation that will be used for the finished product. It is important to design packaging that will allow air within the packaging to readily escape when evacuated during EO processing. Materials considered for packaging design should also take into account the design of the device. Packaging material must be strong enough to prevent any breaks, tears, or pin-holes that may be caused by the device during normal transport. Materials that can be used for EO sterilisation will vary (see **Table 6.9**).

Table 6.9 A summary of some materials used for packaging with EO sterilisation

Material	EO compatibility	Comments
Tyvek®	Excellent	Highly porous material allows EO and moisture to pass readily. Minimises additional stresses on seals. However, should never be allowed to get wet, because microbes in a wet material may pass through
Clean peel transfer	Excellent	-
PETG	Excellent	Excellent choice for trays used in kits
Mylar	Bad	Considered impervious to EO
PU	Good	High affinity to EO
Plasticised PVC	Good	High affinity to EO
PE film	Excellent	Low-density recommended since higher EO and moisture permeability
Polyamide	Excellent	-
PETG: Polyethylene terephthalate glycol		

6.7.3 Packaging for Radiation Sterilisation

Numerous materials can be used for packaging in radiation, only a few are not recommended. Some examples of effects of irradiation on packaging materials are:

- PVC film may discolour, odour, and leach.
- PP may crosslink and get stiff and break.
- PE may give off odours and many polymers may give off odours after treatment with irradiation, requiring additional package materials that are porous to allow odours to evaporate or diffuse out of interior of package, to be reduced, such as with Tyvek®.
- Glass will discolour.

Typically radiation sterilisation processing means that the package design does not require permeation to gas or moisture as is needed with EO, as the only requirement, is the release of odours. Sealed packaging containing inert gases may be utilised to reduce the effects of oxidation. Massive package sizes or high-density products surrounded by low-density wraps/foams should be avoided as they can result in large variances in the maximum to minimum dose delivered to a product. However, packaging materials should be selected to avoid undesirable discoloration, material damage or increases in seal strength due to crosslinking.

During the radiation sterilisation process, all environmental conditions such as humidity, pressure and so on, virtually remain constant and packages may only be subjected to a slight increase in temperature. Although temperature, moisture and pressure changes may affect the package seal integrity, these are not of consequence with radiation, but changes in materials' physical properties may affect the package seal strength.

Sealed packaging containing inert gases may be utilised to reduce the effects of oxidation or, breathable packaging such as Tyvek® may be utilised to allow for dissipation of obnoxious odours or for off-gassing of some polymers. Therefore, odour reduction can be accomplished through the use of gas-permeable packaging (i.e., Tyvek®, paper) and/or elevated temperature conditioning.

Massive package sizes or high-density products surrounded by low-density wraps/foams should be avoided as they can result in large variances in the maximum to minimum dose delivered to a product. Packaging materials should be selected per the guidelines given next to avoid undesirable discoloration or increases in seal strength.

The dimensions and density of packaged product are requirements for radiation processing. The orientation and density of the product within the package is critical in electron beam sterilisation.

6.7.4 Packaging for Hydrogen Peroxide (with and without Plasma)

In general packaging for low-temperature H_2O_2 (and other gas) plasma sterilisation should:

- Allow sterilising plasmas to penetrate packaging materials.
- Be compatible (i.e., non-degradable, non-absorbable) with the sterilisation process.
- Be constructed of a material recommended by the steriliser manufacturer, and
- Be used according to the packaging manufacturer's written instructions.

Low-temperature gas plasma sterilisation is affected by absorbable packaging materials (e.g., cellulose-based packaging material, textile wrappers, paper-plastic pouches, or porous wrap), both the packaging and steriliser manufacturer's written instructions should be followed.

The absorption of the plasma sterilant (e.g., H_2O_2) by paper-plastic pouches or porous wrap could have an adverse effect on the effectiveness of the sterilisation process. Pouches used in low-temperature gas plasma sterilisers should be made of all plastic (e.g., Tyvek or PP). Not all containment systems are compatible with low-temperature gas plasma, the user should obtain the manufacturer's technical data verifying that the containment device has been validated for use in low-temperature gas plasma. If the containment device requires a filter, the filter should be made of non-cellulose material.

Due to the oxidative nature of H_2O_2 vapour, use of certain materials is not recommended. Also because of the deep vacuum required for sterilisation, the packages to be sterilised must be able to withstand the pressure changes. Some packages utilise special venting to allow pressure equalisation between external and internal spaces. Physical damage to the package and/or seals may occur if they are not capable of withstanding both deep vacuums and the rate of pressure change from a given cycle. There are numerous possible compatibilities of packaging material to vapourised H_2O_2 sterilisation as well as some materials that are not recommended (see **Table 6.10**).

The previously mentioned sterilisation packaging materials relates to thermally formed blister packs, sterilisation wraps, peel pouches, trays, tray accessories (silicone mats, medical device holding-devices), and re-usable sterilisation containers. Since packaging material usually involves high surface area/volume ratio, unique considerations

should be given to select packaging materials intended for H_2O_2 vapour sterilisation methods. Packaging materials that require particular scrutiny prior to application include silicone elastomers, plasticised PVC, polyaryl sulfone, polyetherimide, PU and Polyamide. Packaging materials that have been demonstrated to be particularly compatible to H_2O_2 are metallic laminates, Tyvek®, PET/PE laminate film and PE film.

Table 6.10 Some effects of H_2O_2 and vapour plasma on packaging materials

Material	H_2O_2 vapour/plasma sterilisation method
Tyvek® - spun olefin	Excellent
PET/with a laminate film	Excellent – PET may need to have an equivalent area made of a permeable material such non-woven polyolefin
PE film	Excellent
Metallic laminates	Excellent – may need to have an equivalent area made of permeable material such non-woven polyethylene
PETG	Good – may need to have an equivalent area made of permeable material such non-woven polyolefin
Acrylic (XT Polymer)	Medium – may need to have an equivalent area made of permeable material such non-woven polyolefin
Acrylonitrile-methyl acrylate copolymer (Barex®)	Medium – may need to have an equivalent area made of permeable material such non-woven polyolefin
Silicone elastomer	Grade dependent – discuss with equipment manufacturer
Plasticised PVC	Not recommended – high absorption
Polyaryl sulfone (Radel®)	Not recommended – high absorption and limited durability
Polyetherimide (Ultem®)	Not recommended – high absorption
PU	Do not use – very high absorption
Polyamide	Do not use – very high absorption and limited durability
Paper and cellulosic materials	Do not use – absorb and may breakdown materials, and peroxide

6.7.5 Packaging for Ozone Sterilisation

In general, packaging for O_3 sterilisation should comply with the steriliser manufacturer's written recommendations. Packaging not intended for use in O_3 sterilisers may compromise the sterilisation process. Packaging materials suitable for O_3 sterilisation include uncoated non-woven material, Tyvek or PE pouches and commercially available anodised aluminum containers using non-cellulose disposable filters.

O_3 is a strong oxidising agent. There is limited material compatibility information published on O_3 terminal gas sterilisation. It is a strong but selective oxidising agent that requires moisture to provide microbial kill. As well as screening for oxidative material degradation, materials that are sensitive to moisture need to be scrutinised prior to application.

The selection of materials for use with O_3 sterilisation needs to be carefully considered since O_3 is one of the most powerful oxidising agents known. It is not recommended to use O_3 for materials such as polyamide (Nylon), natural rubber, cellulose acetate, and some metals such as zinc, steel, and magnesium. It is also not recommended for continuous use of some materials with moderate O_3 capability or resistance, such as polyacetal (Delrin), polyester (Hytrel), PP, and some synthetic rubber (Neoprene). Materials such as polycarbonate, polytetrafluoroethylene (Teflon), polyvinylidene fluoride (Kynar), polychlorotrifluoroethylene, fluorosilicone, polyetheretherketone, glass, and titanium are highly O_3 resistant and O_3 sterilisation has no or minimum effect on them.

Since packaging material usually involves a high surface area/volume ratio, unique considerations should be given to selecting packaging materials intended for use with O_3 or H_2O_2 vapour sterilisation methods, especially for the in hospital use of the H_2O_2 vapour plasma method.

Packaging materials should be resistant to oxidation and moisture, as well as pressure changes.

Packages not acceptable for H_2O_2 use may be acceptable for O_3. Paper or celluolusics may be more resistant to O_3 than to H_2O_2 and consequently may serve as packaging materials for O_3 sterilisation.

Packages that are not permeable to O_3 may be acceptable, if the O_3 is created within the sealed package. One novel approach would be to create a sterilising environment in the package which would eventually become non-toxic. A non-thermal process using O_3 for the destruction of pathogens could be considered. Given its anti-microbial properties and lack of residual substances, O_3 is both effective and safe. In-package ozonation is a technology, for generating O_3 within/inside a sealed package environment. O_3 is created in a relative simple process between two electrodes operated under adequate voltage, frequency, and geometry. In-package ozonation may use only 40 W of power, for a single package, the equivalent to a weak light bulb. Reactive O_2 species can be generated, which will react with one another, and with O_2 molecules, resulting in the formation of O_3. O_2 species generated as a result of this process can include: O_3, oxides (O_2^-), singlet O_2 (O or O^-), peroxides (H_2O_2), and hydroxyl radicals (OH^-). The treatment can generate a purple plasma field of O_3.

Most reactive O_2 species have very short half-lives (in the range of milliseconds), making them very difficult to work with. O_3, however, can have a much longer half-life, ranging from minutes to days depending on conditions (e.g., >2,000 ppm O_3, >15 min, at low temperature (e.g., ~5 °C), low humidity (e.g., ~37-39% RH). The concentration of O_3 (ppm) increases within packages after varying ionisation treatment times (seconds). A package for example could be exposed to a ionisation treatment process at 18 kV (using a variable autotransformer and copper plates) for up to 5 min. O_3 is more efficient at lower concentrations and treatment times than more standard sanitisers, such as chlorine.

6.7.6 Chlorine Dioxide

There is limited information published on packaging material compatibility with ClO_2 terminal gas sterilisation and reusability. It is not a readily accepted sterilant, and its use as a novel sterilant may require approval from the US Food & Drugs Administration (FDA) for example. It is a very strong but selective oxidising agent that requires moisture to provide microbial kill. As well as screening for oxidative material degradation, materials that are sensitive to moisture need to be scrutinised prior to application

6.7.7 Liquid Sterilants

For re-used devices and healthcare products, the effects of repeated liquid chemical exposures on the device or packaging materials must be evaluated, unless information is provided by the manufacturer(s). At times a product is packaged that has been sterilised by a liquid sterilant, although aseptic handling may be involved because of transfer from the liquid sterilant to the final package.

Active ingredients in commonly used liquid chemical sterilants (high-level disinfectants) are aldehydes such as glutaraldehye and oxidising agents such as H_2O_2 and peracetic acid (PAA). *Ortho*-phthalaldehyde is a relatively new liquid chemical sterilant (FDA approved in 1999). Glutaraldehyde has the potential to crosslink with unsaturated structures and H_2O_2 and PAA can be corrosive. In addition, leaching of device material additives such as plasticisers can occur. Some liquid sterilised products have been sterilised, and processed with containers with end cap filters so the liquid sterilant or preservative can be flushed before use of the product.

A major application of liquid chemical sterilisation in industry is for the sterilisation of biological devices made from animal or human tissues, which cannot be subjected

to other methods of terminal sterilisation. The devices may be packaged in a container with the sterilant, which is intended to be rinsed off before use.

6.7.8 Sterilisation of Containers and Packages with Liquids and Cycles with Differential Pressure Control

When selecting sterilisation cycles for a package or container with liquid and defining the process for a new product, a number of variables must be considered, such as internal liquid and air pressure under increasing temperature, which has to be correlated to the exterior process parameters of steam and temperature. This discussion aims to provide help for the optimal selection of parameters for the process cycle and the package.

6.7.8.1 The Need for Pressure Control

Sterilisation cycles with differential pressure control between the interior and exterior of the package are used for the sterilisation of liquids in closed packages. Sterilisation can be performed in autoclaves with a superheated water shower (sometimes referred to as a raining water system) or with a mixture of air and steam using fans to make this environment uniform. In addition to the liquid, there is always a certain amount of air inside the packages being sterilised. The pressure inside the package before contents are added and sealed, should be assumed to be equal to the pressure of the autoclave chamber before the start of the sterilisation cycle.

6.7.8.1.1 Heating

The first stage of the sterilisation cycle consists of heating the product. To minimise heating time, it is sometimes beneficial to increase the chamber temperature as quickly as possible. This quickly establishes a temperature differential between the chamber and the product. When the chamber temperature is increased, the chamber pressure increases simultaneously due to the expansion of air and the increase in water vapour pressure. For autoclaves operating with a mixture of steam and air, this is due to the direct introduction of steam to the chamber, whereas with water shower autoclaves it is due to the evaporation of a portion of the re-circulating water.

The container or package receives heat energy from the chamber atmosphere and the temperature (conduction) increases; its temperature during this stage is lower than the temperature of the chamber. The increase in temperature also results in an increase in the pressure within the package due to the vapour pressure and the expansion of

air. Other reasons that contribute to the variation of the interior pressure of a rigid package will be explained later in this discussion.

Since the temperature inside the container package is lower than the temperature of the chamber, if no action were taken the chamber pressure could be higher than the theoretical value within the package during the entire heating process.

To avoid an excessive pressure difference between the chamber and the interior of the package, it may be necessary reduce the chamber pressure, depending on the type of package. In reality, if the package is rigid (e.g., glass) the interior pressure increase as explained previously can be calculated if the liquid temperature is known. This is achieved by use of a sample package, identical to those being sterilised but having a temperature sensor inserted into the liquid. If the package is semi-rigid or soft (for example, plastic) the anticipated increase in pressure may initially be lower and an increase of the package's volume may occur due to the expansion or deformation of the plastic.

6.7.8.1.2 Sterilisation

Initially the product or container may be at a lower tempertaure, until it reaches the sterilising temperature of the chamber, this is the difference between heat-up time and come-up time. Once the product has reached the selected sterilisation temperature, the control system must maintain the chamber temperature and the constant pressure until completion of the set sterilisation period, otherwise problems will occur (bursting expansions, deformation, exploding, breakage of seals, peeling, creeping and so on).

6.7.8.1.3 Cooling

During the cooling process after sterilisation, it is also necessary to maintain control of the chamber pressure in relation to the product temperature. During the heating process there are no abrupt temperature and pressure variations, whereas at the start of the cooling process, due to the abrupt condensation of the steam present in the chamber, a sharp decrease in pressure may occur that could put the integrity of the package at risk.

The objective of a cycle at a controlled differential pressure is to maintain the pressure in the chamber at a level that prevents the rigid packages from exploding and the non-rigid packages from becoming deformed. The optimal condition would be to have the chamber always at the same pressure as the package if it were rigid. This is easy to achieve during the heating and sterilisation process, but is more difficult

when beginning the cooling process for the reason stated previously. The reduction in vapour pressure in the chamber must be compensated by an increase of air pressure. However, the loss of vapour pressure in the chamber can be so abrupt that it can be difficult to provide sufficient airflow to compensate. The solution is to address the problem before it arises by increasing the chamber air pressure after completing the sterilisation but before beginning the cooling process.

6.7.8.2 Types of Package

6.7.8.2.1 Rigid Packages

A glass package can support differences in pressure in both directions, in other words, the external pressure can be higher or lower than the internal pressure. If the external pressure is greater than the internal pressure, under normal circumstances nothing will happen. However, if the internal pressure is greater, problems may arise.

Under the action of a specific pressure differential, the outward deformation of the rubber stopper can be sufficient to break or deform the security tab of an aluminum cap. If the differential is substantial, depending on the package size, the thickness of the glass and its mechanical resistance, it is possible that it may not be able to support the pressure increase and will explode. In addition, when a vial explodes, the neighbouring vials will typically explode as well.

Normally, during the sterilisation process a slightly higher internal pressure than external pressure can be tolerated. The aluminum cap is normally the limiting factor. Inside the package there are two volumes. One volume is occupied by liquid and the other, which is referred to as the head-space, is filled with a mixture of air and water vapour. When heating the glass package, five physical phenomena occur simultaneously:

- The glass expands, increasing the total volume of the vial. Consequently, the head-space increases but the pressure may decrease.
- Part of the water evaporates, which increases the pressure.
- Although only slight, the volume of liquid water decreases because part of it evaporates, increasing the head-space and lowering the pressure.
- The water that has not evaporated expands, increasing its volume, and consequently the head-space decreases and the pressure increases.

- The air expands, therefore, increasing the pressure.

As the water coefficient of expansion is much higher than the coefficient of the glass, and the amount of evaporated water is very low, the result of the combined action of the five phenomena is an increase in pressure that is higher than the value calculated without taking into account the expansion of the glass and the water, which in turn will depend on the proportions of liquid and head-space.

The autoclave control system typically calculates the theoretical pressure inside the package using the temperature read from the sample package, but since the relative proportions of liquid and head-space are not known, the calculation only allows for the effects of water vapour and the expansion of air. The result is fairly close to the actual situation if the initial head-space is substantial. A package that has 50% head-space will reach a pressure that may only be 2% higher than the pressure calculated, taking only the vapour pressure and the expansion of air into account. However, if the head-space is decreased to 15%, the actual pressure may be 17.3% higher than the pressure calculated and if the head-space is reduced to 10%, the actual pressure will be 37.7% higher than the pressure calculated. In other words, a glass bottle or vial that is 90% full of liquid in which the normally calculated theoretical pressure is 334 Pa (absolute) will actually be 459 Pa (absolute).

It can therefore be concluded that in rigid packages, the chamber pressure can be maintained slightly above the theoretical value calculated without representing a danger to its integrity.

6.7.8.2.2 Semi-rigid Containers or Packages

These are the most problematical, since to provide a certain level of rigidity, there are moulded features on the sides and base. This asymmetry, together with the variation in plastic thickness at the corners and other parts, results in expansion properties and mechanical resistance that are not constant over the surface.

If the internal pressure is substantially higher than the external pressure, the base of cylindrical-type packages (vials and ampoules of round and oval cross-section) tends to deform outwards and after cooling does not return to its original shape. As a consequence, they will no longer stand upright when placed on a flat surface. The bases of such packages before and after incorrect sterilisation can be deformed outwards.

Those that have a rectangular shape tend to have very weak corners (trihedrons) where the three sides meet. The injection and blowing process results in the plastic being thinner in those areas than in other parts of the surface and consequently excess

external pressure may deform the corners. The ideal processing conditions for all of these packages are those that maintain the chamber pressure equal to the theoretical pressure inside the package at all times. As a result, when the internal and external forces are equal, the package will always retain its shape.

6.7.8.2.3 Soft Containers or Packages

Normally these are bags or pouches and as the material is very flexible, the theoretical increase in internal pressure causes the material to stretch and the bag or pouch to inflate.

If the external pressure is greater than the internal pressure, the volume of the head-space will reduce due to compression of the air. It is difficult for a high pressure differential in this direction to damage the bag because it does not stretch the material or compromise its welded joints. However, a low chamber pressure will cause the bag to inflate which, if it results in stretching beyond the elastic limit, will lead to non-recoverable deformation or failure at a joint or seal.

Consequently, the best processing condition for this type of product is to always maintain the chamber pressure a little above the theoretical pressure inside the package.

6.7.8.3 Temperature Control

If the process requires that the product be heated to 121 °C and the chamber is set to 121 °C, then the product will either never reach temperature or it will take a very long time. To rapidly heat the product to the selected sterilisation temperature, the chamber is initially set to a temperature that is slightly higher than the required product temperature. As the product temperature approaches the sterilisation temperature, continuing with the same temperature control would cause the product temperature to over-shoot. Consequently, from a certain product temperature value onwards the chamber temperature should be reduced. The closer the product is to the sterilisation temperature, the lower the differential between the sterilisation temperature and the chamber temperature must be. The objective is for the product to reach the sterilisation temperature (or some tenths of a degree above it) and for the chamber to reach some tenths of a degree above the product to compensate for the thermal losses of the equipment.

To achieve this control profile, three temperature parameters related to the sterilisation temperature may be investigated and selected:

- Seal packages at a slightly elevated temperature, so there is less air to expand during sterilisation.
- Increase the chamber temperature compared to the sterilisation temperature during the heating up process.
- Gradually equalise the product temperature to the higher chamber temperature during the sterilisation exposure.

In a water shower sterilisation autoclave, there is an amount of water re-circulating in the chamber, which depending on the chamber size and load, can represent 45-75% of the product volume. Suppose that in order to speed up the heating process the chamber water has been heated to 124 °C. This water has a high calorific value. If at a point when the product is close to the sterilisation temperature (for example, 120.5 °C), heating of the chamber water is stopped, the product will continue to be heated through the thermal inertia of this water and may reach a temperature of 121.6-122 °C. Depending on the type of product, this may or may not represent a problem.

The amount of water introduced into the chamber of a particular water shower sterilisation autoclave is always the same. Due to the geometry and distribution of the load in the trays, the greater the size of the product package, the greater the total amount of product that can be accommodated within the same chamber. Consequently, the thermal inertia of the water in the chamber will have a decreased effect on the final phase of product heating with a batch of large packages when compared to another batch where the packages are smaller.

When using a sterilisation autoclave with a mixture of steam and air, the amount of surplus heat in the chamber as the product temperature approaches the sterilisation temperature is of little importance due to the low density of the steam. The thermal inertia is much lower than that in a water shower autoclave.

The product heats up *via* heat transferred by conduction across the package, with the layers of liquid in contact with the package heating first followed gradually by the centre of the liquid. If an excess of temperature would damage the product, the overheating temperature of the water cannot be very high otherwise the surface layers of liquid in contact with the package could be damaged.

A low volume package, heats and cools quicker than a high volume package due to the relationship between the surface and the volume of the package. Comparing two similar shaped packages of different sizes, the smaller package has a greater surface to volume relationship than the larger one. The heat to be supplied must pass through the surface to heat the entire volume and consequently, a complete liquid sterilisation cycle will be shorter when small packages are processed as opposed to large packages.

In addition, the shape of the package influences its heating and cooling time. The lesser the distance from the centre of the package to a point on the surface of the package, the more rapidly it will heat-up.

Only an empirical study with each type of product and batch will correctly confirm these parameters, which, with the help of the guidelines included herein, should be easier to specify. The effectiveness of microbial thermodynamic inactivation rests with heat transfer from steam to the microbial spore, provided that there is a temperature difference between the two parts of the system. Heat will flow from one part to the other part by two or all three of the heat transfer mechanisms:

- Conduction - heat by conduction is transferred from one substance to another by the vibrational energy of atoms of molecules. There is no mixing of the substances (only steam and air). Transfer of heat from the autoclave to and through the walls of a container or package material occurs by conduction. For liquids in the container further heat transfer by conduction is minimal.

- Convection - this only occurs in fluids. Heat transfer is by warm fluids mixing with cooler fluids. This is of importance for sterilisation of fluid loads in autoclaves, but less so for solid products.

- Radiant heat – this transfers heat energy moving through space by means of electromagnetic waves. If radiant energy comes in contact with an object, heat is absorbed by the object or conducted through it. Typically radiant heat does not make any significant contribution to heat transfer in autoclaves, but is important during dry heat sterilisation. Heat penetration into items being sterilised by saturated steam begins from the outside of each item, which has a layer of condensed steam adhering to it which then transfers heat in it. The transfer of heat is by conduction from the steam, to the condensate, to the walls of the container and/or package, and then on to the fluid or interior. Each stationary boundary layer presents its own resistance to heat penetration. This resistance can be minimised by technologies that improve heat transfer such as movement of the fluid within the container or package, or turbulence within the autoclave (e.g., fan or recirculation). Liquids typically have low thermal conductivities, but convection currently caused by local temperature gradients lead to continuous movements within the fluid; thereby reducing the thermal resistance of the innermost boundary layer of the system. It is not usual to find steriliser loads being agitated except if rotary washers or autoclaves are used. It is reasonably usual to find turbulence within the steam in the autoclave being achieved by fans or recirculation.

6.7.9 Steam – Air Mix within Package

For the steam and air mixture, the total pressure of a mixture of gases (steam and air) is made up by the sum of the partial pressures of the components in the mixture – this is known as Dalton's Law of Partial Pressures.

Consequently, the total pressure in a mixture of steam and air can be expressed as:

$$Total\ P = P_a + P_s \quad (6.2)$$

Where:

- P_a = partial pressure of air.
- P_s = partial pressure of steam.

6.7.9.1 The Partial Pressure in a Mixture of Steam and Air

If the air is not properly removed from the package and displaced by the steam, the steam space becomes filled with a mixture of air and steam. If the pressure of the steam space under these conditions is measured, it will be the pressure of the mixture of air and steam; the pressure shown on the pressure indicator will not be the steam pressure.

The partial pressure is the pressure exerted by each component as if it was occupying the same volume of the mixture. The effective partial pressure of the steam can be expressed as:

$$P_{s_effective} = \nu_s/V\ P\ (abs) \quad (6.3)$$

Where:

- $P_{s_effective}$ = effective steam pressure (absolute).
- ν_s = volume of steam.

- V = volume of mixture.
- P = pressure (absolute).

Reducing part of the steam pressure reduces the effective steam pressure. Increasing the part of steam pressure (to 100%) increases the effective steam pressure.

6.7.9.2 Examples - Mix of Air and Steam

The effective pressure in a steam/air mixture made up by 3 parts steam and 1 part air, with a total pressure 500 Pa (absolute) can be expressed as:

$$P_{s_effective} = \text{(3 parts)/(3 parts + 1 part) (500 kPa absolute)= 375 kPa (absolute)} \quad (6.4)$$

Since the steam has an effective pressure of 375 kPa instead of the pressure of 500 kPa absolute, the mixture would have a temperature of approximately 139 °C rather than the expected saturation_temperature of 152 °C. This has a major effect on the heat transfer capability of a heat exchanger.

The resulting temperature in an air and steam mixture is shown in **Table 6.11**.

Table 6.11 Resulting temperature in air and steam mixture(s)

Mixture pressure (kPa)	0% Air (pure steam) (°C)	5% Air (°C)	10% Air (°C)	15% Air (°C)
14	104	102	101	99
34	108	107	106	104
69	115	114	112	110
138	126	124	122	121

So in this case the pressure of the saturated steam in the chamber is lower than the pressure of the mixture in the package.

Theoretically, the internal pressure of a package is a function of temperature, with other influencing factors such as vapour pressure of the liquid, the coefficient of thermal expansion of the liquid, the fraction of the container volume occupied by

expansion of the contained material and the sterilisation temperature.

The typical internal pressure during a 121 °C cycle in a sealed container of water with air is approximately 343 kPa. This is 131 kPa greater than the saturated steam pressure at 121 °C within the chamber.

Since the liquid in the package/container is less than 70%, substantially higher internal pressures will not typically result, from a rigid container [13]. But this may not be the case, but while internal pressure is slightly higher than the chamber pressure, it is a result of heated air as well as steam vapour, and what we have is a mixture of steam and air, where the heating efficiency of steam has decreased.

The reading on the pressure indicator for the mixture might lead one to expect that the temperature will nearly match the saturation pressure, but the actual steam pressure is lower so the internal temperature of the mixture will never get as high as the expected steam value. If one fails to realise that there is a mixture of gases at play, a curious phenomenon seems to occur - the pressure indicator shows the near designated pressure but the temperature just will not rise. Consequently, when a pressure indicator is used in the package, parallel multiple temperature sensors in parallel are also needed in the package.

The assumed temperature of a known mixture of steam and air attained within a package are given in **Table 6.12**.

Assuming no air is removed from the package, and the air pressure in the package at ambient conditions is 101 kPa, then as heat is applied to the moisture in the scrolls, within the package, then additional pressure (of heated air and steam) will be added to the 101 kPa.

But, despite the additional amount of steam, created in the package with no discharges of air, there will initially be stratification of the steam air within the package, because the density of the steam is ~11 kg/m^3; while the density of air at the same pressure and temperature will be 1.9 kg/m^3.

We know that air can act as a heat insulator, because air conducts heat very poorly. In this regard, it is no exaggeration to say that removing air from the steam space is the first step in the effective use of steam. Since, however, we will not actually be removing air from the package, effective steam within the steam-air mixture will not fully occur, unless the air in the package diffuses out somehow, and to a lesser extent the steam and air eventually mix.

Table 6.12 Temperature relationship of (unsaturated) steam pressure added to atmospheric air

Amount of steam added to the air (kPa)	Resulting temperature within the package (°C)
34	72
69	90*
103	100
138	109
172	115
207	121

These pressure conditions without discharge of air but with addition of steam were taken from J. Perkins, *Principles and Methods of Sterilisation*, Charles C. Thomas, Springfield, IL, USA, 1956 [12].

*Note, with a package pressure of 138 kPa, with no air discharged, a package temperature of only 109 °C may be achieved throughout the package, if steam and air are eventually mixed, however, since stratification initially occurs, the higher or upper area of the package will have a higher temperature >109 °C; while the lower position of the package without moisture would be at a lower temperature <109 °C, until mixing eventually occurs. So depending where the BI and temperature probe is positioned will determine the killing effect within the package. Consequently multiple BI and temperature positions will be needed to determine worse case locations to sterilise. Similarly, temperature indicators located in the upper part of a non-discharged air package may incorrectly indicate sterilising conditions, while at the bottom of the package without moisture, lower temperature and non-sterilising conditions may occur.

The presence of air greatly reduces the eventual temperature of the steam in the package to well below that of pure saturated steam in the chamber. Throughout the normal period of sterilisation the temperature of the lower areas of the package will be substantially lower than the upper areas of the package because of the differences in specific gravities and the reluctance of steam and air to mix, unless with the artificial use of fans and so on, or extremely long times to allow for eventual slow mixing. The mixing of steam and air within a non-turbulent package is typically uncertain, however, we know that the air and steam will eventually mix, resulting in a uniform gas make-up of steam and air, in which part of the heat contained in the steam will have been absorbed by the air.

But nevertheless, the effect of heating efficiency of steam will be reduced by the presence of air. As steam and air is mixed, only the steam content of the mixture heats by the condensation process. The air has no useful penetrating power. The heating or penetrating power of the mixture is reduced in accordance with the proportion of air present in the mixture.

6.8 Leaving Instruments Unpackaged for Sterilisation

All steam sterilisers legally marketed in Europe and United States must prove to the regulatory agency that the 'wrapped' or 'packaged' cycles on their steriliser indeed achieve sterilisation of packaged instruments. Sterilisation is generally defined as the killing of high levels of resistant bacterial spores. The regulatory agency also clears sterilisation packaging material to assure it allows penetration of the indicated sterilant, serves as a microbial barrier and maintains sterility of the instruments inside after processing through the steriliser. Thus, steam (as well as dry heat and unsaturated chemical vapour) can penetrate appropriate packaging materials. The rationale for packaging instruments prior to sterilisation is to protect the instruments from re-contamination after sterilisation and before they are opened at the bedside. Beware of using non-regulatory agency cleared packaging materials such as freezer bags, cloth, heavy paper, aluminum foil or closed metal trays, for they will probably not allow penetration of the sterilant or will be poor microbial barriers. For some products that are sterilised in hospitals, clinics, or dental offices without packaging for immediate-use, using steam (emergency) sterilisation or flash sterilisation, the initial sterility delievered may be compromised by external adventitious contamination because of the lack of packaging.

6.9 Re-using Sterilisation Packaging Material

Most, if not all, of the appropriate sterilisation 'flexible' polymer packaging material is indicated for single use (disposable), unless approved as a re-usable container, or package. Re-using most flexible polymer packaging material may result in failure of one or more of its key properties, compromising sterilisation or the maintenance of sterility as mentioned previously. Check with the material's manufacturer and vendor about any intended re-use (see **Sections 6.6** and **6.12**).

6.10 Strength and Integrity of a Package

There typically appears to be some confusion regarding the strength of a package *versus* the integrity of a package. Package strength is concerned with the force required to separate the two components of the package. It could be the force to separate two flexible components of a pouch, or a flexible lid and a thermoform tray. These forces may be measured in pounds per inch width, as in the seal peel test, or in pounds per square inch, as in the burst test method. Alone, the values of these tests of package strength do not necessarily prove the integrity of the entire package. For example, since the seal peel test per ASTM F88/88M [3] evaluates only a 2.5 cm segment of

the package, there may be other areas of the package that are not sealed adequately to prevent contamination of the product. In fact, the seal width that was actually measured may be within the strength specification but may have a channel leak that could breach the package and negate its integrity.

Likewise, ASTM F1140 [14] burst test method as referenced by ISO 11607 [1, 2] also has its pitfalls. This method evaluates the whole package by applying pressure to all areas of the package, however, the pressure is not applied equally at all points as a result of package irregularities and distortions. This can lead to a relatively high degree of variability between tests. Further, the burst test may not detect breaches in the package, such as pinholes and channel leaks, even though the burst test values have met the performance specification. Even though the package strength specifications may be confirmed, package integrity is not confirmed and not necessarily proved.

6.10.1 Packaging Integrity

Package integrity is defined by ISO 11607 [1, 2] as an unimpaired physical condition of a final package. Seal integrity is defined as the condition of the seal, which ensures that it presents a microbial barrier to at least the same extent as the rest of the packaging. Neither definition refers to the strength of the seal. Package integrity is independent of package strength, although a strong package seal is a convincing indicator of a safe package. Furthermore, if the entire seal area is proved to be homogeneous and continuous, then one could say that the package seals provide integrity. However, this says nothing about the package surfaces that may have pinholes or leaks not detected by seal strength tests. Other mechanical tests may be appropriate for determining package seal homogeneity.

6.10.2 Seal Strength

Seal strength is very important in the overall scheme of developing the package process, but the seal strength performance specification is used most effectively to monitor the process, not to determine ultimate acceptance. Seal strength is also an important determinant for establishing package process parameters. In fact, the ISO 11607 [1, 2] standard requires that the seal strength shall be determined at the upper and lower limits of the defined critical sealing process variables and shall be demonstrated to be suitable for the intended purpose. To restate, seal strength is an important performance attribute for the package and provides suitable guidance in establishing statistical process control limits, but is not the absolute determinant of the acceptability of the package for its intended use. Package integrity at the point of final use is the main

acceptance criterion for a sterile medical device package. However, both performance attributes are essential to the package design and development process.

While a package may be based on the seal and burst test values of packages produced from a specific validated production line, these are not tests of integrity. The strength tests are performed using standardised test methods developed by the ASTM. The seal strength test procedure is described in ASTM F88/88M [3]. This test covers the measurement of the strength of a seal of a given width at a specific point of the package. It does not measure the seal continuity. Other methods such as the 180° peel test may be used to determine the seal continuity or peeling characteristics. The seal strength test is performed by cutting a 2.5 cm wide strip from the seal of the package. The strip is placed in the tensile test machine by clamping each leg of the sample in the grips, aligning the specimen so that the seal is perpendicular to the direction of pull. The seal is pulled apart at a rate of 25-30 cm/min. The peak force required to pull the seal completely apart is recorded. It would be appropriate to perform the test at several points of the package, including the manufacturer's seals (seals produced by the vendor of the package), and the production seals (seals produced by the manufacturer of the product). Typical seal strength values lie in the range between 0.5-1.8 kg. The optimum seal strength varies according to the type of package being tested and its specific applications.

6.10.3 Burst

The burst test procedure is given in ASTM D1140 [14]. This method covers the determination of the ability of package materials or seals to withstand internal pressurisation. Since packages may be produced from sub-standard materials or with inadequate seals, or both, package integrity may be compromised during production, distribution, or storage. Burst testing may provide a rapid means of evaluating overall package quality during production, and overall package integrity after dynamic events associated with shipping and handling.

Two methods of burst testing are provided in the standard: the open package test and the closed package test. The open package test is performed in a fixture that clamps the open end but provides a means for pressurising the package. The pressure is increased in the package at a rate greater than the permeability of the porous package component, until a failure occurs. The type and location of the failure is recorded as well as the maximum pressure at which failure occurred. The open package test is most useful as a quality assurance procedure on incoming materials to ensure that the supplier of the material is meeting pre-established specifications for seal strength.

The closed package test is performed on production samples as an internal quality assurance procedure. This method is performed by inserting the pressure source through a component of the package and then increasing the pressure until a failure occurs. The pressure at failure and location and type of failure are recorded. Burst test values typically fall in the range between 3.4-20.7 kPa. No correlation has been made between the burst test value and seal strength values. A recent study has shown that unrestrained pressure testing may lead to inconsistencies in test results while more consistent test results are achieved by restraining the test specimen between parallel plates [15]. A creep test at 80% of burst can determine time to failure.

6.10.4 Package Integrity

To maintain sterility of the product after sterilisation, microbial impermeable packaging is necessary. There are a variety of ways to test for package integrity. One category of package integrity test methods has been available for over 10 years and involves microbial challenge and product sterility. As shown later in this chapter, these are not the only means of determining package integrity and these methods are coming under tighter examination, as alternate test procedures are developed. In fact, the FDA has recognised ISO 11607 [1, 2] as a consensus standard, which states, 'The manufacturer shall demonstrate the integrity of the package by testing the package. This can be accomplished by physical tests.' Examples of physical tests as described in the ISO 11607 standard include: internal pressure test, dye penetration test, gas sensing test, vaccuum leak test. All of these methods have their advantages and disadvantages.

6.10.4.1 Some Microbial Challenge/Product Sterility Test Methods

There are really two types of microbial barrier test: those performed on materials and those performed on whole packages. Microbial barrier tests on materials are performed by packaging manufacturers to ensure that their materials are impervious to micro-organisms while allowing sterilant gases to permeate for product sterilisation purposes. These tests are typically performed using ASTM F1608 [16]. Microbial barrier testing of materials is significantly less controversial than microbial testing of whole packages, since this methodology lends itself to some level of standardisation and control. Determining the microbial barrier characteristics of materials is very different from the methods required for a whole package. A whole package is significantly more complex than a single material.

6.10.4.2 Aerosol Challenge

With the risk of oversimplifying the procedural demands of microbial testing, here is a summary of how a microbial challenge/product sterility test is performed. There are two types of whole-package microbial barrier test currently in use.

One method takes a sterile finished primary package containing an actual device and fixes it into a vacuum chamber. The chamber is loaded using a specific configuration and then the performance is qualified to establish a homogeneous distribution of the indicator organism prior to the actual test runs. After the performance qualification, the test packages are subjected to a microbial challenge of a high concentration of bacteria, which is nebulised into an aerosol and circulated in the chamber for a specified period of time. Next, the outer surfaces of the package are decontaminated and the product is aseptically extracted from the package. The product may even need to be manipulated further at this point to facilitate the sterility test. The product sterility test determines if any of the indicator micro-organisms were able to breach the package and contaminate the product.

Although this method would appear to be the best at determining package integrity since it is a direct indicator of product sterility or non-sterility, it has several deficiencies:

- It is very expensive to perform the test using an adequate sample size while providing statistical significance.
- It is prone to false positive results due to the high precision necessary for laboratory technicians to aseptically handle and manipulate the packages and products.
- Each package type, configuration, and size must be prequalified in the chamber.
- Several well known studies have indicated that it may not be even able to detect obvious breaches in the package integrity.
- There is no standardised method to ensure the reliability and repeatability of the test.
- Spores may not be able to penetrate as motile microbes.

6.10.4.3 Dust/Talc Challenge

The other whole package microbial method involves a similar concept of challenging the package with a high concentration of micro-organisms and then performing a product sterility test. This method uses talc or dust mixed with a specific micro-organism. The package is exposed to the dust by shaking in a chamber. Similarly,

the outer package surfaces are decontaminated prior to product removal and sterility testing. This method has deficiencies similar to the aerosol method.

The microbial methods are still in use to evaluate package integrity mainly because the regulatory agencies may still be asking manufacturers for data using these methods, or because medical device manufacturers have always evaluated their packages for integrity and they are hesitant to change their protocols. However, there are alternative methods.

6.10.4.4 Liquid Immersion

This is a test that is typically performed on a package that should not normally leak liquids or vapours. The package is immersed in a soup of microbes that typically have flagella and thus, are motile. Inside the package is an artifical liquid medium to grow microbes that somehow can pass through the package. This is typically the method used on parenteral or pharmaceutical products, but ideal for a package that is sealed tight and not allowed to lose moisture (as vapour).

6.10.5 Physical Test Methods

Many physical test methods have been available for many years as published in the ASTM standards. More recently, however, industry has taken a closer look at the validity and effectiveness of these tests and have developed new methods for evaluating package integrity.

6.10.5.1 Visual Inspection

ISO 11607 [1, 2] handles visual inspection for package integrity in Section 6.2, which is very detailed in the requirements and procedures. ASTM Sub-committee F2.60 on Medical Packaging recently published standard ASTM F1886/F1886M [17], to help further detail a method for visual inspection. This standard describes a method to visually detect channel defects in package seals down to a width of 67 μm with a 60-100% probability, depending upon the package type and size of the channel. It provides attribute data (accept/reject) for package integrity of finished, unopened packages. It is generally not effective in detecting pinholes and minute tears in package substrates.

In addition, visual inspection cannot be used for packages with two opaque substrates, as transparency of the seal surfaces is essential to the inspection. Its most applicable

attribute is for monitoring package quality in production to detect any significant changes in heat-sealing process parameters, which may provide the first indication that the process is out of control. Additional testing using more sensitive methods for leak detection of packages under suspicion of having defects may be required to confirm whether the channel or void is in fact an unsealed area. Visual inspection is not considered to be the only means by which the manufacturer should evaluate package integrity.

6.10.5.2 Internal Pressure Test

ISO 11607 [1, 2] describes the internal pressure test as applying an internal pressure to the sterile package while it is submerged in water and then noting any escaping air bubbles. A Flexible Packaging Association (FPA) committee, of the Sterilisation Packaging Manufacturers Council (SPMC), has published several standards for testing packaging. One of those standards, FPA/SPMC Standard 005–96 [18], details the internal pressure test method. The advantages of using this method for determining package integrity are that it is very easy to perform the test. In addition, it is inexpensive to test a large sample size and obtain statistical significance in the test sample set. The equipment costs are low, since all that is required is a pressure source and a water bath. Another method is ASTM F2096 a test for detecting leaks in packaging by the Bubble Test [19].

This method has not been validated by round robin testing, and no precision and bias statement has been made as to its repeatability and reproducibility. Nor is its sensitivity for detecting leak size known. However, independent verification has proved its usefulness in detecting gross leaks in packages. Gross leaks in packages occur most often as a result of handling and distribution hazards that cause tears, gouges, and punctures. Package validations most often fail as a result of the rigors of shipping and distribution. This test is sufficiently sensitive to detect those types of defects caused by the hazards of distribution. Leaks in seals and in material surfaces can be detected using this method.

The method can be used for both porous and non-porous packaging materials. For packages made with porous materials, the porous material substrate is sealed using a label or coating to reduce the porosity of the material. This facilitates the pressurisation of the package and reduces the interpretation of what constitutes a leak and where a leak is occurring in the package. The porous material is not evaluated for leakage, as the coating may mask or block leaks. However, pinholes, tears, gouges, and channel leaks are readily apparent under an internal pressure that does not begin to the separate the seals. Validation of the method for the package under investigation must be performed to determine the proper internal pressure, and to evaluate the

ability to detect channel and pinhole leaks over the permeation of air through the porous substrate.

6.10.5.3 Vacuum Leak Test

The vacuum leak test is similar in concept to the internal pressure leak test in that the result is a pass/fail for the detection of bubbles emanating from the package while submersed in a water bath. The method is described in ASTM D3078 [20]. The pressure differential may be obtained by evacuating the chamber, causing the package to expand. The difficulty in using this method for porous packages is that the pressure differential may not reach a point at which air passes through a channel or material leak before air passes readily through the porous material. Lowering the porosity of the material by coating it with a lacquer or other means could reduce this problem. This test is more suitable for non-porous packages that will expand under vacuum and create an internal pressure adequate to force air through leaks.

6.10.5.4 Dye Penetration Test

The ASTM F02 Committee has approved a dye penetration test method. The new standard, designated F1929 [21], finally provides a standardised method for conducting leak testing of package seals using a low surface-tension solution and dye indicator. The basis of the test is that, when the test solution comes in contact with a channel or breach in the package seal, it will flow through the channel by capillary action. The leak will be indicated by a blue streak visible in the seal and/or a profuse and consistent flow of the dye through the channel.

This test method is generally considered to be more sensitive than the whole-package microbial challenge methods discussed earlier in this chapter. In a study on Tyvek®-to-plastic pouches, seal defects down to 38 µm may be readily detected with a blue dye solution. The published test standard has verified by round robin testing that the smallest channel that can be reliably detected is in the order of 50 µm. In fact, the detection rate for breathable pouches and trays with breathable lids was found to be 98-99%. It was discovered during the testing that significant reductions in test performance can be observed when indicator dyes other than toluidine blue were used. Also, the round robin results are specific for the wetting agent (Triton X-100) used for the solution.

The most effective application for the dye penetration test method is for detecting breaches in the seals of transparent packages, since seal defects must be easily observed. It is possible to use this method for opaque packages, however, observation of the

seal leak must be made at the seal's outside edge and the exact location of the leak may be difficult to ascertain. One characteristic of this test methodology is that it is difficult to use for detecting leaks in the surfaces of package components.

That is, pinholes, gouges, or abrasions of the materials cannot be detected, since the dye cannot be easily contacted with all of the package surfaces. So, although the dye penetration test is a sensitive leak indicator for seals, it is not a good whole-package integrity test. Other means must be used to detect material leaks, such as the bubble emission leak test. Other characteristics of this test method must be considered before incorporating it into a package validation protocol. The method is difficult to use for packages having a paper component, as the dye solution can destroy the material in a very short time - maybe even faster than the dye would travel through a channel. Other porous packages may allow the dye solution to wick through, causing difficulty in distinguishing a true leak from the permeation or wicking of the solution through the material. Since the dye solution is injected into the package, the method is destructive to the package and, in many cases, also to the product.

6.10.5.5 Gas Sensing Test Method

Typically there has never been a cost-effective means of performing this type of test. The introduction of a new technology that allows a trace gas (helium) to permeate through the porous component of a package has made non-destructive package integrity testing possible. The test is performed by first placing the test package into a specially designed housing.

This system is ideal for thermoformed trays with porous lids and flexible pouches with one porous side. The test has been shown to detect leaks as small as 50 µm. Guidant (now part of Boston Scientific) and Medtronic have demonstrated the reliability of detecting leaks in blind tests and have quantified 100% of the purposely manufactured leaks in thermoformed trays [15]. In addition, there were no false positive readings in any of the unaltered packages.

The test method is suitable for testing packages for package validation in which the package system is being designed and developed. In the short term, it is thought that this test methodology could replace the whole-package microbial challenge test methods, as it provides greater reliability, reduces the risks of false positives, and is similar in cost. In the long-term, since the test housing is designed and manufactured for the package, this test methodology could be incorporated into a quality assurance programme to validate the integrity of each and every package being manufactured.

An ongoing 100% inspection or lot-to-lot sampling programme would ensure the

efficacy of the package process. Quality assurance during the packaging operation may include the following:

- Verify labels, their lot number, expiration date(s), bar codes (if applicable). Verify counts of labels (if applicable).
- Verify inserts (within the package) and that they are correct and clean and all together.
- Verify packaging materials.
- Verify all packaging components (e.g., stoppers, containers, films, lids, seals and so on.
- Verify the contents of the package visually through the package, as well as sampling a certain number of product packages to be further evaluated.
- Verify the absence of foreign materials, dirt and particulates within the package.
- Verify that the labels are not defaced, do not have smeared printing, missing information, are dirty or torn.
- Verify intermediate shipper or secondary package (if applicable) has proper labelling and physical appearance.
- Verify that the outer shipper (if applicable) is properly labelled.
- Verify counts of products to be sterilised against the number of actual sterilised products.
- Verify that there are no holes, scratches, adhesive skips, blooms, clarity/haze or gloss issues, no open seals, no distortions of trays or containers.
- Verify that there are no tears on opening the packaging.
- Verify that there is no illegible printing, ink smearing, discoloration, stains and so on.

The risk of a non-sterile package finding its way into the operating room should be virtually eliminated. As indicated previously (see **Section 6.9**) package seal strength does not of necessity equate to package integrity.

These two attributes and qualities of a finished device package are separate considerations in proving the efficacy of the package. Industry has developed tests for seal strength testing that are used to validate the packaging process.

Although package seal strength is an important performance attribute and characteristic, the ultimate acceptance of the package is based on its complete integrity against microbial penetration and loss of product contents. As described previously there are several tests available for evaluating the integrity of sterile packages. The application of a particular or specific integrity test, described previously, depends upon many factors, including the type of package, material of construction, size, desired sensitivity, and/or objective of the test (e.g., maintaining internal moisture, product content).

6.11 Sterile Packaging Storage

While packages may have been qualified through stability and shelf-life studies, it is always wise to store the package properly. For example packages should not be stored near water, or wet spots, because Tyvek®, paper and some wraps can allow for the penetration of moisture of water through it, allowing for motile or water microbes to contaminate the product within the package. The typical recommended temperature for all sterile storage areas in hospitals of healthcare facilities is typically 24 °C. Such areas require at least four air exchanges per hour in a controlled relative humidity that does not exceed 70%.

Sterile items should typically be stored in hospitals on or in designated shelving, counters or carriages separate from non-sterile items. Sterile packaged items in hospitals should be stored properly. Some examples of good storage are:

- At least 45 cm below the ceiling (or level of a sprinkler head) because adequate space is needed for air circulation and to ensure the effectiveness of the sprinkler systems.
- At least 20-25 cm above the floor to prevent contamination during cleaning.
- At least 5 cm from the outside walls because of condensation that may form on the interior surfaces of outside walls.
- The bottom shelf should be solid or contain a physical barrier between the shelf and the floor.
- Heavy packages, e.g., instruments, should not be stacked due to the possibility of compression and package damage.
- Outside shipping containers and corrugated cardboard boxes are exposed to unknown and potentially high microbial contamination and should never be allowed in the sterile storage area.

- Wet packaging should not be stored and should be removed, because many of the medical device packages if wet could allow microbes to permeate the package material.

Sterilised products ought to be stored in a manner that preserves their integrity of the packaging material, seal or closure. Storage practices can be either date- or event-related. Although some facilities continue to date every sterilised package and use shelf-life practices (first in, first out), other facilities have switched to event-related practices. This approach recognises that the product should remain sterile until some event causes the item to become contaminated, (e.g., a package becomes torn or wet). The quality of the packaging material, the conditions under which items are stored and tran sported, and the amount that they are handled all affect the chances that the package and its contents will remain sterile. All packages containing sterile items should be inspected before use to verify barrier integrity and dryness. Any package that is breached, wet, torn, dropped on the floor, or damaged in any way should not be used. The products may be recleaned, packaged in a new wrap, pouch, container and so on and then sterilised again.

Even for event-related packaging, the date of sterilisation should be placed on the package. When multiple sterilisers are used in the facility, the steriliser used should also be indicated on the outside of the packaging material. This information can facilitate retrieval of processed items in the event of a sterility or sterilisation failure.

6.12 Transportation of Sterile Items

Sterile items should be transported in covered containers or enclosed carts with solid bottom shelves. If transported by hand, sterile packages that contain instrumentation and equipment and so on, should be kept parallel to the floor.

Contaminated or returned items should be contained and transported separately to the decontamination areas or soiled utility areas in containers, devices or carts labelled as biohazard and should be sent away as soon as possible. Dirty items should be separated from clean and sterile supplies.

Items that need to be kept moist in a transport container should be provided with a moist towel (water not saline) or using a foam, spray or gel product specifically intended for such use. Transport vehicles used for offsite transportation (motorised or manual) should be totally enclosed and leak free, and constructed of a material that allows for proper decontamination processes. Such transport vehicles should not be used for both contaminated infectious wastes and sterile uncontaminated packages of product.

6.13 Aseptic Packaging

Aseptic assembly or filling is a way of putting together sterilised parts, components, products, liquids or solutions within sterile packaging under a strictly controlled environment. Packaging must be pre-sterilised. Sterile packaging provides a means of assembling or filling products that cannot be terminally sterilised as a finished product in a package. Aseptic sterile packaging can incorporate different methods of terminal sterilisation (**Table 6.3**) such as chemical sterilisation (H_2O_2 vapour), dry heat, EO, irradiation, and steam sterilisation.

Within aseptic packaging systems, packaging is pre-sterilised by a variety of sterilisation methods (e.g., dry heat, H_2O_2, irradiation), in order to kill all microbes contained on the packaging before insertion of product or filling. Dry heat, in particular dry heat tunnels, are frequently used to pre-sterilise glassware (e.g., vials) prior to filling. The heat tunnel moves the product from a non-sterile area into a sterile environmental space or facility. Plasma has also been introduced into vials to inactivate microbes. H_2O_2 with concentrations up to 30% and 80 °C with contact times of up to 15 seconds, with or without a wetting agent have also been used. The final product must contain less than 0.5 ppm. Plastic tubes, flasks and containers may be pre-sterilised with UV light on carousels. UV lights have been used as a surface decontamination agent, sterilising tubes, vials, and flasks for special surfaces for cell cultures that would otherwise be adversely affected by other sterilising processes such as EO with toxic residues. In line Electron beam treatment may be another approach but uniformity of dose delivered to pre-formed containers must be provided. Economic considerations may inhibit the use of Electron beams for such an application. Combination of UV light irradiation and particle irradiation to sterilise the outer surface of packaging as well as the inside of a package for aseptic processing may be a useful approach, because UV light can sterilise surfaces but cannot be relied upon to sterilise through materials, while particle irradiation can penetrate.

Some aseptic packages may be made of several laminated layers of materials with aluminum to olefins to guarantee an O_2 barrier. Other processing materials may be paper and carton processing materials and an external moisture barrier (metallised surface) may improve the durability of the container under chilled conditions. Such a laminated aseptic package may be pre-sterilised with dry heat.

6.13.1 Purpose of Aseptic Sterile Packaging

The main purpose of aseptic sterile packaging is to ensure the safety of sterilised devices or healthcare materials and prevent them from being contaminated. The sterile pack must act as a barrier against bacteria and provide an aseptic or clean environment

for the equipment and devices until they are used in hospitals or by other healthcare providers. A major concern for all hospitals and other healthcare providers is the risk of infection, especially during surgical procedures, where there will be contact of instruments or devices with a patient's blood or tissues. One measure to control the risk of infection is to pre-clean the packs before opening them. A sterile state of devices is maintained through sterile packaging. It is very important to understand the design and material of the device in order to use a compatible packaging material.

6.13.2 Guidance on Aseptic Sterile Packaging

European standards for sterile packaging and international standards have been developed to deal with the requirements for sterile packaging in order to improve the safety and lessen the hazards to patients or the users during use.

EN 868-1 indicates the general requirements and test methods required for all the packaging of medical devices that are to be sterilised terminally. All the other standards in the EN 868 series provide information about the requirements for the commonly used materials and systems for sterile packaging.

ISO 11607 [1, 2] for packaging for terminally sterilised medical devices developed in 1997, gives the basic requirements for developing and validating the sterile packaging process and evaluating its performance.

ISO 13485 [22] and ISO 14644-1 [23] are among the standards typically applied to controlled environments or cleanrooms for heightened hygienic manufacture of packaging, prior to sterilisation.

Medical devices that are regarded as accessories, such as packaging materials that are applied in a medical environment to maintain the sterility of medical devices, must have a CE mark and be in compliance with the Medical Device Directive.

6.13.3 Sterile Packaging for Hospitals

The aseptic sterile package should be pre-sterilised properly in order to prevent contamination during aseptic assembly or processing, and subsequent nosocomial infections with healthcare facilities (e.g., hospitals). The safe and suitable choice of a packaging material for hospital application is typically a 'see through package'. Such packages are made of medical grade materials and are readily available in the form of pouches or tubing compatible with various biomaterials and devices in hospitals. The most commonly used sterilisation process for these materials are the steam and

gas sterilisation (see **Table 6.3**). The main advantage of this kind of packaging is for easy identification of and transparency of the biomaterial and/or devices and their condition. The packaging also acts as an barrier in preventing bacteria and other micro-organisms entering the system allowing a clean and aseptic environment to prevail until the package is opened.

6.13.4 Some Sources of Failure

The main reasons for the failure of pre-sterilised aseptic packaging are:

- Absorption of chemicals (e.g., H_2O_2 or EO) which may damage the material or leave toxic residues.
- Material damage (e.g., heat or irradiation) - some materials cannot tolerate high temperatures of either dry heat or moist (steam) heat.
- Other materials may be damaged by sterilising doses of irradiation (e.g., PP, PVC, glass and so on).

Some irradiated materials such as PE may give off offensive odours and these will need to escape after sterilisation. The polymers that most often exhibit post-irradiation odours are PE, PVC, and PU:

- When the packaging materials are exposed to high temperatures or heat (during sealing or the sterilisation process), most of the plastic materials undergo a process called crystallisation. This crystallisation allows the materials to undergo phase change and thereby making the materials harder and brittle. This may also occur with irradiation. In today's world, however, with the help of multi-layer film technology, high temperature and irradiation resistant materials can be obtained along with good sealing properties.
- Damage to the package during the opening - occurs due to low tensile strength of the package materials.

6.14 Opening of Packages – Asepsis and Identification

One of the weakest links in the sterility chain, lies in the opening of the package. It may be difficult to determine the degree of contamination that may occur during the opening of a package, but it must be easy to open the packaging and it must be user friendly. The product should be easy to remove and handle without introducing contamination.

One way to reduce contamination is to double package the entire product, so that the inner package surrounding the product will not have any exterior contamination on it when it is opened. This is often applied for implantable products. Another way is to provide a double seal to open the package, so that one seal is required to be opened before the second seal can be peeled. Peelable packages have been improved, so that there is no fibre tearing, such as with polymer packages.

Another approach is to create a controllable rupturing of seals with fibre packages such as paper. To minimise the 'bellows' effect of flexible package opening, which could cause an aerosol of contamination, rigid containers, trays and so on could be used, which may have better characteristics over flexible packages such as better physical/mechanical protection and means of opening which enable the contents to be removed without contamination.

Regardless of the type of package, the package must permit or allow ease of opening, and also allow for the checking of the product within the package by being transparent. Some laboratory simulations are to open the package to ensure that the properties of the processed package are the same as those of controls (not processed). In this simulation evidence of material failure are made such as tearing of lids, fibre generation of paper and delamination of plastic laminate during opening. Printed material(s) must be compared to the control to make certain that processing has not affected printing legibility, colour or clarity.

Identification of the product may be ensured through transparent materials as well as labelling.

It is important to label what the packaged product is. Using the right description is important so that both the handler and the user gets what they want. To ensure proper labelling, it is important to ensure outdated 'print' material is segretated and destroyed. This is true in hospitals as well as in manufacturing. Documentation in industrial manufacturing must accompany such activities. Labels printed in foreign languages must be proof read by someone familiar with the printed language. Small defects in printed material can alter meanings. When multiple languages are used on labels they must be given special attention. Using proper symbols and colours is also important.

6.15 Future of Sterile Packaging

With increasing concerns about environment, safety and healthcare waste management, hospitals and healthcare providers are expressing an interest in environmentally friendly packaging or so-called 'green packaging'. Nearly 70-75% of the hospitals'

wastes are municipal wastes such as paper, plastics from packaging and so on. Green packaging is made from green materials which are sourced from renewable materials or eco-friendly materials that can be re-used, recycled or destroyed without any harm to the environment. However, various restraints such as costs, regulatory concerns and lack of powerful demand from the consumers and end-users have restrained their use in the medical packaging industry. Recycling and sustainable eco-friendly packaging are ultimately going to be the key to the future of the healthcare and medical packaging industry. As time goes by, the regulations and legislation are going to become more stringent for environment and safety. Manufacturers will be pushed to look for novel eco-friendly and safe materials for packaging.

The ideal package for sterilisation, would be a completely sealed package, that would not allow any microbe to penetrate, as could possibly occur with a porous package. The ideal package would have a positive pressure compared to the ambient environment. Consequently, if the package were inflated, no contamination could go in the package. A sterilised package that is inflated and stays inflated could indicate that the package is sterile. One novel approach would be to create a sterilising environment in the package which would eventually become non-toxic, and yet not burst under negative pressure (e.g., in an airplane luggage carrier).

6.16 Safety, Reusability, Waste and Disposal

At times the biocompatibility considerations must be assessed for packaging, because it can have contact with the product, as well as the user, and the environment. It may be necessary for the package user to obtain Material Safety Data Sheet information for materials, inks, and dyes used in the packaging. In some cases, a risk assessment may indicate that the package itself will not need to be tested for biocompatibility, but the product normally needs to be evaluated after being in contact with the package. Besides chemical toxicants, particulates may be of consideration and concern, but this are principally of concern for the product that is implanted or in contact with sensitive tissues such as the eye.

Relative to disposal, consideration of ageing, toxicants and the environment must be assessed. The package may end up in the disposal stream with blood contaminated waste and, therefore, may require expensive treatment. This can lead to medical wastes that cannot be re-used or recycled. However, careful planning during package design/selection and at the point of use can often reduce the disposal issues to standard disposal and recycling issues. For example, the cost of separating laminated packaging films with multiple layers to meet recycling goals may end up being prohibitive for many manufacturers. Whenever possible, designers are using less material that cannot be re-used or recycled in order to reduce medical waste.

Some packages, for example, wraps and metal trays have been designed for 'repeated' use in the hospital environment. In these applications, devices can be placed within such a package and the presence of sterilant (steam, gas or vapour) can make them permeable and give it access to microbes, inactive them, and yet the package still maintains a sterile integrity after sterilisation, until reused.

If the cost of a product is less than the cost to clean and re-package, then disposal of a single use device completely avoids the risks associated with the transfer of contaminated blood, however, on the flip side of this are concerns related to package disposal. One of the final requirements of polymers used in packages relates to their disposal. Disposal of medical waste plastics presents the standard substantial challenges of solid waste disposal as well as the additional very sensitive challenge of infectious waste disposal. In the USA, it is estimated by the Environmental Protection Agency that approximately 20% of the nearly 1 billion pounds of hospital waste is plastics. While the cost of waste disposal in general is increasing, the cost of disposing of infectious waste may typically be 2-5 times or more the cost of standard solid waste disposal. Plastic waste is likely to continue to increase at a substantial rate, despite its environmental impact, due to the substantial value of minimising the risk of infectious diseases through the use of single use, disposable plastic products.

It is, therefore, incumbent on both the device manufacturer and the material vendors to participate in overall waste management strategies. Regulation in this activity has been seen around the world.

6.16.1 Waste Strategies

There are a number of waste strategies and these are covered in the next sections.

6.16.1.1 Source Reduction of Unnecessary Waste

Packaging has been the area with the greatest potential impact since it has the highest volume. Environmentally friendly polymers that are biodegradable may also have an impact. Reduction in use of overwraps can be helpful. Reusability of wraps, trays, and others can be beneficial (reduce source materials) in this respect.

6.16.1.2 Use of Reusable Packaging

Reusable wrap type packaging such as is used in hospitals will definitely reduce the cost of single use, polymer use. Thick paper or double paper may be used in place

of polymers if properly designed, and it can be biodegradable. Reusable solid plastic trays with single use lids, instead of flexible films may reduce costs, or reusable plastic trays with solid, reusable, breathable lids may be another solution. Reusable woven textile materials should be laundered between every use for rehydration. Re-sterilisation without relaundering may lead to superheating and could be a deterrent to achieving sterilisation. Over-drying, heat-pressing, and storage in areas of low humidity may also lead to superheating and sterilisation failure. When woven textiles are not rehydrated after sterilisation, and/or if repeated sterilisation is attempted, the textiles may absorb the available moisture present in the steam, thereby creating a dry or superheated steam effect.

Polymeric materials used in reusable packaging must be carefully selected to assure that repeated use, handling, cleaning and re-sterilisation does not adversely affect the functionality and safety of the device. The device must also be designed to assure that cleaning and re-sterilisation can be effective. There may be labelling claims and liability issues that may be considered. For example, some key concerns about material vendor liability were abated in 1998 in the US when biomaterial legislation HR 872 [24] was passed. This legislation stated, in part, that suppliers of raw materials and component parts, meeting defined criteria, could not be brought into product lawsuits against manufacturers, alleging harm caused by the products. This may lessen concerns about supplying materials for polymer packaging in the aftermath of high profile cases such as the silicone breast implant case that cost the silicone material; however, consideration of regulations and liabilities should always be a consideration.

6.16.1.3 Other Waste Strategies

Other waste strategies worth considering are:

- Recycling to extend life-cycles and as an incentive for an alternative to disposal.
- Incineration with effective emissions controls.
- Optimising landfill waste options, with biodegradable features.

The environment has a major impact on designing more environmentally efficient packaging that may increase the cost of production. In the increasingly cost-conscious environment of healthcare, this is not popular. This leads to the need for the material vendor, device manufacturer and the heath care facilities to work together toward cost-effective solutions.

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7 Statistics, Standards and Validation

Mark Twain, the American writer and humorist, once said there are three ways to lie - lies, damn lies, and statistics!

Sterilisation numbers and statistics do not lie, but the assumptions used to obtain and apply those numbers to statistics can. For example, it is typically assumed that biological indicators (BI) are more resistant than bioburden, but not always so in the presence of proteins and salts, biofilm accumulation, and attenuation of irradiation by some metals. It is typically assumed that microbes are inactivated logarithmically, but many are not. A natural bioburden consisting of many types of microbes very rarely demonstrates a singular logarithmic or first order straight line because a natural bioburden generally consists of multiple types of microbes with varying resistance. It is assumed that viruses are less resistant than microbes to irradiation, but this is not always true either. Bioburden between interval testing may exceed action limits. So let us question what we are not totally sure about.

The statistics of a validated sterilisation process are a significant consideration in the sterilisation processing of diagnostics, drugs, medical devices, and healthcare items today.

Sterilisation is differentiated from other techniques with lesser means of destroying or removing microbes. Terms and techniques such as disinfection, commercial sterilisation, sanitation, pasteurisation, decontamination and clean are not synonymous with true sterilisation, and to use them or apply them as such only leads to the abuse and misunderstanding of the meaning of sterilisation. Sterilisation is defined as a method of inactivating all viable micro-organisms at a selected probability.

Sterilisation statistics and validation are a formal and proceduralised approach to demonstrate that a sterilisation process is truly effective, efficacious and reproducible. It is typical to validate a sterilisation process, by monitoring the process with measurement testing (e.g., temperature, % relative humidity (RH), exposure time, pressure, chemical concentration or dosimetry), and challenging with a BI challenge, dosimeter, and/or performing bioburden, product sterility testing for various test conditions (e.g., sub-process, fractional, half cycle).

Sterilisation agents that predictably and reproducibly kill all micro-organisms from viruses to spores, are amazing 'magic bullets' but their use is not without limitations, precautions (safety), procedures, records, regulations and validations. This is not actually an easy task.

The sterilisation techniques need to be statistically sound, reproducible, and they need to be validated. A validation master plan, or other equivalent document, must be prepared and approved, prior to starting a validation. This typically must be initiated at the earliest practical point and must be reviewed and updated throughout the validation. The validation master plan must address all the relevant stages of the validation such as design qualification, installation qualification, operational qualification, performance qualification and statistics.

7.1 Statistics

Successful sterility assurance and validation is contingent upon adequate application of statistics to the sterilised product and the sterilisation process. To fully appreciate the role statistics plays in the sterility risk assessment and sterilisation validation endeavour, let us start from scratch and begin with the word, sterile.

7.1.1 Definition and Background

Sterile is defined in the dictionary as the complete freedom from all living viable organisms. The term sterile implies an all or nothing condition. All viable organisms are either killed or removed. In reality sterile is a probability function, a relative term. However, there is a tendency at times to mistakenly use sterile in the wrong context. For example, in early medicine, sterile meant to destroy only organisms causing disease. In the home, baby bottles boiled in water have been implied to be sterile, but in reality boiling in water for a short time would not sterilise all the microbes present.

Sterile must always be differentiated from lesser means of destroying or removing microbes. Terms and techniques such as antiseptics, decontamination, disinfection, germicidal, pasteurisation, sanitisation, and clean are not synonyms of sterile and to use them or apply them as such only leads to the abuse and misunderstanding of sterilisation and the term sterile. These terms have been discussed previously in **Healthcare Sterilisation: Introduction and Standard Practices, Volume 1, Chapter 3**.

Sterilisation is applicable to items that have a sterile claim and a significant need to be free of contamination, (e.g., during surgery, materials used to dress wounds, invasive drugs or devices). If a hospital product, device or drug is going to be delivered

invasively for more than 72 h, that product, device or drug should be sterile, have a low likelihood of contamination (e.g., 10^{-6}).

The actual number of sterilising techniques or methods recognised as capable of meeting the criteria of sterility without adversely affecting product quality is few. These are techniques that can be validated. Common traditional sterilisation methods used are:

- Dry heat (see **Healthcare Sterilisation: Introduction and Standard Practices, Volume 1, Tables 2.8** and **7.2**, and **Sections 7.1.8**, **7.1.9**, **7.2.1** and **7.2.2**).
- Ethylene oxide (EO) (see **Sections 7.1.8**, **7.1.9**, **7.2.1**, **7.2.3**, **7.2.3.2**, **Tables 7.2** and **7.7**).
- Filtration (aseptic processing) (see **Table 7.5**, **Sections 7.1.8** and **7.2.9**).
- Radiation (see **Sections 7.2.1** and **7.2.4**, **Tables 7.2**, **7.7**, **7.8** and **7.9**).
- Steam (see **Sections 7.1.8**, **7.1.9**, **7.2.1**, **7.2.3** and **7.2.3.1**, **Tables 7.2** and **7.7**).

There are a few other, newer, non-traditional sterilising agents that can be accepted and validated:

- Hydrogen peroxide (H_2O_2) (with plasma (see **Sections 7.2.1** and **7.2.5**, **Tables 7.2** and **7.6**).
- Ozone (O_3) (see **Sections 7.2.1** and **7.2.5** and **Table 7.6**).

And several novel sterilisation methods exist that may or may not be accepted:

- H_2O_2 vapour, (see **Sections 7.2.1** and **7.2.5** and **Table 7.6**).
- Chlorine dioxide (ClO_2), (see **Sections 7.2.1**, **7.2.5** and **7.2.6**).
- Glutaraldehyde (liquid sterilisation) (see **Sections 7.2.6** and **7.2.7**).
- Peracetic acid (PAA) (liquid sterilisation (see **Sections 7.2.6** and **7.2.7**).
- Supercritical carbon dioxide ($scCO_2$), (see **Sections 7.2.1** and **7.2.8** and **Table 7.6**).
- Oxides of nitrogen (e.g., nitrogen dioxide) (see **Sections 7.2.1** and **7.2.5** and **Table 7.6**).

These methods are not adequate for all specific applications. All sterilisation methods have their limitations. But all sterilising methods have one thing in common - they must remove or destroy all micro-organisms. If sterilisation is true to its definition, how do we know if a process has completely sterilised a product without evaluating every product? We start by performing a sterility test.

7.1.2 Determination of Sterility

To determine sterility, we must test for it, and we must know what sterile means. Sterile is defined as 100% freedom from all viable micro-organisms under testing conditions. Therefore when we test for sterility there must be no evidence of microbial growth, under appropriate and optimal growth conditions. In general there are a few basic ways to test for sterility (see **Table 7.1**):

1. Product sampling and product sterility testing;
2. The application and use of BI (indirect); and
3. Combined product and inoculated BI on product.

Table 7.1 Types of sterility testing	
Different methods of sterility testing	**Different tests or organisms within methods**
Product sterility - a direct indication	• Membrane filtration • Direct immersion • Product flush
Biological indicators - an indirect indication	*G. stearothermophilus* - moist heat, O_3, H_2O_2, ClO_2 (BI may be variable)
	Bacillus atrophaeus - dry heat, EO, ClO_2 Endotoxin indicator (3 log) (e.g., 10–100 ng *Escherichia coli* lipopolysaccharide) - dry heat depyrogenation
	Bacillus pumilus E601, ATCC 271421 - radiation for a NDA and so on
Combined - product and inoculated BI on product - provides for a direct correlation	Test and compare resistance from product to resistance of BI inoculated on product
ATCC: American Type Culture Collection NDA: New drug application	

BI may be an inoculated product, an inoculated paper strip or other carrier, or as spores enclosed within vials or containers, or part of a more sophisticated process challenge device (PCD) with spores on a specified material or device within a barrier to the sterilising agent.

In brief, product sterility testing is performed by placing a sample of the sterilised product in a suitable bacteriological recovery media and monitoring the bacterial

growth. Alternatively, a drug product or aqueous product is evaluated, by passing through a membrane, which retains the bacteria. The membranes are put in the recovery medium as previously described and evaluated. The product flush sterility test is reserved for products that have hollow tubes, such as transfusion and infusion assemblies, where immersion is impractical and where the fluid pathway is labelled as 'sterile'. This method is easy to perform and requires a modification of the fluid thioglycollate media (FTGM) for small lumen devices. The products are flushed with fluid D (peptone water containing polysorbate 80) and the eluate is membrane filtered and placed into FTGM and soybean casein digest medium (SCDM). This method is selectively used, where appropriate.

Product sterility testing methods for drugs and medical devices are generally described in the United States Pharmacopoeia (USP) [1], International Organization for Standardization (ISO) American Association of Medical Instrumentation (AAMI) standards, ISO 11737-2 [2], ISO 11137 [3-5]) and other compendia.

7.1.3 Biological Indicators

BI are another form of sterility evaluation. BI generally consist of spores of highly resistant microbes which are placed on or in the product load prior to sterilisation. These indicators generally have a high microbial population in excess of what is naturally occurring on the product. The combination of high microbial population and high resistance to a specific sterilisation process make these indicators a fairly reliable tool for the determination of sterility. However, the BI in itself cannot validate that the sterility level of a product is 1 in 10^{-6}. The BI by itself can only indicate that a specified treatment has been delivered. When developing and selecting a BI (internal challenge) and/or PCD one must consider the following:

- Internal challenge – the most difficult.
    - The most difficult to sterilise devices are seeded with a BI in the most resistant areas within a load configuration and most difficult to sterilise material and/or location(s) in the device.
- Process challenge device.
    - An external BI test pack that replaces the internal challenge device.
    - It should be an equal or more difficult challenge to the process than the internal challenge (see previous point).
    - It is typically developed using comparative resistance studies.

- Protocol or specification must detail the number and location of all samples in load.

The type of BI or challenge organisms (**Table 7.2**) are matched to the specific sterilisation method used.

Table 7.2 Types of sterilisation methods and corresponding biological indicators or challenge organisms

Sterilisation methods	**Different BI organisms**
Saturated steam, O_3, H_2O_2 or oxides of nitrogen (e.g., nitrogen dioxide)	*G. stearothermophilus* ATCC 7953
Saturated steam (industrial, drug)	*Clostridium sporogenes* PA 3679 or ATCC 11437
Saturated steam (industrial, drug)	*Cl. sporogenes* PA 3679 or ATCC 11437
Saturated steam (industrial, drug)	*Bacillus smithii* Nakamura, Blumenstock and Claus (ATC 51232) or *B. coagulans* Hammer *FRR B666* [SLS 37]
Saturated steam (industrial, drug)	*Bacillus subtilis 5230 or ATCC 35021*
ClO_2	*Bacillus atrophaeus ATCC 9372* may be more stable to ClO_2 than *G. stearothermophilus*
Dry heat and EO	*Bacillus atrophaeus* ATCC 9372 or NCTC 10073, not ATCC 6633
CO_2	*B. atrophaeus* (probably) 1A-M 1069, Institute of Molecular and Cellular Biosciences (Tokyo), *B. atrophaeus* ATCC 9372 - very resistant
Radiation	None, except for *B. pumilus E601* or ATCC 2142 in NDA*. (*Deinococcus radiodurans, Bacillus sphaericus,* some *Clostridium* and small viruses**)
Filtration	*Brevundimonas diminuta* ATCC 19146
Liquid chemicals (general), for EPA registration	*Bacillus subtilis* ATCC 19659 and *Cl. sporogenes* ATCC 3584 in the AOAC sporicidal test
Liquid PAA	*G. stearothermophilus*
Gaseous PAA	*Bacillus circulans* ATCC 61

*When applied to NDA or other regulatory approval.
** Some resistant microbes
Note: B. pumilus may be more resistant in the aqueous or anoxic state.
Note: B. pumilus SAFR-032, isolated at spacecraft assembly facilities of the NASA Jet Propulsion Laboratory, is difficult to kill with H_2O_2.
Proposed nanobacteria (which remain under investigation) may be more resistant to H_2O_2 and irradiation than other microbes.
Similar to the product sterility test, the BI or challenge (inoculum) is placed in an optimal bacteriological recovery medium and observed for growth.
AOAC: Association of Official Analytical Chemists
NASA: The National Aeronautics and Space Administration

7.1.4 Product Sterility

In product sterility testing of finished devices there is a statistical relationship between the sample size and the probability of passing an unsterile product, as described in **Healthcare Sterilisation: Introduction and Standard Practices, Volume 1, Table 1.1**.

For example, if a lot contained 3.4% contaminated product and 20 units were tested for sterility there is a 50% chance that no growth will occur and the lot will pass. If there was a 13.9% contamination, there is only a 5% chance that no growth will occur and the lot will pass. With 60 samples tested from a lot containing ~1.1% contamination, there is a 50% chance of not finding contamination. If the lot consisted of 10,000 units with 1.1% contamination, there are 110 units that may be contaminated, with a 50% chance that no contamination could have been detected.

Variations in statistics do exist, as the next table (**Table 7.3**) shows, for some probabilities of acceptance of lots from sterility testing with different sample sizes as compared with varying contamination rates on the samples.

Table 7.3 Some probabilities of acceptance* of lots with varying assumed degrees of contamination compared to sample sizes*****

Sample size***	Percentage of contamination in lot(s) (varying degrees of contamination)				
	0.1%** contaminated	0.5%	1%	5%	10%
10***	0.99 or 99%* Probability of acceptance without detection	0.96	0.91	0.60	0.34
20	0.98 or 98%	0.94	0.82	0.35	0.11
100	0.91 nor 91%	0.61	0.37	0.01	0.00

*: Probability of accepting the lot, for example 0.99% or 99% (see above).
**: Assumed degree of contamination, for example 0.1% (see above).
***: Sample sizes, for example, 10 (see above).

A sterility test on 20-100 samples is not even close to reliably measuring a failure rate of one out of 1,000,000 devices, or less devices with less contamination. It shows the futility of depending upon sampling for assurance of sterility of a lot or a test. The sterility sample may not even detect a low level contamination of a highly irradiation resistant microbe or pathogenic microbe.

What **Table 7.3** indicates is that even with a sterility sample of 100 under sub-processing conditions, a small number of samples may still have resistant microbes in

large product builds or lots such as greater than 1,000 units. Under such conditions, it may be worthwhile to perform an additional bioburden and/or sterility test. Knowing the bioburden may well be a better way of judging the appropriateness of a sterility test of a lot or a test.

Sampling should be performed three times on the same large production lot (e.g., random or non-random - beginning, middle, and end), if the bioburden and/or sterility testing can be performed without accidental contamination. Where accidental contamination may potentially occur in the bioburden or sterility test of a product, it is better to perform the test under a sterile isolation hood with a surface (only) sterilant agent on the unopened product package.

Table 7.3 shows that there may always be a greater likelihood of contamination surviving in larger lot sizes due to the number of possible higher contaminants left undetected and with lower rates of contamination.

A common statistical assumption is that all microbes die in a logarithmic order, however according to Rahn's logarithmic death model this only applies to 40% of the curves where a straight logarithmic line occurred. So while a sub-process sterility test, based upon imaginary bioburden D-values, with no positives exists, there may always be the possibility of a non-logarithmic situation of inactivation occurring. So it is always important to know your bioburden such as the types of microbes present, whether or not they form spores, environmental conditions (e.g., microbial clumping, anaerobes, facultative and micro-aerophilic), populations, probable bioburden resistance and so on. Selection of a predominant bioburden type or resistance provides a means of looking for the best fit, behaviour, and response to a sterilant.

It is important to remember that the natural bioburden is not static but extremely and inherently variable and physiologically will consist of different modes of growth, pre-spores, spores, dormant spores, young, stationary and old growth. Sometimes it will be pleomorphic genetically as well as morphologically altered. Bioburden can mutate, germinate, form capsules or films, desiccate, replicate or add additional deoxyribonucleic acid (DNA), be hydrated, be anoxic and so on. So judgment of the final sterility test results must extend beyond simple microbiological mathematical aspects (e.g., growth or no growth upon sterility testing).

Another problem, inherent in sterility testing is accidental contamination. When the sample size is increased to detect low-level contamination, the chance of accidental contamination will increase proportionally. Sterility testing depending upon the type of product to be sterilised generally requires careful aseptic manipulation and rigorous sterility techniques. For example if the process being evaluated or validated is radiation, this requires half of the type of sterility media (e.g., casein soybean digest

media, but typically not anaerobic media) (ISO 11737-2 [2] and 11137 [3-5]) as well as less product than stated in the USP [1] (e.g., AAMI ISO Techincal Information Report (TIR) 13409 [6], AAMI TIR 27 [7], AAMI TIR 33 [8] and so on) Consequently their chance of detecting different types of contamination (e.g., anaerobes, fungi, and yeasts) and percentage product contamination may be significantly less, particularly under large production loads (see **Table 7.3**). However, too much reliance on BI or minimal or limited product sterility testing as proof of sterility can be sometimes be misleading. For example, indigenous micro-organisms can sometimes exceed the resistance of BI or detection of the product sterility test used (e.g., *Pyronema domesticum, Propionibacterium,* anaerobes, facultative bacteria, thermophiles, some thermotolerant organisms and yeast). Studies of radiation resistance have not fully taken into consideration the possible effect of various factors (anti-oxidants, reducing agents, micro-aerophilic or facultative anaerobic organisms, or incubation temperature and environment, spore dormancy), which may influence the recovery of the surviving microbes. How can logarithmic death predictions be calculated if the presence of anaerobic or microaerophilic organisms are not even evaluated in many radiation validations - another erroneous assumption. Experimentally, anaerobic spores have been found to deviate significantly from the logarithmic order of death and tailing. Tailing results in high resistance, and non-logarithmic behaviour resulting in incalculable predictable probability of survivors. So if certain microbes (e.g., anaerobes or viruses) are not evaluated during the validation, then this deviation of the logarithmic order of death, or sterility is an extraneous assumption. Heterogenous resistance of microbes have been found. Some processes and product sterility tests are not able to either inactivate or detect small targeted viruses, prions and endotoxins (pyrogens).

Regardless of which sterility test is used, it is necessary to understand the bioburden and environment of microbes on products, and to understand the kinetics of microbial inactivation so that adequate and reasonable statistics can be applied in the design, development and validation of a sterilisation process or product to eliminate the concern of erroneously passing a non-sterile lot.

7.1.4.1 The Suitability and Qualification (Validation) of Sterility Testing of Product

Sterility testing of medical devices is required during radiation sterilisation validation for both gamma and electron beam irradiation. It does not use BI of greater resistance of bioburden. Rather, the irradiation sterilisation uses sterility testing as a direct measure of the adequacy of the sterilisation dose and parameters. Consequently, a knowledge and understanding of sterility testing and its limitation is very critical in terms of designing a radiation validation process. The need to provide adequate and

reliable sterility test data is an important quality control issue. Sterility testing can be a very tedious and manipulative process that must be performed by trained and qualified laboratory personnel. The investigation of sterility test failures is a further process that requires attention to environmental data as well as to many other factors including training and sampling difficulty.

In general, medical device and pharmaceutical sterility testing is an essential part of every sterilisation validation. Sterility testing is an extremely difficult process and technique that must be designed to eliminate false positive results, but it should also maximise the recovery of all microbes that need to be sterilised. False positive results are generally due to laboratory contamination from the testing environment or technician error, and are of significant concern. However, negative sterility test results can be obtained, despite high bioburden counts, or the presence of bioburden microbes that may not grow well in standard sterility test medium (which does not recover all microbes) is related to the limitations of the test medium, incubation period and environmental conditions. The environment must be designed to meet the requirements of viable microbial counts. Growth media used in sterility testing must be meticulously prepared and tested to ensure that it can support not only standard microbial growth, but all microbial growth. The most difficult to sterilise area(s) or most populated areas should be defined for each medical device or pharmaceutical product. Procedures for sampling, testing, and follow-up must be defined in the validation procedures.

One expects that product sterility testing will recover all microbes in order to satisfy the absolute definition of sterility or sterilisation. However, this is not necessarily true. There are many microbes including viruses that may not be recovered under a standard sterility test. For example, the 'Suitability Test' (or growth promotion test) and the 'Qualification (Validation) Test' [bacteriostasis and fungistasis test (B/F)] for sterility media is performed to confirm that each lot of growth (sterility) media used in the sterility test procedure will support the growth of less than 100 viable micro-organisms. If the media cannot support the growth of the indicator organisms, then the test fails. Note the media must support 100 viable microbes or less. Typically test laboratories will perform this test in excess of 10 microbes. Would or could the sterility test demonstrate growth with only 1-10 microbes? Not so with many microbes such as fastidious and slow growing organisms (e.g., *Enterococcus faecalis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium ulcerans, Mycobacterium avium, Mycobacterium kanasasii, Propionibacterium acnes, Peptostreptococcus*) which are not likely to be 'always' recovered in small numbers, and some proposed nanobacteria, which are assumed to be very small microbes that cannot be detected by standard sterility tests. Nanobacteria (which remain under investigation) may supposedly survive under extreme conditions, including radiation (e.g., >25 kGy and may require greater than 150 kGy) to inactivate); and even 11%

H_2O_2 may have no sterility effect on nanobacteria. It is recognised that calcium carbonate crystals, which may be related to nanobacteria, can make normal bacterial spores extremely resistant to most traditional sterilisation methods. But the means to sterilise microbes encapsulated in calcium carbonate crystals is available, and while potential concerns about their presence may be justified, without the means to cultivate and count them, it is impossible to attest to their complete absence, unless there are proven ways to inactivate or eliminate.

Furthermore, the previously mentioned bacteria (*Enterococcus*, *Mycobacterium, Propionibacterium* and so on) are not the standard microbes, used to test the suitability of the sterility media. A further qualification test is used to determine if the test sample will inhibit the growth of micro-organisms in the sterility test media. Stasis, in terms of microbiology, is defined as the inability of a microorganism to grow and proliferate in microbiological media. Media that is bacteriostatic does not necessarily kill bacteria; it simply may retard bacterial growth and proliferation. The qualification test (B/F) must be performed on each product prior and/or during sterility testing. This test determines if the media volumes are valid for the particular product. Some healthcare and medical products contain bacteriostatic and fungistatic compounds that may require special procedures and special media for testing. This test is similar to the suitability test described previously in **Section 7.1.4.1**, however, the product sample is placed in the media along with the microorganisms. Microbial growth in the presence of the test samples is compared to controls without test samples. If microbial growth is present in the sample and control containers, then the test is valid. The next step is to proceed to actual sterility testing. Suitability, qualification and sterility tests can be performed simultaneously. Sometimes the qualification test is performed on sterility test samples after they have been incubated and demonstrated no growth. *Note: Enterococcus, M. leprae, M. tuberculosis, M. bovis, M. ulcerans, M. avium, M. kanasasii* and *Propionibacterium,* are not used to qualify the standard sterility media for B/F testing. So when we say something has been tested and is sterile, without BI which would have greater resistance than those microbes not included in the test, then it (the term) may be lacking and used inappropriately.

For a sterility test to be valid, the technician must be trained (and certified) in how to detect growth during the incubation period. Typically growth is determined by observing the media, which is generally clear and transparent, against a light source. Turbid (cloudy) areas in the media are indicative of microbial growth. Once growth is detected, the suspect vessel is tested to confirm that the turbidity present is due to micro-organisms and not due to disintegration or precipitation of the sample. Sometimes samples produce turbidity because of particulate shedding or chemical reactions within the media. Once a suspect container has been tested, it should be returned to the incubator for the remainder of the incubation period. Samples that render the media turbid are typically transferred on day 14 of incubation and are

observed microscopically and then typically incubated for four more days, however, not all microbes will grow within four days of incubation, particularly slow growers. Positive growth samples require further processing such as identification and storage.

For every positive sterility test, the laboratory should perform an investigation to determine the validity of the positive growth. This investigation encompasses the following items and more:

- Clean room environmental test data.
- Media sterilisation records.
- Technician training records.
- The relative difficulty of the test procedure.
- Control data (open and closed media controls).
- Technician sampling data (microbial counts on gloves and/or garments post-testing).
- Environmental microbes.

The USP [1] allows for a retest of the product if persuasive evidence exists to show that the cause of the initial sterility failure was induced by the laboratory, handling, or the environment. Identification and speciation of recovered microbes and when it was recovered is a significant contributing factor to the final decision. If the first stage sterility test can be invalidated by the laboratory, then the USP allows for a second stage sterility testing. A second stage sterility testing requires that double the original number of samples is tested. The second stage test can be repeated if further evidence exists invalidating the test due to a laboratory error as noted previously.

A detailed investigation may uncover circumstantial evidence to support a final decision. It is recommended that sterilisation cycle data, environmental data, and bioburden data be reviewed prior to making any decision about releasing the product. It is recommended that medical device or pharmaceutical manufacturers qualify the test procedure with non-sterile samples. The probability of a false positive may be calculated, but it is more difficult to assess if the sterility test isolated (or recovered) all the microbes. The probability or potential of false positives may be based upon:

- Sample container diameter.
- The amount of time the container is left open.
- The room particulate count.

Despite a sterility test failing to demonstrate growth of all microbes, sterility testing should require high levels of knowledge of the bioburden, quality control, good laboratory practice, environment (aseptic clean room ISO Class 5 or better), good employee practices, and good assessment. It is essential that a meticulous technique be employed. Sterility testing is an integral part of sterilisation validation as well as a routine quality control. False positive results, as well as negative sterility results (despite high and unusual bioburden) may occur and should be considered and thought out.

7.1.5 Kinetics of Microbial Inactivation

To evaluate a sterilising agent or a product sterilisation, an estimation and measurement of survivors to the agent and product must be made. Knowledge of BI and kinetics of microbial inactivation is required. An evaluation is generally done by performing sterility tests after a series of incremental exposures to the sterilising agent and product. Results will vary depending upon the initial 'natural' bioburden (which may be for example, desiccated, hydrated, sporulated, encapsulated, or enclosed in organic matter or biofilm), the mixture and state of the population, the BI used, environmental conditions (e.g., biofilm accumulation, presence of protein and salts), product configuration (e.g., mated surfaces, lumen length and diameter, restricted flow, moisture and/or gas absorption, metal attenuation of irradiation), and associated sterilising parameters of the specific sterilising agent.

The dynamics of microbial inactivation reveals, in general, that microbes are destroyed in a logarithmic or first order rate. An old explanation of this phenomenon is that the logarithmic order of death is due to an expression of a monomolecular reaction of protein penetration or damage (e.g., one DNA gene) essential to reproduction. It should be realised that the microbial death observed is really a failure of the microbe to reproduce (not necessarily its death) when placed in a favourable environmental and optimal recovery medium. What are favorable conditions can vary. For example, many *Bacillus* species including *Bacillus anthracis* are typically cultured to grow well at mesophilic (30-35 °C) temperatures, however, they could be environmentally adapted to grow at thermotolerant (45-55 °C) temperatures.

The statistics of sterilisation are based on the assumption that all micro-organisms die or are inactivated in a logarithmic or first order reaction rate. This assumption is reasonably true under laboratory or pure environmental conditions. However, exceptions exist. Steam sterilisation characteristically does kill in a logarithmic way with some exceptions (e.g., heat activation, an initial shoulder or hump in a straight logarithmic curve). Radiation has an activation shoulder or initial lag with *B. pumilus* (E601) ATCC 27142 before a logarithmic inactivation decline; but tailing

with *Clostridium* anaerobic spores, after a logarithmic inactivation decline. Dry heat sterilisation may exhibit tailing (non-logarithmic decline) with high populations (e.g., greater than 10^3). Where tailing or other non-logarithmic behaviour is exhibited, possibly a sterility assurance level (SAL) greater than 10^{-6} should be applied, such as 10^{-9} or other 'improved' mathematical approach(es) should be applied.

In reality, most 'natural' bioburden consists of mixed cultures, and micro-organisms in these mixed cultures are in various stages of growth from haploid, diploid DNA, endospores, dormant spores, vegetative stage, encapsulation, mycelium, mould, fungi, virus, aerobic, anaerobic, and micro-aerophilic, to within an anoxic condition, which naturally results in non-logarithmic behaviour - so a logarithmic demonstration of inactivation is not likely to be demonstrated. However, the logarithmic death phenomenon is commonly exploited to predict the probability of survivors.

Whether the logarithmic phenomena is or is not, an accurate explanation is not important only that the kinetics provides a practical way to compute microbial inactivation values, to exploit and to draw conclusions independently of complications so that statistics can tell us if we potentially have a reliable, sterilised product.

The commonly used and recognised mathematical expression of microbial inactivation is the decimal reduction value, commonly referred to as the decimal reduction value (D-value (D_{10} value)).

7.1.6 Decimal Reduction Value

The D-value is the backbone of sterilisation statistics for EO, radiation, steam, dry heat, O_3, ClO_2, and H_2O_2/plasma sterilisation. However, a log reduction value or log removal value (LRV) is used, particularly for evaluating microbial filters.

The LRV can also be used for determining the inactivation factors by subtracting the log of the bioburden from the LRV.

The D-value is defined as the time or dose to reduce a bacterial population by one logarithm or by 90%, to a known sterilising condition, specified for each sterilisation method. A simple mathematical description (Stumbo equation) of the D-value is:

$$D - \text{value} = \frac{\text{Exposure time or dose}}{\text{Log } N_o - \log N_t} \quad (7.1)$$

Where:

- Time or dose is typically an incremental or sub-exposure of a sterilising agent that allows us to have survivors.
- N_o is the initial bacterial/bioburden or spore population prior to exposure to or treatment with the sterilising agent.
- N_t is the population surviving after exposure to the sterilising agent.

The D-value provides a characterisation of the resistance of a particular microbial population to a sterilisation method. Sometimes it becomes difficult to determine a D-value because the microbial population is heterogeneous, the population and resistance is extremely low, and the indigenous population does not follow a perfect logarithmic order of death. In many cases it is easier to perform a D-value for a particular process of bacterial spore populations used in BI because they can be prepared with high and homogeneous population(s), have a high resistance to the sterilising agent and demonstrate an ideal D-value curve to the sterilising agent.

Ideally the D-value allows us to measure, evaluate, and estimate the effectiveness of the specified sterilising condition. For example, as progressively greater sterilising time or exposure is tried, higher levels of the bacterial population are proportionally and logarithmically destroyed. For example if a sterilising process reduces an initial bacteria (spore) population of 1,000 by 90% in x min to 100 surviving organisms, then in a 2x time we would expect a 99% reduction of the initial population to leave only 10 organisms and so with a 3x time, we would anticipate a 99.99% reduction of the initial population to leave only 1 survivor. As we continue we can begin to extrapolate into areas where no microbial survivors can be detected and we can calculate probabilities of survivors occurring at lengthening exposure times.

Up to this point the definition of sterilisation has been the complete destruction of all microbes, however, in reality the term can never be absolute or 100% complete because a certain probability of survivor will always exist due to the logarithmic order of death that occurs with microbial inactivation.

7.1.7 Probability of Survivor (or Sterility Assurance Level)

The determination and estimation of a level of probability of survivor is a useful statistical tool because it permits us to design and validate sterilisation processes. A mathematical formula for determining the probability of survivor is:

$$N_t = \text{Log}^{-1}\left(\text{Log } N_o - \frac{\text{Exposure time or dose}}{\text{D} - \text{value}}\right) \quad (7.2)$$

Where:

- N_t is the probability of survivor at a given sterilising exposure or dose.
- N_0 is the initial product bioburden or BI count at zero time of exposure.
- Exposure time or dose is the sterilisation exposure time or dose delivered to the product bioburden or BI.
- D-value is the time to destroy 1 log or 90% of the product bioburden or BI population.

To estimate or design a sterilisation process it is necessary to know what level of probability of survivor is needed.

In general acceptable levels of probability of survivors vary depending upon the process and the particularly the type of product to be sterilised. A product containing a drug or food that can support microbial growth and has a low sensitivity to antibiotics is typically given a higher standard than that which does not (10^{-11} *versus* 10^{-6}). And a medical device product that is invasive requires a higher standard than one that does not (e.g., 10^{-6} *versus* 10^{-3}, topical). Some possible examples of probability of survivors for various sterilised product, procedures are shown in **Table 7.4**.

Table 7.4 Different probabilities of survivors for different product types

Type of product	**Probability of assurance of sterility**
Canned chicken soup[1]	10^{-11}
Large volume parenterals[2]	10^{-6}
Invasive medical devices	10^{-6}
Topical medical devices	10^{-3}
Small volume parenteral[3]	10^{-3}
Laparoscopic instruments[4]	10^{-2}
Limits of USP sterility test[5]	$10^{-1.2}$

[1] Sterilised food to prevent botulism - 12 logs inactivation, with 10 organisms or 1 log bioburden with a 10^{-11} SAL
[2] Sterilised parenteral solutions - 8 logs inactivation, based on 2 logs of initial bioburden or spores, with a 10^{-6} SAL
[3] Sterile fill, with an assumed SAL of 10^{-3}.
[4] Sterilised with liquid chemicals, not within a terminal package or container.
[5] With 95% confidence, with 20 samples, but with two sterility media (some sterilisation methods may use only one sterility type media for sub-processing).

A sterilisation process can be designed once the level of probability of a survivor that is required for a particular product and process/product is determined or known.

7.1.8 Design of a Sterilisation Process

Design qualification (validation) is a process that ensures that quality is built into the design of the sterilisation process. In general, all sterilisation processes have their limitations, for example, heat may distort and melt certain plastics, but may be compatible with many drugs. EO can sterilise many plastics, but cannot typically sterilise liquids and it leaves residues. Radiation may damage some electronics, some plastics, and drugs, but can sterilise many polymers and very dense materials. Low dry heat may be used to sterilise silicone prostheses and electronics, but may damage many plastics and heat sensitive materials. It must be recognised that a variety of factors (e.g., availability, cost, compatibility, disposability, ease of control and monitoring, environmental concerns, lethality, regulatory and reusability) must be carefully considered for each sterilisation method selected, in order to achieve reproducible and repeatable processes without adversely affecting the product quality and the material's chemical properties and biocompatibilities. Not to be overlooked in the design of a sterilisation process, is biocompatibility (**Section 7.1.8.1**) of the material, polymer product(s) or the items to be sterilised.

7.1.8.1 Biocompatibility

What may physically/chemically appear to be a compatible polymer may not be biocompatible. A polymer listing for a specific sterilisation method may not be an indication that the polymer is compatible biologically. Polymer degradation (biological) and failure may occur individually with some polymers. It is the responsibility of the product manufacturer to determine the suitability and biocompatibility of the polymer for its specific application. *Note:* The presence of additives, plasticisers and stabilisers can significantly affect the stability properties of many polymers, including their suitability for a specific sterilisation method. Additionally, under some conditions a material that is generally thought to be compatible with a technique will not be compatible when evaluated under another condition.

Material selection must meet the stringent requirements of ISO 10993-1 [9]. The materials are tested after exposure to the sterilisation method. Consequently, selecting a sterilisation method that is biocompatible and physico-chemically compatible with a material is key. The biological testing of the polymer is dependent on the intended contact duration. Body contact polymers are characterised according to their

surface contact, how they communicate with the external world, and where they are implanted. Implanted polymers have the most stringent requirements.

7.1.8.2 Pre-qualification Selection of a Sterilisation Method

There are always trade-offs when pre-selecting a method of sterilisation based on the acceptable material's inherent properties for sterilisation. Some pre-qualification steps to consider when pre-selecting a sterilisation method are:

- Identify sterilisation method(s) that appear compatible with product, design, packaging, materials and polymers.
- List alternatives: Cost, in-house *versus* contract, regulatory issues, local codes and environmental regulations.
- Perform feasibility studies to determine gross compatibility (may include biocompatibility) with the pre-selected processes.
- Perform detailed pre-validation studies to demonstrate product, component, package compatibility with the pre-selected process and attainment of pre-required SAL.
- Based upon the previous steps, select the most suitable pre-sterilisation method.
- Availability or sufficient time to develop and gain approval of a specific sterilisation process.
- Volume of product required to be sterilised.
- Environmental issues.
- Packaging or non-packaging requirements.
- Stability or time of use requirement(s).
- Clinical use of the product to be sterilised.

7.1.8.3 A Simple Way to Design a Process Mathematically

There are several ways to design a sterilisation process statistically, to improve product quality and material's compatibilities.

A 'simplified' mathematical expression for designing a sterilisation process is as follows:

$$\text{Exposure time or dose} = D_v(\text{Log } N_o - \text{Log } 10^{-x}) \quad (7.3)$$

Where:

- Exposure time or dose varies depending upon D_v, N_0, and probability, 10^{-x}.
- D_v is the time or dose to destroy 1 log or 90% of a product bioburden, BI or PCD.
- N_0 is the initial product bioburden, PCD or BI challenge population.
- 10^{-x} is the probability of a survivor after exposure to a specified parameter.

The design of a sterilisation process may be approached statistically by other means. For example, a process may be established on the basis of the number logs or microbial inactivation desired or required. Some log levels, which have been suggested are given in **Table 7.5**.

Table 7.5 Criteria of microbial log reductions by different products

Product type	Microbial log reduction criteria
Sterilised food to prevent botulism or an overkill process[1]	12 logs[1]
Sterilised parenteral solutions[2]	8 logs or F_o of 8[2]
Microbial challenge to filters	7 logs/cm^2 of filter
Sterilised devices based upon bioburden[3]	6 logs + log bioburden[3]
Topical devices based upon bioburden[4]	3 logs + log bioburden[4]

[1] 12 logs inactivation by steam is required for botulism, but 12 logs is also typical overkill where BI is 6 logs and an additional probability of a survivor is 6 logs.
[2] 8 logs inactivation by steam is applied where there is an initial population of 100 organisms or less, or 2 logs or less.
[3] 6 logs is the additional probability of survivor for an invasive medical device.
[4] 3 logs is the additional probability of survivor for a topical medical device.
F_o: Heat lethality time at 121 °C

To apply log levels to the design of the sterilisation process, the following simplified mathematical equation is used:

$$E(TCDT) = n(D_v)$$
or (7.4)
$$F_{o(bio)} = D_{121}(\text{Log } N_o - \text{Log } N_t)$$

Where:

- E is the sterilising exposure time or dose at given sterilising parameters, heat temperature, process parameters, or irradiation source conditions (dose).
- TCDT is thermal chemical death time (e.g., EO) or F_{bio}.
- F_{bio} is the notation for thermal exposure equivalent to moist heat at 121 °C.
- n is the number of logs inactivation required or desired - n may equal ($\text{Log } N_o - \text{Log } N_t$).
- or as specified in **Table 7.5**.
- D_v may be the D-value at the specified sterilisation parameters for dry heat, H_2O_2, irradiation, O_3 or EO.
- D_{121} is the D-value notation for death by heat at 121 °C.

Besides designing a sterilisation process on the basis of a desired level of the probability of a survivor or the required log reduction, a sterilisation process can be designed and validated on the basis of an overkill approach or a bioburden approach. The overkill approach is based on establishing a sterilisation process with the use of a BI where spore populations may typically range between 10^4 to 10^6 with a probability of a survivor of 10^{-6}. Prior to AAMI/ISO guidelines the common overkill approach to radiation sterilisation was the use of a minimum radiation dose of 25 kGy, which implied a 12-15 log reduction of *B. pumilus* E601. The traditional overkill approach to steam sterilisation is 12-15 min at 121 °C which implied a 12-15 log reduction of *G. stearothermophilus* spores. However, with a mesophilic or disease causing spore former with only a D-value of 30 seconds, this thermophilic log reduction may be equivalent to a mesophilic 24-30 log reduction. Consequently a typical hospital steam sterilisation process may in reality have an extraordinarily higher astronomical spore inactivation level or sterility assurance, compared to that from industrial or irradiation sterilisation.

7.1.8.4 Pre-validation Product Qualification

During design qualification (validation), pre-validation studies can be conducted using finished products made during pre-validation evaluations and will satisfy

the need for product performance qualification during formal validation. Design qualification should ensure that devices and products conform to defined user needs and intended uses and should include testing production units under actual or simulated use conditions. Original designs and design changes are subject to design control requirements. The results of design qualification are subject to review under the design control review requirements.

The purpose of design product qualification is to demonstrate that the process has not adversely affected the finished product and that the product meets its pre-determined specifications and quality attributes. Product performance qualification and design qualification of the initial finished devices are closely related. According to the design control requirements, design qualification should be performed under defined operating conditions on initial production units, lots, or batches, or their equivalents. Products used for design qualification should be manufactured using the same production equipment, methods and procedures that will be used in routine production. Otherwise, the product used for design qualification may not be representative of production units and cannot be used as evidence that the manufacturing process will produce a product that meets pre-determined specifications and quality attributes.

7.1.9 Other Methods of Microbiological Performance Qualification

7.1.9.1 Bioburden Approach

With the bioburden approach the design of a sterilisation process is established or verified on actual bioburden count and resistance.

The most used example of this bioburden approach has been with radiation, where AAMI ISO has published guidelines to establish the radiation dose based upon computerised bioburden population model counts and resistance. From these theoretical models radiation doses as low as 11 kGy can be established compared to the minimum dose of an overkill approach of 25 kGy (AAMI ISO 11137 Method 2 [10], other overkill methods were AAMI ISO TIR 13409 [6], AAMI TIR 27 [7]).

These validation studies must of necessity be done on product samples prepared under actual manufacturing conditions, environment and exposed to the sterilisation method under its final packaging and loading configuration.

Similarly, the bioburden approach can be performed under other sterilisation approaches [e.g., SO 11135-1 [11], ISO 17665-1 [12] and ISO 17665-2 [13]), by

performing bioburden per ISO 11737-1 [14], selecting the most resistant strain to the method, and determine its D-value by a fractional negative (e.g., Holcomb-Spearman-Karber method, or Stumbo-Cochran procedure). Once the D-value is determined with 3-4 fractional cycles, final qualification can be finished with at least two or three half cycles, in addition to the fractional cycles. Further detail can be obtained in ISO 11138 [15] and/or ISO 14161 [16], and ISO 11135-1 [11] or ISO 17665-1 [12].

7.1.9.2 Survivor Curve Construction

The lethality of the sterilisation cycle is determined by construction of a survivor curve using direct enumeration of survivors. In this case, at least five points using graded exposure times to EO, with all other process parameters except time remaining constant, should be included on the survivor curve. The initial count (i.e., the time zero on the survivor curve) should be determined on BI exposed to all stages prior to a sterilant injection.

At the completion of each graded sterilisation cycle, the BI, PCD or resistant bioburden challenge is removed from the load after a minimum aeration time or post-processing time. BI, PCD, or resistant bioburden challenge should be refrigerated until shipment and should be forwarded to the testing laboratory in a cooler with a cooling media. These BI, PCD, or resistant bioburdens should be put on test as soon as possible, because delays can result in further reduction of the treated spores or microbes, leading to erroneous conclusions.

A test laboratory performs a population enumeration on each PCD, BI or resistant bioburden after it is removed. A population enumeration may typically be performed for example, as follows:

1. Aseptically transfer each BI, removed from the protective glassine envelope (if it still remains) or other carrier, into a separate sterile dilution tube. For example, a dilution tube is a laboratory tube, usually 20 mm volume, that has a screw cap with a Teflon® (DuPont) liner.

2. Add 4-6 sterile glass beads and 10 ml of sterile water to the dilution tube. This is the starting (10:1) dilution tube.

3. Allow the BI to soften for 10-15 min or up to 2 h if refrigerated.

4. Use a vortex mixer, or other means (extraction, maceration, elution, dilution, filtration) to remove microbes from the BI. For example, the BI can be macerated into single fibres and until no clumps remain. The use of a blender for maceration

is not recommended because of the additional kinetic energy that it imparts upon the PCD, BI or resistant bioburden challenge.

5. Add an extra 5 ml of sterile water to the tube to dilute out the BI fibres and to avoid clogging during the serial dilutions. Using a vortex mixer or manually shaking, mix the dilution tube well.

6. Perform five serial dilutions by transferring 1 ml, with a pipette, from the current dilution tube into a new dilution tube containing 9 ml of sterile water. This will create the 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions.

7. Heat shock any dilutions that will be tested. Heat shocking is performed by placing the dilution tubes into a hot water bath that has been equilibrated at 80-85 °C. Some spores (e.g., *B. atrophaeus*) can be heat shocked at 65-70 °C. The tubes should be left in the hot water bath for 10 min at 80 °C or longer at lower temperatures. The temperature is measured from a control dilution tube with a thermometer in it to prevent contamination of the test tube.

8. Immediately remove the dilution tubes from the hot water bath and place into a chilled water bath.

9. Create duplicate pour plates for each dilution level. For example, a pour plate is created by transferring 1.5 ml of the dilution (well mixed) into a petri dish, pouring soybean casein digest agar or trypicase soy agar (TSA) agar into the petri dish until it forms a continuous layer, swirling the petri dish 15-20 times to disperse any organisms evenly throughout the plate, and allowing the agar to harden.

10. Invert the pour plate and incubate it for 48 h at 30-35 °C or 35-37 °C for *B. atrophaeus*, or at 55-60 °C for *G. stearothermophilus*.

11. Count any colonies that appear and calculate the population of organisms that were present on the BI at each exposure period.

12. Multiply the count by the dilution factor to estimate the total count.

Once the population of each BI is determined, a survivor curve is constructed using correlation techniques to plot the linear regression of the survivors *versus* the EO exposure time. The slope of the regression line is then used to estimate the D-value of the microbial population of the PCD.

The D-value multiplied by 6 (for a 6-log reduction), plus 1 or 2 additional log for no survival should be the minimum amount of EO or steam exposure time required for a successful half cycle. The minimum EO or steam exposure time required for a successful full cycle should be the D-value multiplied by 12 (for a 12-log reduction),

or double the half cycle exposure. In practice, when using a D-value study to calculate the half or full cycle EO exposure time, an additional safety factor is added to the exposure time, to ensure no survivals from the BI. For example:

$$\text{Half cycle exposure time} = D_v(\text{Log } N_o + \text{Lg } n(\text{number of BI/PCD}) + 1\text{Log})$$
or
$$\text{Half cycle exposure time} = D_v(\text{Log } N_o + 2 \text{ Log}) \quad (7.5)$$

Where:

- Half cycle is the exposure time to typically inactivate 10^6 spore population on all BI or PCD with some safety factor (e.g., 90% or 99%).
- N_0 is the initial BI spore population.
- n is the number of BI or PCD that all need to be inactivated.
- 1 log = 90%.
- 2 log = 99%.

The resulting data enables one to calculate the time of exposure to EO required to achieve a particular probability of survival of the test organism, such as 10^{-6} on an extended survivor curve. For example, the starting population (N_0) is used as the first point on the Y-intercept of the survivor curve line, followed by the four subsequent graded exposure times. The starting population should be determined using BI that have been exposed to all stages of the process prior to sterilant injection.

7.1.10 Fraction-Negative Approach

Indicators for sterilisation are exposed to graded exposures for the sterilant with all parameters except time remaining constant. After exposure, the test samples are assayed by direct immersion into an appropriate culture medium. A minimum of 5 exposures should be performed, including:

- At least one set of samples in which all tested samples show growth.
- At least two sets in which a fraction of the samples show growth (quantal region).
- At least two sets of samples in which no growth is observed.

- The D-value can be calculated from the results obtained. The exposure time required to achieve a specified probability of the survival of the test organism should be calculated from this D-value.

The indicators can be BI, PCD or resistant bioburden, which are subjected to a time graded exposure with other parameters of a sterilisation method remaining constant. After exposure, the test samples (BI, PCD or resistant bioburden) are assayed by direct immersion into an appropriate culture media. The samples are scored or averaged by the proportion of the samples showing growth or no growth after incubation. D-values can be calculated. Further details are provided in ISO 11138 [15] or ISO 14161 [16]. The Holcomb-Spearman-Karber procedure requires a minimum of five exposure conditions covering at least one set of samples in which all test samples demonstrate growth, at least two sets of samples in which samples show a fraction of growth, and at least two samples in which no growth is observed. Testing is performed as described next.

At the completion of the sterilisation cycle, the BI, and PCD are removed from the load after the minimum aeration time or post-processing period. The BI, PCD, resistant bioburden challenge should be tested as soon as possible, or they should be refrigerated until shipment to a test laboratory and should be forwarded to the testing laboratory in a cooler with cooling media.

Upon receipt, the test laboratory performs a PCD, BI, or bioburden sterility test on each sample after it is removed from the load. A BI, PCD, or bioburden challenge sterility test is performed as follows:

- Aseptically transfer each BI, PCD or bioburden challenge, from whatever the protective item it is within, into a sterile tube containing 30 ml of optimal media (e.g., SCDM for *B. atrophaeus*).

- Incubate the tubes for seven days at the optimal temperature of the BI, PCD, or resistant bioburden challenge (e.g., 30-35 °C, for *B. atrophaeus*).

- At the end of seven days, check the tubes for any signs of growth. Further incubation may be attempted to look for slow growth.

- Record the number of growths and no-growths for each run.

When the interval between the EO exposure times and the number of replicates at each exposure is a constant, n, the Spearman-Karber equation is typically used to calculate the D-value.

Exposure time to inactivate the 10^{-6} SAL can be determined on the basis of the D-value obtained in this procedure. For example:

$$\text{Exposure} = \text{Log } N_o + \text{Log } n + \text{Log } 10^6 \tag{7.6}$$

7.1.10.1 The Stumbo-Murphy-Cochran Procedure

When the interval between the EO exposure times or the number of replicates at each exposure is not constant, then the Stumbo-Murphy-Cochran equation is typically used to calculate the D-value.

The formula for the Stumbo-Murphy-Cochran procedure requires one result in the fraction-negative range consisting of time (t), the number of units negative for growth (r), the number of replicates (n), at one exposure time within the fraction-negative range, and the initial number of micro-organisms per replicate (N_0).

The formula for the Stumbo-Murphy-Cochran procedure is:

$$D = \frac{\text{Exposure time}}{\text{Log } N_0 - \log N_t} \tag{7.7}$$

Where:

- Exposure time is the time to demonstrate 1 log or more of spores on a BI or PCD or bioburden on a product.
- N_0 is the initial spore population or bioburden population.
- N_t is the number of spores or bioburden surviving after exposure.

N_t can be determined by counting the number of spores or bioburden directly on the samples or by the Halvorsen-Zieglar (most probably number) equation where N_t = log n/r where n is the number of total samples evaluated, and r is the number of sterile samples.

To obtain valid data using this procedure as per ISO 11138-1 [17] the D-value needs to be calculated as the average of at least ‘three’ runs in the fraction-negative range in order to confirm reproducibility. For further guidance, see ISO 14161 [16].

The exposure time required to inactivate 10^{-6} SAL can be determined on the basis of the D-value obtained in this procedure, for example:

$$\text{Exposure (process time)} = \text{D} - \text{value} (+\ 2\ \text{SD})\ (\text{Log N}_0 + \text{Log n} + \text{Log } 1/10^{-6})$$

$$\text{Half cycle} = \frac{\text{Exposure (Process time)}}{2} \tag{7.8}$$

Where:

- The D-value is the decimal reduction value of BI, PCD, bioburden.
- SD is the standard deviation of the D-value that may be applied, if significant.
- N_o is the initial spore number of the bioburden population used in the challenge.
- n is the number samples of BI, PCD, an/or product that will be applied to the cycle run.

$$\text{Sub}-\text{process or fractional cycl} = < \text{half cycle exposure time} \tag{7.9}$$

7.1.11 The Overkill Approach or Method

This method involves determination of the minimum time of exposure to dry heat, EO, and moist heat with all other process parameters except time remaining constant. In this method there are no survivors, after a half cycle exposure. The purpose of the half cycle 'overkill' method is to demonstrate a 6 log spore reduction at half the time of the proposed routine cycle, therefore showing that at the proposed routine exposure, the BI will be reduced to a 12 log spore reduction.

Two further experiments (half cycle runs) should be performed to confirm the minimum time, for demonstration of three consecutive runs with no survivors. All should show no growth from the BI, PCD, or resistant bioburden challenge. The specified (full cycle) exposure time should be at least double this minimum time. A cycle of short duration from which survivors can be recovered should also be run to demonstrate the adequacy of the recovery technique.

An overkill approach involves using the most resistant microbe (typically spores) to a sterilisation process or method at a population typically of 10^6. In general, the overkill approach assumes the most resistant spore is more resistant than any bioburden organism. This is generally true with moist heat sterilisation using *G. stearothermophilus*, however, the overkill approach in other methods (e.g., EO, H_2O_2, O_3) may not have the safety factor (e.g., a significantly greater spore resistance than bioburden resistance), that moist heat has.

Typically, the sequence for an overkill method, is to run initially bioburden and BI population, then a sub-process cycle (less than half cycle), to demonstrate recovery of the BI and calculate a D-value, for information, and then followed by three consecutive half cycles, and one to three full cycles. One full cycle may be acceptable, if the three half cycles can demonstrate reproducibility of a critical cycle parameter. The sequence and detail of the overkill method proceeds as follows:

- Perform initial bioburden and BI population.
- Then run sub-process (one or more), to demonstrate recovery.
- Followed by three half cycles.
- Then to verify complete process reproducibility run 1-3 full cycles. *Note*: only one full cycle is needed if the previous three half cycles are run consecutively at the same cycle parameters, as the full cycle (except exposure time).

A modified approach of either the overkill or bioburden approach has been the sterilisation of parenteral solution where an equivalent time to sterilise at 121 °C for 3.5 to 8 min has been accepted with sterilising temperatures of only 105-115 °C that is compatible with many parenteral drug solutions. Similarly, spacecraft sterilisation has demonstrated using dry heat sterilisation parameters to as low as 105-135 °C for 12 or more hours. In these sterilisation processes, time is established by integrating heat lethality during heat-up, exposure, and cool-down times at or less than 121 °C or whatever temperature is applied. For example, where lethality has been adjusted to this temperature through the statistical use of the temperature difference required to cause a 120-fold change in the D-value (Z-values). The Z-value is defined as the temperature difference required to cause a 10-fold change in the D-value. The Z-value may be derived from the following equation:

$$z = \frac{T_x - T_0}{\text{Log } D_o - \log D_x} \tag{7.10}$$

Where:

- D_0 is the D-value at the initial temperature, T_0.
- D_x is the D-value at a later temperature, T_x.

A typical Z-value of *G. stearothermophilus* spores, for example, is 10 °C, for moist heat. However, the Z-value for dry heat with *B. atrophaeus* will be much greater,

such as >20 °C. The application of the Z-value, to determine a F_o value, for moist heat is typically represented as follows:

$$F_o = \sum_{t_o}^{t_i} L\,(dt) \tag{7.11}$$

Where:

- F_o is the equivalent time to sterilise at 121 °C.
- S is symbol for summation or integration.
- L is the lethality value = $\dfrac{T_{(t)} - 121\ °C}{z}$
- dt is the interval time variable from initial time t_0, to final time t_i.
- t_o is the initial time.
- $t_{(t)}$ is the final time.

In practice the applied sterilisation of a product is based on both killing highly resistant spores of *G. stearothermophilus* or the slightly lesser resistant spores *Cl. sporogenes, B. coagulans* or *B. subtilis* 5230, and bioburden where the bioburden population and/or resistance is performed concurrently.

It should be recognised that the number of items to be sterilised can have an impact on sterility statistics and risk. If 1 million devices are to be sterilised, and the probability of a survivor is one in a million or 10^{-6}, then out of a million devices treated one device may be contaminated or non-sterile. So one should consider not only the bioburden on the product, but the number of products to be sterilised by an individual process.

Once a sterilisation process has been designed, the process must be validated. In general process validation may consist of performing a sub or fractional cycle, to verify the appropriateness of the BI to the bioburden) and/or qualify/verify with three half cycles (overkill), and a series of full validation cycles at established sterilising parameters. However, radiation validation is an exception to this, it does not necessarily require three half or three sub-processes, but typically only one sub-process following three bioburden tests. Its reproducibility can determined by three dose maps, where dosimeters of irradiation are distributed through the sterilisation load and irradiation measured to demonstrate uniformity.

7.1.12 In Review

In review, statistics play a significant role in sterility risk assessment and sterilisation. To appreciate its role we began with a definition of the word, sterile. Sterile is defined as the complete removal or destruction of all micro-organisms, but the means of testing for sterility is complicated and methods of detection have to be taken into consideration. Therefore, sterile is not an absolute term, but a relative one, requiring the application of statistics, as well. The kinetics of microbial sterilisation has been described as a logarithmic phenomenon. The backbone of sterilisation statistics typically is described as a D-value, the time to inactivate one log or 90% of a known population.

However, going beyond D-values, it should be recognised that statistics can be misleading, particularly if the result of microbial inactivation is not logarithmic, but non-logarithmic. However, if you can measure what you are expressing, in numbers, you demonstrate you know something about it. However, when you cannot measure in numbers, your expression is minimal or meager. If a sterilisation process treats more than million device items, the opportunity of one non-sterile unit out of 1,000,000 SAL becomes increasingly possible. Consequently what is more significant than a SAL is the level of safety that the sterilisation process can deliver to a treated load of hospital product, beyond the mere stated SAL.

Careful planning of a validation study is essential to ensure that the process is adequately validated. The plan should include design reviews. The plan for the validation study is documented in the validation protocol. A copy of the protocol and validation results are placed in the design history file or quality system record file. The operational, monitoring, and other production-related procedures are part of the device master record (DMR). Planning for the validation should include the following elements as well as any other relevant issues that must be addressed to conduct the validation study:

- Identification of the process to be validated.
- Identification of device(s) to be manufactured using this process.
- Criteria for a successful study.
- Length and duration of the study.
- Assumptions (shifts, operators, equipment, components).
- Identification of equipment to be used in the process.
- Identification of utilities for the process equipment and quality of the utilities.

- Identification of operators and required operator qualifications.
- Complete description of the process.
- Relevant specifications including those for the product, components, manufacturing materials, the environment and so on (may reference the DMR and quality system files).
- Any special controls or conditions to be placed on the preceding processes during the validation.
- Process parameters to be controlled and monitored, and methods for controlling and monitoring.
- Product characteristics to be monitored and method for monitoring (Code of Federal Regulations - Title 21: Food and Drugs - 820.70(a)(2) [18]; 820.75(b)(2) [19]; and 820.80(c) [20]).
- Any subjective criteria used to evaluate the product.
- Definition of what constitutes non-conformance for both measurable and subjective criteria.
- Statistical methods for data collection and analysis (Code of Federal Regulations - Title 21: Food and Drugs - 820.250 [21]).
- Consideration of maintenance and repairs (Code of Federal Regulations - Title 21: Food and Drugs - 820.72(a), 2012 [22]).
- Conditions that may indicate that the process should be revalidated (Code of Federal Regulations - Title 21: Food and Drugs - 820.75(b) [23]).
- Stages of the study where design review is required, and
- Approval(s) of the protocol.

The validation plan should also cover the installation and operation qualification of any equipment used in the process, process performance qualification, and product performance qualification.

A 'full' validation programme may generally consist of several major steps as shown in **Figure 7.1**.

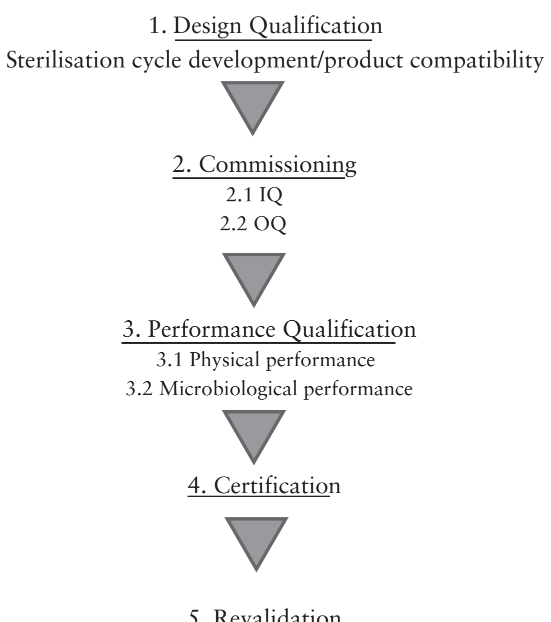

Figure 7.1 A general validation outline for sterilisation. IQ: Installation qualification and OQ: operational. qualification

7.2 Sterilisation Validation

Sterilisation validation is a formal procedure to demonstrate that a designed process can reliably sterilise a specific product. Validation may be defined as establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality. It is a systematic approach to gathering and analysing sufficient data, which will give reasonable assurance (documented evidence), based upon scientific judgment, that a process, when operating within specified parameters, will consistently produce results within pre-determined specifications. There may be other different types of validations, depending upon the circumstances. The types of validation are, for informational purposes:

7.2.1 Retrospective Validation

A type of validation of a process for a product already in distribution, based on accumulated production, testing, and control dates. It is a summary of existing

historical data. This type of validation makes use of historical data and information which may be found in batch records, production log books, lot records, control charts, test and inspection results, customer complaints or lack of complaints, field failure reports, service reports, and audit reports. Historical data must contain enough information to provide an in-depth picture of how the process has been operating and whether the product has consistently met its specifications. Retrospective validation may not be feasible if all the appropriate data was not collected, or not collected in a manner, which allows adequate analysis.

Incomplete information mitigates against conducting a successful retrospective validation. Some examples of incomplete information are:

- Customer complaints, which have not been fully investigated to determine the cause of the problem, including the identification of complaints that are due to process failures.
- Complaints which were investigated but corrective action was not taken.
- Scrap and rework decisions that are not recorded, investigated and/or explained.
- Excessive rework.
- Records that do not show the degree of process variability and/or whether process variability is within the range of variation that is normal for that process, for example, recording test results as 'pass' or 'fail' instead of recording actual readings or measurements, results in the loss of important data on process variability.
- Gaps in batch records for which there are no explanations (Retrospective validation cannot be initiated until the gaps in records can be filled or explained).

If historical data is determined to be adequate and representative, an analysis can be conducted to determine whether the process has been operating in a state of control and has consistently produced product which meets its pre-determined specifications and quality attributes. The analysis must be documented.

After a validated process has been operating for some time, retrospective validation can be successfully used to confirm continued validation of that process if no significant changes have been made to the process, components, or raw materials.

7.2.2 Prospective Validation

Prospective validation is conducted prior to the distribution of either a new product, or product made under a revised manufacturing process, where the revisions may

affect the product's characteristics. Concurrent validation (see next) is typically a subset of prospective validation and is conducted with the intention of ultimately distributing product manufactured during the validation study.

7.2.3 Concurrent Validation

It may be a combination of retrospective and prospective validation. It is performed against an approved protocol but the product is released on a lot-by-lot basis. Usually used on an existing product not previously validated or insufficiently validated. Concurrent validation is typically a subset of prospective validation and is conducted with the intention of ultimately distributing product manufactured during the validation study. Concurrent validation is feasible when non-destructive testing is adequate to verify that products meet pre-determined specifications and quality attributes. If concurrent validation is being conducted as the initial validation of a new process or a process which has been modified, product should be withheld from distribution until all data and results of the validation study have been reviewed, and it has been determined that the process has been adequately validated.

Concurrent validation may be conducted on a previously validated process to confirm that the process is 'still' validated. If there have been no changes to the process and no indications that the process is not operating in a state of control, product could be released for distribution before revalidation of the process is completed. There is some risk to early release of product in that subsequent analysis of data may show that the process is not validated.

Concurrent validation is typically carried out during normal production. This method is effective only if the development stage has resulted in a proper understanding of the fundamentals of the process. *The first three production batches must be monitored as comprehensively as possible* [1]. The nature and specifications of subsequent in-process and final tests are based on the evaluation of the results of such monitoring.

This careful monitoring of the first three production batches is sometimes regarded as prospective validation.

Concurrent validation together with a trend analysis including stability should be carried out to an appropriate extent throughout the life of the product.

7.2.4 Revalidation

Revalidation is to validate after a specified period of time (e.g., annual) or after

a change in equipment, packaging, formulation, operating procedure, or process that could impact product safety, efficacy, or potency. It is important to establish a revalidation programme for critical equipment to maintain validity.

A standard or typical validation programme for dry heat, EO and/or moist heat sterilisation generally consists of several major steps and an example is shown next:

1. Sterilisation cycle development/product compatibility
2. Commissioning
    2.1 IQ
    2.2 OQ
3. Performance qualification
    3.1 Physical performance
    3.2 Microbiological performance
4. Certification

- Sterilisation cycle development: this predetermines what type of sterilisation process is best for the material(s) used in the product for eventual qualification. Some sterilisation cycle development may be incorporated into eventual validation (performance qualification), if predetermined per development and validation documents. Sterilisation cycle qualification may require material/product qualification for the specified process. Sterilisation cycle development may or may not occur before commissioning or installation of the steriliser, because it may include the following:
    - Exploratory sterilisation method or cycle evaluation.
    - Product (compatibility), material, packaging, and/or load test runs.
    - Pre-microbiological qualification tests - D-value studies, bioburden analysis.

The statistical worse case conditions should be applied in the final sterilisation development. Commissioning typically follows cycle development and includes IQ and OQ.

Commissioning can consist of two steps, IQ and OQ:

- Installation qualification: this establishes that the steriliser and related equipment (including instruments) is received as designed and specified, that it is properly

installed in the selected environment, and that this environment is suitable for the operation and use of the instrument. IQ is the process of obtaining and documenting the evidence that the equipment has been provided and installed in accordance with its specification. IQ may also consist of:

  - Facilities/utilities evaluation.
  - Equipment calibration.
  - Equipment testing.
  - Equipment documentation.
  - Examining equipment design and supplied documentation.
  - Determining installation requirements.
  - Establishing any environmental controls and procedures needed.
  - Assuring that the work area has sufficient space to perform the processing and associated activities.
  - Installing the equipment.
  - Verifying correct installation.

- Operational qualification: this is the process of demonstrating that a steriliser and related equipment (including instruments) will function according to its operational specification in the selected environment. OQ is also the process of obtaining and documenting evidence that the installed steriliser and related equipment operates within predetermined limits when used in accordance with its operational procedures. This may include one empty run or several or a product run, depending upon what is known and what is not known. Additional items to be considered are:

  - Establishing manufacturing procedures for the monitoring, operation, and control of the equipment including the minimum number of operators.
  - Determining calibration, cleaning, maintenance, adjustment, and expected repair requirements.
  - Identifying important elements of the equipment that could affect the output or the finished device.
  - Verifying that the system or sub-system performs as intended throughout all anticipated operating ranges.

- Documenting the previous information.

Subsequent to IQ and OQ, performance qualification is made to provide assurance by obtaining, documenting, and interpreting results that compliance to the predetermined specifications can be achieved.

- Sterilisation performance qualification: is the process of demonstrating that the steriliser consistently performs according to a specification appropriate for its routine use. Performance qualification (PQ) is the process of obtaining and documenting evidence that the steriliser and the equipment, is installed and operated in accordance with operational procedures, consistently performs in accordance with predetermined criteria and thereby yields a product meeting its specification. PQ is verified using two main steps, the PQ and the microbiological qualification (MQ). PQ and MQ may be typically performed in parallel or the PQ may be performed first to determine the microbiological challenge that may be required.
- The physical qualification requires three consecutive runs to demonstrate the reproducibility of the processing of the product and that the acceptance criteria are met throughout the load for the duration of the proposed routine process. It confirms that the process is operating within the required parameters and that the product and load is reacting properly. This is confirmed by reading the load and product sheets, which are produced by the sterilisation equipment and by using sensor, and test equipment. The loads must contain the proper amount of temperature sensors and humidity sensors (as required). The required amount is based on the volume of the product load.

The microbiological PQ will demonstrate that when BI, PCD or product exposed to the sterilisation process meets the requirements for sterility, the load will be representative of the most challenging to sterilise, routine load. This is demonstrated using the biological testing previously described. This is confirmed using BI. The required amount is based on the volume of the product load. In addition to the BI, several other microbiological tests are required.

Microbiological qualification cycles consist of one or more of the following runs:

- Fractional or sub-process cycles
- Half cycles
- Full cycles

Typically the validation process for EO, moist heat and so on is broken up into three steriliser loads. The first load generally consists of a sub-lethal cycle, half cycle and

full cycle. The second and third loads may each consist of a half cycle and a full cycle, or if three consecutive half cycle can demonstrate cycle parameter reproducibility, then only one full cycle may be required. Generally two types of BI/PCD may be used, internal and external, which are used to demonstrate that the external BI/PCD are more resistant than the internal BI/PCD so that only external BI/PCD need to be run on routine runs. Internal BI/PCD are placed in the hardest to sterilise positions of the device and external PCD are placed externally to the load and will be used to monitor routine processes. When performing a sub-lethal cycle with partial BI/PCD positives and partial negatives, it is intended to achieve the use of less BI/PCD product on a routine basis, when internal BI/PCD survivors demonstrate less resistance than external BI/PCD BI survivors. This confirms that the internal BI/PCD and the product are less resistant to the sterilisation process than an external PCD, which may be used on routine runs to justify sterilisation of a less resistant product. Some typical microbiological qualification methods are shown in **Table 7.6**.

Table 7.6 Microbiological qualification methods

Bioburden (irradiation)	**Survival Curve (direct, F/N)**	**Fraction negative (F/N)**	**Overkill**
Assay bioburden (1-3 lots)	3-5 different exposures	5 exposure times	3 half cycles
Select sub-dose	Graded exposures	1 set (all positive)	Consecutive half runs
Run sub-dose (1-3)	1 run - zero exposure to demonstrate survivors	2 sets/runs demonstrate F/N growth	3 half cycles - no growth
0, 1, >1 growth	• Stumbo (F/N), Average the D-values of different exposures • Direct count, graded	2 sets (no growths)	1 sub-process to show recovery

- Certification is the final step of the validation study. It should specifiy the established cycle process parameters that are required for post-sterilisation monitoring. Certification certifies that the steriliser and sterilisation process intended for use will maintain operational specifications and will perform within the required process parameters necessary to achieve sterilisation of specific product(s). Certification is verification of the validation that may include the following:
    - Written protocol - IQ, OQ and PQ.

- References of specifications and procedures used.
- Documentation of calibration.
- Compilation and documentation of data.
- Documentation showing that processing specification are met.
- Successful results of tests, for example, sterility of BI or product (in the case of irradiation and acceptable dosimeters), residues (if EO residue dissipation rates), dose or temperature, RH%, mapping/distribution, pyrogens (if fluids), any biocompatibility requirements, device functionality, package integrity and strength and impact of re-sterilisation (optional).
- Analysis and interpretation of data and results.
- Documentation control system of standard operating procedures, including operation, preventative maintenance and calibration.

Statistics play a primary role in steps 1 and 5, starting with step 1, process development where D-values may be preliminarily be performed, statistics considered and process parameters are shown to be compatible with the product/material to be validated. During sterilisation, the results of half cycle runs or sub-process verification runs are performed that verify the results of D-value calculations by showing complete or nearly complete inactivation of micro-organisms on the product, to indicate that the desired probability of survivors is established. These runs eliminate the need for complete destruction of all products to prove sterility. Full cycle or nominal cycles are subsequently applied during the performance qualification phase merely to show repeatability, and/or critical process parameter distributions (e.g., temperature and humidity distribution, dose mapping).

The last step in validation is certification, which is purely documentation, formal review and approval. However, during the review of the validation, it is acceptable to confirm, and calculate the probability of finding survivors for the process. From this information, a sterilisation process can reliably be shown to sterilise.

Validation of sterilisation is a documented procedure demonstrating that a prescribed specification has been met, by obtaining data, recording, and interpreting results that show the process will consistently produce a product free of micro-organisms with a high degree of assurance and confidence.

Validation can be considered to be a total programme. This programme encompasses a parallel qualification of product and package, a determination of sterilisation effectiveness of micro-organisms, effect of process on product samples (i.e.,

irradiation), a qualification of equipment upon installation or commissioning, process performance qualification, and certification. Once a process is completely validated, the process and equipment are typically revalidated periodically or annually. A completed validated process allows for routine processing and releasing of product.

To effectively establish a validation programme requires an overview of the entire sterilisation system. Sterilisation begins with an understanding and control of the environment under which the product is manufactured. Sterilisation matrices can describe the various interactions of sterilisation within the manufacturing process and subsequently for the release of product.

In the past few years considerable effort has been exerted by AAMI, The Food & Drugs Administration (FDA), and others to devise international standards under the auspices of the ISO, to obtain harmonisation with the European Community (EC). This task has not been easy. Differences between countries and sterilisation methods exist. There are several standard setting organisations involved in sterilisation: Parenteral Drug Association (PDA), Health Industry Manufacturing Association, USP, AAMI, Community for European Normalisation (CEN), ISO and so on.

In this discussion, we will try to deal with just a few basic sterilisation validation standards for dry heat, radiation, steam, and EO, from AAMI, CEN and ISO.

The ISO standards are generally characterised by pertinent regulatory agencies, such as the FDA, Department of Health and Social Security, and the international (global) community. AAMI standards have long been used by the FDA as guidelines in assessing good manufacturing practice standards, for example. The CEN standards have been established most recently for the EC in 1992.

In contrast to these standards the US strategy is to set new sterilisation standards through AAMI that influence the ISO standards in order to bring about harmonisation of requirements.

There are detailed requirements for the various sterilisation methods that include physical/chemical qualifications.

The microbiological qualification step possibly constitutes one of the most important aspects of process qualification for many companies because most of them deal with contract facilities and sterilisers, today.

Qualifications are generally not extensively repeated unless significant changes occur. With most manufacturers' new or significant alterations of equipment, product, packaging or material changes are reasons for repeating qualification studies. Once a process has been qualified, it will undergo requalification periodically or annually.

In microbiological qualifications, all sterilisation methods are concerned with the demonstration of inactivation or elimination of viable micro-organisms under sub-processing conditions.

The purpose of all standards is to establish minimum technical criteria and requirements for sterilisation, development, validation and routine control of sterilisation processes. The standards also address the conditions necessary for optimum performance, operation, testing, safety and maintenance of sterilisation.

The cited standards include, besides ones for sterilisation, related subjects to give a complete overview of the subject. These include: standards for bioburden (ISO 11737-1 [14]) biocompatibility (ISO 10993-1 [9] and ISO 10993-7 [24]), BI (ISO 11138 [15], dosimetry (ISO 11137-3 [4]), material compatibility (AAMI TIR 17 [25]), sterility (ISO 11737-2 [2] and packaging (ISO 11607-1 [26])

Furthermore, the goal of procedures and standards, is to provide harmonisation among regulatory and notified bodies, countries and companies. All sterilisation standards and recommended practices are voluntary (unless, of course they are adopted by government regulatory or procurement authorities. Standard or recommended practice reflects the collective expertise of a committee of healthcare professionals and industrial representatives.

Some examples of standards related to sterilisation are given in **Table 7.7**.

7.2.4 A Total Approach toward Sterilisation Validation when a New Product, Polymer or Package is Required to be Sterilised

Elements of sterilisation of validation will vary:

- Protocol:
  - Installation/commissioning qualification
  - OQ/PQ
  - PQ
  - Reference of specifications and procedures
  - Documentation of calibration, preventative maintenance, operation
  - Data generation and acquisition
  - Collection, compilation, and documentation of data

- Analysis and interpretation of data/results
- Report/completed protocol
- Certification

- Documentation control system (standard operating procedures (SOP):
    - Operation
    - Preventative maintenance
    - Calibration

Table 7.7 Various standards related to sterilisation and its methods

Standard number	Item covered	Sterilisation standard or related standard	Method(s) used	Reference
ISO 11135-1	Medical devices	EO	General requirements	[27]
ISO 10993-7	Medical devices	EO Residuals	Biological evaluation – EO sterilisation residuals	[24]
ISO 11137-1	Healthcare products	Radiation	General requirements	[5]
ISO 11137-2	Healthcare products	Radiation	Establishing the sterilisation dose	[3]
ISO 11737-1	Medical devices	Microbiological methods	Estimation of population of microorganisms on products	[14]
ISO 11737-2	Medical devices	Microbiological methods	Validation of a sterilisation process	[2]
ISO 17665-1	Healthcare products, medical devices.	Moist heat	Development, validation and routine control of sterilisation	[12]
ISO 20857	Healthcare products, medical devices	Dry heat	Development, validation and routine control of a sterilisation process	[28]
ISO 11607-1	Packaging for medical devices	Terminal sterilisation	Materials, sterile barrier systems and packaging systems	[26]
ISO 11607-2	Packaging for medical devices	Terminal sterilisation	Validation requirements for forming, sealing and assembly processes	[29]
ISO TS 11135-2	Healthcare products	EO	Guidance on the application of ISO 11135-1	[30]
ISO 11137-3	Healthcare products	Radiation	Guidance on dosimetric aspects	[4]
ISO TS 17665-2	Healthcare products	Moist heat	Guidance on the application of ISO 17665-1	[13]
ISO 14937	Healthcare products	General (e.g., for new methods- H_2O_2, O_3, ClO_2, $scCO_2$, or oxides of nitrogen and so on.	Guidance on development, validation, routine control of the sterilisation process in general	[31]

The steps, measurements and standards may vary with different sterilisation methods (**Table 7.8**).

When approaching a validation the following topics need to be considered:

- Determine what standard to use.
- Select SAL and label.
- Select sterilisation method, cycle and/or parameters.
- Insure appropriate packaging for the product.
- Determine worst case load.
- Determine BI/or PCD and/or bioburden assessment – consider internal BI/PCD, external BI/PCD and PCD or BI.
- Select validation approach:
    - BI or bioburden release
    - Parametric or dosimetric (release)

Table 7.8 Validation aspects of traditional sterilisation techniques

Methods - required steps	**Dry heat/steam**	**EO**	**Radiation**
Process parameters or specifications	Time and exposure	Preconditioning	Dose only (for gamma irradiation)
	Pressure (for steam)	Initial vacuum	Conveyor speed and dose (for electron beam irradiation)
	Optional (steam) initial evacuation and/or steam pressure pulses	Leak test	-
	Come-up time and pressure (steam)	Humidification - %RH, temperature and time	-
	Exposure - time and temperature	Gas injection - time and rate	-
	Cool-down	Exposure - time, temperature and pressure	-
	Drying period (steam)	Post-exposure - vacuum and air washes	-
		Vent to atmosphere - period and rate	
		Aeration – time and temperature	

Microbiological	BI/PCD routine	BI/PCD routine	Dosimeters
	Bioburden (qualification and requalification	Bioburden qualification	Bioburden and sterility at sub-process (qualification and requalification)
			Quarterly bioburden
BI	• *G. stearo-thermophilus* (steam) • *B. atrophaeus* (dry heat) • Endotoxin for depyrogenation	*B. atrophaeus*	Not applied, except for NDA
Performance qualification (micro)	• 1 to multiple D-value and/or 3 half cycle(s) • 1-3 Full cycles • F_o/F_h applied	• 1 to multiple D-value and/or 3 half cycles • 1-3 Full cycles • EO residuals	• Single or multiple sub-processes • Full dose established
Standards	• ISO 17665-1 [12] (steam) • ISO 20857 [28] (Dry heat) • ISO 11737-1 [14], ISO 11737-2 [2] • ISO 11138 [15] (appropriate part)	• ISO 11135 [27, 30] • ISO 11737-1 [14], ISO 11737-2 [2] • ISO 10993-7 [24] and AAMI TIR 19 [32] • ISO 11138 [15] (appropriate part)	• ISO 11137-1 [5], ISO 11137-2 [3], ISO 11137-3 [4] • ISO 11737-1 [14], ISO 11737-2 [2] • TIR 33 [8]
Monitors, traceable calibration	Temperature sensors, pressure monitors, timers, and flow meters	Temperature, %RH, pressure, gas concentration (optional) and timers	Dosimeters, conveyor speed timer, spectrometer and timer
Other	Leak test, steam quality, filters, boilers and/or heaters	Wall temperature, leak test, filters, EO gas background monitoring, scrubbers, gas cylinders and humidifiers	Radiation source, radiation background measurement

Typically validations will include at least three half cycles or sub-cycles, except for radiation which will vary.

There are several steps toward achieving a ‘full initial’, successful validation, when a new product, polymer or package is required to be sterilised:

- Specify specific considerations and limitation of the new product, polymer or package to be sterilised.
- Select specific sterilisation method and/or procedure, technical review and design considerations.
- Provide definitions, references, and appropriate standard(s) for the protocol.
- Provide responsibilities of various personnel and departments in the protocol.

- Status of commissioning/installation of steriliser.
- Status of operation of steriliser and related equipment.
- Know the metrology systems required.
- Performance of developmental cycles or processes, to assess.
- Minimum (microbiological inactivation) and maximum (product tolerance).
- Write a sterilisation validation plan.
- Create process and procedural criteria and acceptance criteria.
- Discuss performance and review of bioburden.
- Assess comparative resistance of bioburden to BI or process challenge device or dosimeter, and adequate recovery.
- To deliver a SAL (e.g., 10^{-6}).
- Performance of full cycle(s)/process(es), to verify reproducibility.
- Analyse and review data, results and documentation, and acceptance criteria.
- Resolve any issues or further considerations or repeat steps as necessary.
- Approve validation and provide certification.
- Maintenance of validation.

The performance and development phase addresses product and packaging materials evaluation, as well as sterilisation parameters and/or dose determination. The commissioning/installation phase deals with equipment testing, calibration, mapping, and documentation by the sterilisation facility. The manufacturer and sterilisation facility should then address process qualification, which includes establishing a product loading pattern, followed by parameter or dose mapping for the identification of minimum and maximum parameters/dose within the product load. Once the data have been collected for the three phases (installation, operation and performance qualification) of validation, the documentation is certified in accordance with ISO 9001 [33] or other criteria. Routine validation maintenance ensures the validity of the sterilisation parameters or dose, equipment, and BI, calibration or dosimetry systems.

Revalidations (or maintenance of validation) will vary. For example, typical requalification requires at least one-half or sub-process, or sub-dose per year or every two years for steam, dry heat or EO sterilisation. For radiation, a quarterly

bioburden is required for revalidations based upon the bioburden approach. For aseptic processing, two re-validations per year are required.

7.2.5 Dry Heat Sterilisation Validation

The validation for dry heat is similar to that for steam and EO, but with fewer measurements or monitoring control(s), and it may be as easy and flexible as baking a cake. Yet dry heat requires no more process parameters to measure than radiation, and there is a tremendous microbial heat resistance history from spacecraft monitoring as there is for radiation. Reducing the temperature, can result in many more polymers and materials that can be sterilised. Only heat (temperature) and exposure (time) have to be measured.

7.2.5.1 Dry Heat instead of Steam Sterilisation

For dry heat we look for inactivation of micro-organisms after their exposure to dry heat, in areas often impenetrable and damaging to steam. Validation by dry heat is controlled by ISO 20857 [28].

Dry heat is used sometimes for sterilisation in place of the more efficient steam sterilisation because some materials are sensitive to moisture, and to ensure that glass or other materials are free of pyrogenic materials.

The sterilisation within a chamber, hot air tunnel, or liquid is a critical process and there is a requirement for validation of the process and its equipment. While validation requirements for dry heat sterilisers and processes may vary in detail, the common theme is to show reproducibility that sterilisation is achieved.

For dry sterilisation, the validation includes measuring the temperature at critical locations with the chamber, tunnel or liquid throughout the process and load, but not humidity. In dry heat tunnels sterilisation with laminar flow is widely used in high-speed aseptic manufacturing. Typically, laminar flow tunnels contain three sections: pre-heating, heating and cooling. Sterilisation may occur at temperatures higher than 300 °C in the heating section. After sterilisation, cooling is necessary before filling the containers. It is therefore very important to keep conditions sterile in the cooling section (up until a filling station is reached) by keeping the cooling section at a slight positive pressure towards the tunnel room, e.g., 2-3 Pa.

Dry heat sterilisation temperature criteria can vary between 105-400 °C with varying exposures. The steriliser is required to heat all parts of the load up to the specified

temperature for a specified period long enough to achieve the desired sterility. The confidence of the degree of sterility can be expressed by the lethality formula:

$$F_o = \sum_{t_o}^{t_i} L\,(dt) \tag{7.12}$$

Where:

- F_h is used instead of F_o as for steam, and the equivalent time to sterilise at 160 °C, for example.
- S is a symbol for summation or integration.
- L is the lethality value = $\frac{T_{(t)} - 160}{z}$
- 160 is the temperature in °C.
- dt is a time variable from initial time, t_0, to final time, t_i.

In practice the applied kinetics of sterilisation of a product is based on both killing the highly resistant spores of *B. atrophaeus*, or the use of indigenous spores which have the different resistance and where bioburden resistance is performed concurrently.

7.2.5.2 General Aspects of Dry Heat Sterilisation Validation

Commissioning is required to demonstrate that the sterilisation equipment intended for dry heat use will maintain operational specifications and will perform within the required parameters necessary to achieve sterilisation of the specific product item. Process qualification provides assurance by obtaining, documenting, and interpreting the results, thereby showing continued compliance to the pre-determined specifications.

Most often, the validation of a dry heat cycle follows the half-cycle or 'over-kill' method using BI and product. This method demonstrates that the resistance of the microbiological challenge test system is equal to or greater than the product bioburden.

The appropriateness of the BI - 10^6 *B. atrophaeus*, should be evaluated. In some cases the most resistant strain of bioburden may be the BI in a bioburden validation approach, but nevertheless the appropriateness of this resistant strain should be evaluated. This can be done by characterisation of the natural bioburden and using this information to determine D-values; determining that the bioburden level is for

example ≤1000 colony forming units (CFU), indicating a lesser challenge than the BI; or, if characterisation is not performed and the bioburden level is for example, >1000 CFU, by performance of a fractional exposure cycle using BI and product, followed by comparison of any positive response(s) yielded from the BI *versus* the product.

A half-cycle, run three times, at the minimum exposure to dry heat which yields no surviving micro-organisms with all process parameters, except exposure time, remaining the same, demonstrates a 6 log reduction. Additional half-cycle experiments are performed as confirmation, for revalidation. A full cycle, providing a 12 log reduction (10^6 BI), is performed for product release purposes. Continued assurance that the original process parameters remain effective is shown through revalidation, usually performed on an annual basis.

Typical revalidation is shown by performing one half-cycle and one full cycle, as well as a review of the original validation data and any subsequent revalidation records to confirm that no changes have taken place. However, the appropriateness of the BI can be determined with a fractional cycle demonstrating a greater BI resistance than bioburden resistance:

BI> bioburden

A schedule of routine bioburden testing monitors any changes in product components, environment, packaging, or manufacturing process that could have potential impact on the product bioburden and its resistance to the sterilisation process.

Testing to be considered when validating a dry heat sterilisation cycle includes:

- Bioburden - sterilisation must begin with control of the bioburden and the environment.
- Bioburden validation for recovery efficiency.
- Test of sterility for BI and product.
- BI population and BI resistance confirmation at the appropriate standard for example, ISO 11138 [15].
- B/F – validation of a product test of sterility.
- Bacterial endotoxin test (BET) for depyrogenation.
- Inhibition enhancement – validation of the BET.
- The BI can consist of inoculated product, inoculated spore strip or unit, inoculated simulated product, or inherent natural product bioburden as the challenge.

7.2.5.3 Dry Heat Validation Plan

A brief validation plan for dry heat sterilisation, is needed which reviews any previous design control work, in order to further clarify the plan and supplement it as applicable and appropriate.

- Create definition section(s) - separate validation into installation, operational and performance qualification.
- Thermal lethality (F_h) (physical) data can be calculated for all runs and input into tables.
- F_h physical data and F bio should be reviewed and compared.

  Note: in the pharmaceutical industry, the typical BI and the F_h or F_{bio} may be replaced with a 3 log endotoxin indicator challenge, in order to demonstrate the greater depyrogenation.
- Tables should have the product and/or total cycle times.
- All product heat-up times and/or total cycle times will be reviewed in comparison to other data (e.g., cool-down).
- All dry heat steriliser temperature data collected will be summarised for temperature distribution and reviewed.
- Dry heat steriliser temperature distribution will be determined and/or performed to determine any cold zones, as necessary, and the data obtained and its variations will be analysed if significant. Prior to microbial challenge testing of the dry heat sterilisation, heat-penetration studies must be completed in order to identify the cool spot in the entire load.
- Product heat penetration data and temperature distribution will be reviewed to look for hot and cold locations in product immersed in the dry heat steriliser. Supplemental product heat penetration study may be performed to find product cold location (if not found previously), and reviewed to determine what correction factor to existing heat penetration data is needed, if significant. Small size of containers and dry heat steriliser may not show significant differences.
- Description of critical cycle parameters will be determined for any supplemental validation (see **Section 7.2.2.5**).
- Review maximum product/material tolerances and biocompatibility data.
- Review and clarification any previous information that may be used toward further supplementation.

7.2.5.4 Biovalidation

Bioburden estimations will be performed as instructed in ISO 11737-1 [14] under worse case conditions, with determination of correction factor, aerobe bioburden; numbers of anaerobes, spores, yeast/mould will also be determined. Data will be reviewed to see if there is a need for further bioburden characterisation and further testing (e.g., further bioburden resistance and/product sterility testing could be performed with further BI worse case locations under fractional cycles (if necessary), and integrated lethalities [e.g., F_h (physical) and F (biological)] would be calculated and demonstrated under worse case conditions.

The 'appropriateness' of the BI to bioburden resistance will be demonstrated. Under the fractional cycles, temperature distribution and temperature penetration studies, bioburden and BI resistance testing, will be performed. The appropriateness of the BI (*B. atrophaeus* spores) resistance will demonstrate greater resistance than the product bioburden resistance.

For example, *B. atrophaeus* will be inoculated on the product or placed in an equivalent or greater resistant PCD to demonstrate greater resistance than the natural bioburden on the product.

Correction factors, when necessary, will be determined for the bioburden and inoculated spores. *Note*: BI spores (for use) will be equilibrated at standard environmental conditions (e.g., conditions that simulate production conditions). Typical conditions in the environment should be recognised.

Use of bioburden will determine aerobes, anaerobes, spores and yeast mould. Resistant survivors will be further identified and consideration for their potential 'relative resistance' to the BI will be determined. The interface, non-fluid path of product (e.g., sealed lumen end) or mated surfaces will be inoculated with 10^3 spores or higher if the bioburden is higher, as a worst case scenario. These areas are assumed not be invasive, but topical in relation to the user or patient. Such considerations may not meet all regulatory requirements, internationally, but require a 10^6 challenge instead.

Half cycles will be reviewed and supplemented (as needed) with BI, temperature distribution and heat penetration testing, considering worst case conditions and calculations of F_h (physical) and F_h (biological) (as necessary). Half or sub-process cycles for each product type that will demonstrate a 10^{-6} SAL of product and selected 10^6 *B. atrophaeus* BI when considered under full cycle exposure conditions.

One to three full cycles will be reviewed and supplemented (as needed) with BI, temperature distribution, product heat penetration with BI applying worst case

conditions (as necessary) and analysing them for process consistency, reproducibility. A singular full cycle may only be required if the half cycles can demonstrate cycle parameter reproducibility.

Perform additional tests as necessary, for example, in the pharmaceutical industry, endotoxin indicator and evaluation with the *Limulus* amebocyte lysate (LAL) pyrogen test as an endotoxin challenge. For example, the endotoxin challenge in dry heat sterilisation – may be to inoculate commodity samples (e.g., glass vials) with a known amount of endotoxin (e.g., 10–100 ng *Escherichia coli* lipopolysaccharide) throughout the vessel. Thermocouples should be placed in commodities adjacent to those containing endotoxin for temperature monitoring and correlation with LAL for test results. Endotoxin destruction should be ascertained and verified at the coolest location of the load.

Supplementation may include verification of a validated cycle specification, if necessary.

Supplementation may include a bacterial immersion and/or helium leak testing for product package integrity and contamination (particularly for product in a vial).

Post-sterilisation testing may clarify and supplement environmental control, cleaning and control steps of product to minimise bioburden and sources of contamination, if necessary.

After validation is successfully completed, each product lot is released by BI, cycle parameters and/or dry heat dosimetry (e.g., F_h).

7.2.2.5 Validation

The validation process covers the product sterility assurance requirement. USP testing cannot achieve this level of assurance because it is only conducted on a sub-set of the total sterilant-exposed samples. While the results are accurate for the samples tested, they cannot be extrapolated to determine the SAL of the remainder of that lot. For example by performing sterility testing on samples, we obtain the following results:

- For 10 samples tested, a SAL of 10^{-1} is demonstrated.
- For 20 samples tested, a SAL of $10^{-1.3}$ is demonstrated.
- For 100 samples tested, a SAL of 10^{-2} is demonstrated.
- For 1,000 samples tested, a SAL of 10^{-3} is demonstrated.

- For 10,000 samples tested, a SAL of 10^{-4} is demonstrated.
- For 100,000 sample tested, a SAL of 10^{-5} is demonstrated.
- For 1,000,000 samples tested, a SAL of 10^{-6} is demonstrated.

Since the test results do not demonstrate the desired SAL for an entire lot that is not tested, product sterility testing cannot be used *in lieu* of validation, to prove sterility.

Once a validation is conducted, product lot-release sterility testing is not required as long as the manufacturer periodically conducts audits to verify continued validity of the minimum sterilisation dose. Barring the need for other release tests, such as pyrogen testing, the product is ready for release if the BI, cycle parameters, and/or dosimeters indicate that the required minimum sterilisation dose has been achieved. Although there is no rule that says that validation cannot be augmented with USP sterility testing, there are issues that render this impractical. Because a USP sterility test requires a 14 day test period, its use can create a two week delay for a product otherwise ready for release. Also, devices used in a USP sterility test cannot be sold, and, thus, are a sunk cost. If the product is expensive to manufacture, product sterility testing adds significant unnecessary expense to lot release.

The backbone of all terminal sterilisation methods is the decimal reduction value, commonly referred to as the D-value. The D-value is the time or dose that the sterilisation process takes to inactivate a microbial population one logarithm or 90%. The approaches toward applying D-value information varies slightly with different sterilisation methods. One of the major differences is the application of D-values from the bioburden, which consist of naturally occurring micro-organisms, on or from the product or manufacturing environment: BI/challenges that consist of selecting resistant micro-organisms for a specified sterilisation method.

7.2.6 Steam and Ethylene Oxide Sterilisation Validation

In EO and sometimes steam sterilisation the BI or overkill approach is the microbiological qualification approach. Combinations of methods are an alternative approach that facilitate the reduction of exposure times and EO concentration or steam pressure. The bioburden approach is the most involved and rigorous approach from an environmental control perspective. Validation evaluations must be performed on product samples prepared under actual manufacturing and environmental conditions, and ultimately are exposed to the sterilisation method in its final packaging and loading configuration.

7.2.6.1 Steam Sterilisation Validation

Steam sterilisation validation has many similar characteristics to dry sterilisation validation (see **Section 7.2.2**), except for addition characteristics such as including steam, steam pressure, steam sterilisation kinetics, and drying (see **Table 7.8**).

The IQ process is intended to demonstrate that an installed autoclave meets all the specifications for proper installation and that a supporting programme (SOP, maintenance sheet) is in place. The IQ includes the following checks: supplier or manufacturer name and address should be checked. Any deviation observed should be informed to the supplier or manufacturer through the purchasing department for corrective action. Equipment name, make and model number should be noted. In-house blue prints should be allocated to check the proper location of installed equipment. Mechanical equipment specification (chamber, valve, filters, vacuum pump), site specification/utilities, construction material, change/spare parts, operating and maintenance manuals, and a preventative maintenance programme.

The OQ process is intended to demonstrate the functionality of the equipment. The OQ includes the following checks: operational tests (operator modes, emergency stop, doors, display checks, switch, interlock checks and programmable parameters), saturated steam check, filter sterilisation, leak/air removal test, and power loss recovery test. Several utilities need to be verified such as the clean steam generator, air filtration system, power source and cooling water.

PQ heat-distribution studies – these include two phases:

1. Heat distribution in an empty autoclave chamber, and
2. Heat distribution in a loaded autoclave chamber.

The places where the thermocouple wires are soldered should not make contact with the autoclave interior walls or any other metal surface.

Heat-distribution studies – these may use thermocouples at the cool spot in the chamber. The principle is to locate any cool spot and the effect of the load size and/or configuration has on the cool spot location. The difference in temperature between the coolest spot and the mean chamber temperature should typically be not greater than 2.5 °C. Greater or varying temperature differences may be indicative of equipment malfunction.

Heat penetration studies - This is the most critical component of the entire validation process. The main purpose is to determine the cold spot inside the product. The container cold spot for containers ≥100 ml is determined using container-mapping

studies. Thermocouple probes are inserted within a container and repeat cycles are run to establish the point inside the container.

Thermocouples will be placed both inside and outside the container at the cool spot location(s), in the steam exhaust line, and in constant-temperature baths outside the chamber. The difference in temperature will be calculated based on the temperature recorded by the thermocouple inside the container at the coolest area of the load.

Microbiological challenge studies (biovalidation) are used to provide supplementary assurance that adequate lethality has been delivered to all parts of the load (see **Table 7.7**). Calibrated BI used as bioburden models provide data that can be used to calculate F_o. The micro-organisms used to challenge moist heat sterilisation cycles are *G. stearothermophilus* and/or *Cl. sporogenes*.

After the sterilisation cycle is complete, the inoculated items or spore strips are recovered and subjected to microbiological test procedures. Typically spore strips are immersed in a suitable growth medium (e.g., SCDM or TSA) and incubated for up to seven days. The F_o value for *G. stearothermophilus* is typically 12 min F_o at 121 °C.

For more details and information on parenteral drug steam sterilisation qualification see Rogers [34].

7.2.6.2 Ethylene Oxide Sterilisation Validation

Commissioning for EO sterilisation demonstrates that the sterilisation equipment intended for use will maintain operational specifications and will perform within the required parameters necessary to achieve sterilisation of the specific product or item. It may include:

- Equipment specifications/diagram.
- Calibration records.
- Profiles for pre-conditioning (temperature and RH), aeration rooms (temperature) and empty chamber temperature distribution.

Process installation and performance qualification provides assurance by obtaining, documenting, and interpreting the results, thereby showing continued compliance to the predetermined specifications.

Physical qualification of EO sterilisation may consist of the following:

- Profiles within loaded preconditioning and aeration areas.

- Loaded chamber temperature and %RH distribution studies.
- Diagrams showing load configuration, thermocouple, %RH monitors and BI placement.

Typically the microbiological qualification of an EO cycle follows the half-cycle or ‘over-kill’ method using BI and product. This method demonstrates that the resistance of the microbiological challenge test system is equal to or greater than the product bioburden. Some of the results of this qualification approach may include the following:

- Records of performance runs (sub-lethal, half, and full cycles).
- Diagrams of load configuration with BI and thermocouple placement.
- BI test result.
- Bioburden results and % recovery.
- Sterility test result of product.
- B/F testing.

Other approaches may be a combined bioburden and BI method, or an absolute bioburden approach (see **Table 7.7** and ISO 11135-1 [11]). In the combined BI and bioburden method, BI and bioburden testing are at times performed simultaneously, and fractional cycles are performed, instead of half cycles. The absolute bioburden method is seldom used for EO sterilisation.

The appropriateness of the BI (10^6 *B. atrophaeus*) should be evaluated. This can be done by characterisation of the natural bioburden and using this information to determine D-values, showing that the bioburden level is for example, ≤100 CFU, indicating or suggesting a lesser challenge than the BI. If characterisation is not performed and the bioburden level is >100 CFU, use a fractional exposure cycle using BI and product, followed by sterility testing for comparison of any positive response(s) yielded from the BI *versus* the product.

Fractional negative method is a series of multiple sub-process cycles of graded exposures to EO.

The difference between the half cycle – overkill method are the number of cycles recommended and the number of survivors in terms of a positive BI. Typically it requires a minimum of seven cycles to be used in the fractional negative method as compared to only three or more for the half cycle overkill approach. The fractional negative cycle method provides a better picture of the kinetics and statistics of the

BI and bioburden (if applied). If bioburden and BI are incorporated it is possible to determine if logarithmic inactivation is occurring.

For the *overkill method*, a half cycle is the minimum exposure to EO, which yields no surviving micro-organisms, typically demonstrating a 6 log reduction of spores, within acceptable process parameters. Two additional half-cycle experiments are performed as confirmation. There should be three successful consecutive half-cycle results (see **Table 7.7**). A full cycle, providing a statistical 12 log reduction or a 10^{-6} SAL is performed for product release purposes. In the overkill approach the following typically occurs:

- Demonstrate a SAL of 10^{-6}.
- Assume bioburden has a lower population and resistance than BI/PCD, by evaluating the bioburden. It is generally and historically assumed that the BI has a greater resistance when the bioburden is less than 100 CFU/device. A fractional cycle may be run to demonstrate greater resistance of the BI or PCD, if the bioburden is greater than 100 CFU.
- Need a $\geq$12 log spore reduction of the BI.
- Three half cycles with no BI survivors.

In case parametric release is planned for the validation, the following considerations must be realised:

- It is a more complicated validation than BI release (e.g., overkill; see TIR 20 [35] and ISO 11135 [11, 30]). There is a minimum of 6 or 7 sub-lethal cycles.
- Microbiology requirements.
- Equipment requirements for pre-conditioning, sterilisation and aeration.
- Product considerations - load configuration becomes more critical and so pallet configuration and product density have to be allowed for.
- Parameter establishment for routine monitoring - there must be direct measurement of EO, RH, temperature, exposure and decisive process parameters.

Continued assurance that the initial, process parameters remain effective is shown through revalidation, usually performed on an annual or a biannual basis.

Typical revalidation is done by performing one-half cycle and one full cycle, as well as a review of the original validation data and any subsequent revalidation records to confirm that no changes have taken place.

A schedule of routine bioburden testing, monitors any changes in product components or materials, environment, packaging, or manufacturing processes that could have potential impact on the product bioburden and its resistance to the sterilisation process.

Some testing to be considered when validating an EO sterilisation cycle includes:

- *Bioburden*: The bioburden of the product should be determined and evaluated. For most products, aerobic bacteria and fungi bioburden may be appropriate, however the standard indicates anaerobe testing too. Also microaerophilic microbes or may grow both aerobically and anaerobically. To characterise resistance, presence or absence of spores should be evaluated. AAMI ISO 11737-1 [10] recommends testing 10 samples from each of three production lots. It is most appropriate to sample the production lots that will be used in the three validation cycles. The bioburden samples may be selected to represent various production or packaging times. Typically samples are collected immediately prior to sterilisation.

- *Bioburden validation for recovery efficiency*: This evaluation determines if it is necessary to apply a recovery factor to routine bioburden test results. The bioburden validation may use the repetitive (exhaustive) method (five non-sterile samples are recommended: a minimum of three samples are required) or the bioburden validation recovery can be determined by a product inoculation (typically spores) method (five sterile samples are recommended: a minimum of three samples are required).

- *Test of sterility of BI and product*: This test involves total immersion in an appropriate recovery bacteriological media that is tested at appropriate temperatures and incubation times, to determine if any survivors occur. Failure to reproduce and grow indicates sterility, under appropriate conditions.

- *Confirmation of BI population and resistance* (appropriate ISO 11138 [15]): This test confirms the stated population for the BI spore strip or inoculum, and resistance is typically stated on the label by the manufacturer.

- *B/F*: The B/F procedure is used to verify acceptable product sterility testing. This testing ensures that false negative results will not occur in the sterility test. A false negative result allows a non-sterile sample to appear sterile due to inhibition of the microbial growth. This type of reaction is caused by certain materials utilised in some medical devices, but can be overcome by modifications to the sterility test procedure. Sterile samples are required for B/F testing. If sterile samples are not initially available, the B/F samples can be run with the fractional or half-cycles.

- *EO residuals and biocompatibility as necessary, for different patient use*: EO residual levels are evaluated using the recommendations in ISO 10993-7 [24].

Typical EO residuals are EO and ethylene chlorohydrin. Some conditions may include levels for ethylene glycol. Simulated and/or exhaustive extractions *via* immersion or fluid path extraction are performed on products based upon the duration of exposure and contact to patients. Extracts are typically analysed by gas chromatography.

- *Bacterial endotoxin test*: This test measures the presence of specified endotoxin levels depending upon label claim and the specific area contacted in the body. Endotoxins are toxins that are released from gram-negative organisms, and the tests determine whether these organisms are present (alive or dead) through the presence or lack of those toxins. Tests can be by evaluation of extracts on rabbits or the LAL test. The LAL test, can use a gel-clot, chromogenic or turbidimetric technique. The LAL can be utilised to demonstrate that a product is free of bacterial endotoxins (pyrogens). The LAL test can be used as an alternative testing method to the rabbit pyrogen test. The gel clot method is used to test raw materials or end products for the presence of endotoxins. This method uses components found in the blood of the blue horseshoe crab (*Limulus polyphemus*), which forms a gel-like clot when incubated in the presence of endotoxins. This method is used to determine if products or materials are endotoxin free. The sensitivity or detection limit of the endotoxin test is 0.06 eu/ml (endotoxin units). Products and materials can be certified to a sensitivity of 0.03 eu/ml upon request. Upon receiving the product to be tested at a laboratory, a product extract is made and then exposed to and incubated with the horseshoe crab LAL. The product extract is incubated along with a standard series of the control standard endotoxin as the positive control and the unexposed extract fluid as a negative control. After the incubation period, the tubes containing the controls and the extract are observed for the presence of the gel clot. If it clots, endotoxin is present. If no clot is observed, the product is free of endotoxins.

- *Inhibition enhancement – validation of the BET*: This test ensures there is nothing present that would inhibit or enhance the actual endotoxin present on a product. The degree of product inhibition or enhancement of the LAL procedure should be determined for each product, as necessary. At least three production batches of each finished product should be tested for inhibition and enhancement.

- *Temperature and %RH distribution*: Temperature and %RH are monitored throughout the chamber of the steriliser, but not within the product or load.

- *Temperature and %RH penetration*: Temperature and %RH are monitored throughout the load of the product.

- *Pressure measurements, recording and documentation*: These are additional measurements, records, and documentation that are required in the protocol.

- *Loading pattern(s)*: The loading pattern must be defined and specified, because variation in the load could lead to failures.

For validation cycles, the maximum quantity of product representing the densest load configuration that will be processed in full routine cycles must be loaded in to the EO steriliser chamber. The product with BI test samples must be selectively or widely distributed throughout the steriliser chamber. A placement diagram must be prepared, typically by the contract sterilisation facility in consultation with the device manufacturer. The diagram must indicate the location of the product with BI test samples plus temperature and humidity sensors in the loaded chamber.

One of the greatest concerns in sterilisation process qualifications/validations today is what level of probability of survivors or SAL will be acceptable throughout the world.

In Europe the absolute minimal SAL is 10^{-6}. In the US there is essentially a dual SAL standard of 10^{-3} for topical products and 10^{-6} for invasive products. The alternative SAL is essentially an economic necessity for radiation sterilisation, because it allows for many materials to be irradiated without deleterious effect. Harmonisation of world-wide sterilisation requirements was an important issue. This harmonisation was difficulty. It was tested by the world sterilisation community, but at the end of the test, some aspects of sterilisation were harmonised while others were not. For example much of medical device sterilisation was harmonised but not drug and pharmaceutical sterilisation.

For other details and further information on EO sterilisation validation, see article by Rogers [36].

7.2.7 Radiation Validation

When choosing irradiation to sterilise medical devices, manufacturers consider the European Medical Devices Directive. AAMI members have been working with ISO and the American National Standards Institute (ANSI) to produce voluntary, harmonised guidance for validation and testing methods. The European Union has also enacted mandatory standards for its members. Although efforts toward harmonisation are still ongoing, there have been sufficient changes in the regulatory guidance to generate questions as to how to achieve simultaneous compliance with these various standards.

7.2.7.1 Guidance Standards

Section 820.752 of the FDA's quality system regulation requires that 'all processes

used to produce medical devices be validated'. Manufacturers may follow accepted sterilisation validation guidelines by selecting SAL of various product types (**Table 7.6**), and standards based upon specific sterilisation methods (**Table 7.7**).

All radiation validation methods are typically bioburden based:

- All micro-organisms have a defined resistance to radiation.
- Validation of a dose is based on the resistance of bioburden (collection of micro-organisms).
- Radiation dose needed for sterilisation is not related to the pathogenicity of an organism.

In the following sections, a history of various radiation standards is provided to see when and where different sterilisation and sterility outcomes might have occurred or will yet occur. Radiation standards have resulted in numerous changes and revisions over a relative short period of time. Previous standards have been superseded by new standards and sometimes, significant changes and practices have resulted. The difference between the earlier historical Kilmer method and the final standard(s), have both resulted in virtually the same traditional dose of 25 kGy.

Both have low sample sizes and both have a remote chance of finding low-level contamination in a lot. However, the change(s) in standards and sample sizes between this historical Kilmer standard and the VD_{max} method of the latest ISO 11137-2 [3] have been profound to justify 25 kGy dose. For example, a review of the different sterilisation methods to justify 25 kGy is shown in **Table 7.9**.

Table 7.9 Different radiation validation approaches

Samples	**Methods**			
	Kilmer	**AAMI ISO TIR 13409 [6]**	**AAMI ISO TIR 15844 [37]**	**ISO 11137 Method 3 [3-5]**
Initial sample sizes	10	66-306	66-306	10
Sample sizes for audit	10	20-100	20-100	10

Note: AAMI TIR 35 [38], provides alternative sampling plans to those provided in ISO 15844 [37], to economise in relation to the number of product units required while maintaining assurance of attaining the desired SAL [38].

Is the past a prologue to the future, or is it that, the more things change, the more they remain the same? The best and worse may yet be discovered. Accuracy of bioburden

type and/or resistance may be the best, while the futility of sample size and sterility may be the worse, for what it can detect and what it cannot detect.

7.2.7.1.1 ANSI/AAMI/ISO 11137-1, Sterilisation of Health Care Products - Radiation - Part 1: Requirements for Development, Validation and Routine Control of a Sterilisation Process for Medical Devices, 2006

ISO 11137-1 specifies requirements for the development, validation and routine control of a radiation sterilisation process for medical devices. Although the scope of ISO 11137-1 is limited to medical devices, it specifies requirements and provides guidance that may be applicable to other products and equipment. ISO 11137-1, covers radiation processes using irradiation with the radionuclide ^{60}Co or ^{137}Cs, a beam from an electron generator or a beam from an X-ray generator. ISO 11137-1 does not:

- Specify requirements for the development, validation and routine control of a process for inactivating the causative agents of spongiform encephalopathies such as Scrapie, bovine spongiform encephalopathy and Creutzfeldt-Jakob disease or virus infectious agents.
- Detail specified requirements for designating a medical device as sterile.
- Specify a quality management system for the control of all stages of production of medical devices.
- Specify requirements for occupational safety associated with the design and operation of irradiation facilities.
- Specify requirements for the sterilisation of used or reprocessed devices.

Part 1 does emphasise the attention to be given on other aspects of the sterilisation process from raw materials to final sealed, processed product packaging, such as:

- The microbiological status of raw materials.
- The validation and routine control of any cleaning and disinfection procedures used on the product.
- Exertion of control of the environment in which the product is assembled, manufactured and packaged.
- The control of equipment and processes.
- The control of hygiene and personnel.

- The materials and manner in which the product is packaged.
- The environment and/or conditions under which the product is stored.

7.2.7.1.2 ANSI/AAMI/ISO 11137-2, Sterilisation of Health Care Products - Radiation – Part 2: Establishing the Sterilisation Dose, 2012

ISO 11137-2 has been developed to establish the correct sterilisation dose for medical equipment. This international standard takes an in-depth look at the methods aligned with the two approaches in ISO 11137-1, including dose setting to get a product specific dose; and dose substantiation to confirm a preselected dose of 25 kGy or 15 kGy. These methods are based on a probability model that demonstrates the inactivation of microbial populations after radiation – such as gamma and ionising radiation – and involves performance of tests of sterility on medical instruments. ISO 11137-2 helps the user to choose and test the right product for the verification of sterilisation doses. It also looks at the manner of sampling to ensure quality control, as well as microbiological testing. This standard includes various procedures for dose setting using bioburden information (Method 1), and dose setting using fraction positive information from incremental dosing (Method 2), and maximum verification, VD_{max} (Method 3).

ANSI/AAMI ISO 11137-2 specifies the methods of demonstrating the minimum dose required to achieve a specified requirement of sterility and methods to substantiate dose(s) of 25 kGy or 15 kGy, as the appropriate sterilisation dose to achieve a SAL of 10^{-6}, typically. It also specifies methods of dose auditing to determine the continued effectiveness of the sterilisation dose. ISO 11137-2 also defines product families for dose establishment and dose auditing.

Before, ANSI/AAMI ISO 11137-2, the historical ISO standard regarding irradiation of medical devices was simply ANSI/AAMI/ISO 11137, *Sterilisation of Healthcare Products - Requirements for Validation and Routine Control -Radiation Sterilisation*. It did not provide for the use of BI but used evaluation of bioburden, unlike for drugs, which accepts the use of *B. pumilus* E601 (ATCC 27142), as a BI. *B. pumilus*, a spore former, was used for many years as a BI to test for sterility. Its use today has been discontinued for medical devices, but not necessarily for drugs. The radiation resistance of *B. pumilus* may be generally lower than the dose required to achieve a 10^{-6} SAL at 25 kGy, unless manipulated in anoxic conditions or other conditions. BI may not accurately represent the natural form of bioburden on a product, however, some bioburdens consisting of spores (e.g., *Bacillus odysseyi*, *B. sphaericus or B. subtilis*) which could be made into BI on dense surfaces (e.g., metal wires, metal cores) could be more resistant than paper strips or their natural bioburden, or routine

dosimeters as such. Other spores such as natural *Clostridium* species which tail non-logarithmically may be more accurate or definitive due to their inactivation behaviour than dosimeters, depending upon the SAL level and the initial bioburden counts.

The current medical device standard for radiation relies on bioburden, not BI, however, radiation sterilisation dose setting experiments use bioburden information cited under the AAMI standards. Very early radiation qualification used the Kilmer method with allowed one to qualify a 25 kGy dose with a small number of products and little bioburden information. This was replaced by AAMI Method 3A - dose setting for infrequent product (25 kGy) and Method 3B - dose setting for small lot sizes and infrequent production under the earlier AAMI ISO 11137 [10]. These were replaced by either ISO 13409 [6] or AAMI TIR 27 [7].

Some early techniques developed were the AAMI ST 31 [39] and ST 32 [40] that were adopted into ANSI AAMI ISO 11137 [41]. The validation method in ANSI AAMI ISO 11137 was referenced in EN 552, *Sterilisation of Medical Devices—Validation and Routine Control of Sterilisation by Irradiation*.

Additional approved ISO and European Normalisation standards (EN) focus on test methods to support validation programmes. These documents address bioburden enumeration (ISO 11737-1 [14]; EN 1174-1 [42]) and sterility testing (ISO 11737-2 [1]). Three further EN standards - EN 1174-2 [43], and EN 1174-3 [44], provided specifics on sampling methods, validation of test methods, and test methodology. Technical comparison of these documents shows some uniformity in testing methods, including incubation times, media selection, and temperature conditions used during validation and routine audit, bioburden and sterility testing.

The current AAMI/ISO documents setting(s) standard have more than three dose setting approaches they are: Method 1, Method 2 and Method 3 (see **Table 7.10**).

Table 7.10 Various guidance documents used for radiation sterilisation process validation

Document	ISO standard
Estimation of population	11737-1 (2009) [14]
Microbiological sterility	11737-2 (2009) [2]
Validation sterilisation - Methods 1 and 2	11137-2 (2012) [3]
Validation sterilisation - small lots and single batch - VD_{max} dose	Method VD_{max} 15 kGy and 25 kGy incorporated in ISO 11137-2 (2012) [3] Method 3

- *Method 1*: Dose setting using bioburden information outlined in ISO 11137-2 [3]

Method 1 (from ISO 11137-2) determines a lower sterilisation dose to be necessary for determining the bioburden population. This method should be used when the lowest possible sterilisation dose is desired due to cost considerations, because of materials sensitive to irradiation, or when the bioburden count is above 1,000 CFU. It requires demonstration of the average bioburden from three consecutive lots and a verification dose experiment is required, from one of the lots or from the one that is the highest. In order to verify a dose for a SAL of 10^{-6}, one million products would need to be irradiated and sterility tested. Method 1 requires that for a SAL of 10^{-2} 100 products must be used for the verification experiment.

Sensitivity to radiation may vary considerably from one micro-organism to another. This basically depends on the type of bacteria or virus (the species or strain). Radiosensitivity is numerically evaluated using the D-value. This corresponds to the radiation dose required to reduce the initial bacterial population to 10%. Most bacterial species have D-values below 10 kGy. The more resistant bacteria (spore-forming bacteria) can have D-values as high as 30 kGy.

To perform bioburden measurements accurately a recovery efficiency test needs to be performed. It measures the ability of a specified technique to remove micro-organisms from the product.

To perform a valid sterility test, a B/F test is required to be performed with selected micro-organisms to demonstrate the presence of inhibitory substances or conditions, that affect the multiplication of these micro-organisms. This test must be re-done if any changes are made to the product or the condition of the product. It is recommended that even without changes that the test should be repeated every one to three years to account for any changes in raw materials or suppliers. The number of samples required for this testing is typically is between three and six. As the bioburden recovery validation generally involves three to six samples, it determines the efficiency of the bioburden testing method. Based upon the materials used for manufacturing the device and the complexity of its design, there is a possibility that a specific bioburden test can only remove a fraction of the existing micro-organisms from the device for bioburden counting. The recovery test makes an allowance for the residual organisms remaining on the device after the assessment, yielding either a percentage recovery or a recovery factor which is used to adjust the bioburden counts. Because the results gained from the AAMI 11137 [10] method 1 rely on mathematical calculations based on the device bioburden, using the correct bioburden count is critical. An underestimated bioburden results in a lower verification dose, risking validation failure and product recall.

For example, if a bioburden test results yield 750 CFU per device, knowledge of the method's efficiency may be critical to the selection of the validation test method. If the 700 CFU total is a non-adjusted or uncorrected number, but a parallel recovery study of the device were to indicate that the bioburden test method recovers only 40% of the organisms on the device, then the assayed bioburden must be divided by 0.4, or a correction factor, resulting in an adjusted bioburden of 1,750 CFU, which would be well over a maximum limit of 1,000, for example.

Method 1 is typically called the bioburden method because the number of organisms on the product must be determined prior to sterilisation. Ten samples from each of three lots are tested for a total of 30 samples. The bioburden results are used to calculate an experimental radiation dose called the verification dose, which is anticipated to yield an SAL of 10^{-2}.

An additional 100 samples from a single production lot are exposed to this dose and sterility tested. A B/F test is also conducted with selected micro-organisms to examine whether the presence or absence of various other substances inhibits their growth. Additional samples are required for this test.

In the verification step, if there are no more than two positive (non-sterile) cultures in the 100 sterility test samples, the validation is considered successful, and a routine SAL sterilisation dose is calculated based upon the original bioburden data.

Method 1 generally requires 136–146 samples and is usually considered the method of choice because Method 2 requires a much larger number of test samples.

The steps in Method 1 are:

- Select the SAL and select 10 product samples from three independent production batches (or 30 samples) The samples must be representative of routinely sterilised products.
- Determine the average microbial load of the three batches of 10 items (method based on ISO11737-1 [14]).
- Obtain the verification dose (referring to table 5 of ISO11137-2 [2]).
- Conduct verification dose experiments on 100 irradiated pieces (method based on ISO 11737-2 [2]).
- Interpret the results.
- Establish the sterilisation dose based on the results (maximum of two positives out of 100 pieces).

The advantage of this method is that it enables any sterilising dose to be validated.

When a large or costly device is used, the manufacturer may not have to use the full number of samples noted previously. For example, instead of using a full complement of complete finished devices, a large device might be divided into five portions equal in anticipated bioburden makeup, both in overall number of organisms and number of types. For example, if 20 such devices were be cut into five pieces, yielding 100 portions, the sample item portion would be equal to 0.2% of the original device.

If a large device consists of several dissimilar components, each with a different level or mix of bioburden organisms, this practice will not work. There are, however, often other ways to reduce the total number of devices needed to fulfill Method 1 requirements. If a sample item portion of less than 1.0 is chosen, it must be validated to document the bioburden equivalency by performing a sterility adequacy test with 20 non-sterilised units that yield at least 80% positives.

- *Method 2*: Dose setting using fractional positives

Method 2 is used to determine the radiation sterilisation dose necessary to achieve a chosen SAL for the resistance of micro-organisms to radiation as they occur on the product (see **Table 7.9**) for examples. No assumptions are made regarding the resistance of the microbes contaminating the product, and no initial bioburdens are required. The process is comparatively complex and a number of calculations are used to determine this dose.

In method 2 the bioburden resistance method requires the manufacturer to determine the radiation resistance of the organisms actually resident on a product. In Method 2, there are two sub-methods (Method 2A and Method 2B), the former is more general while the latter is used for products with small, low and consistent bioburden. In this method, there are two device units from each of three production lots, which are exposed to incremental radiation doses (e.g., groups exposed to 2, 4 and 6 kGy, and so on) and then sterility tested. The results are used to determine a verification dose expected to yield an SAL of 10^{-2}. A group of 100 devices is then exposed to this verification dose. For example, if 0-15 of the 100 units are non-sterile or positive, the data are used to calculate a routine SAL sterilisation dose.

A very brief outline of the process is given next.

A minimum of 280 samples from three separate batches (840 in total) must be available for analysis. Twenty product items are irradiated at each dose level from each of three batches. A series of not less than nine dose levels, increasing by 2 kGy increments (e.g., 2, 4, 6, 8, 10, 12, 14, 16 and 18 kGy) are used, and each of the delivered doses is monitored. All the irradiated product items are then individually sterility tested.

Using the sterility test results obtained, the calculation outlined in the Standard is used to determine an initial estimate of the dose required to achieve a SAL of 10^{-2}. The sterility test results also determine which batch is to be used for further sterility testing. A sample of 100 product items from this batch are irradiated at the calculated verification dose and individually sterility tested. The results of all the sterility tests are used to calculate the required minimum sterilisation dose.

Method 2 may be chosen because of its capability to validate a lower dose than with Method 1 (e.g., 11 kGy compared to 15 kGy). Method 2 is determined by the natural micro-organisms' average resistance to radiation on a product, whereas Method 1 is based on a theoretical model population that may or may not be comparatively resistant to radiation as the organisms being studied. For example, based on the ability of DNA ligase to repair radiation caused DNA damage, it could conceivably take a smaller dose of radiation to destroy the less sensitive organisms on the actual device than it would to inactivate the model population used to establish the Method 1 doses. Thus, a Method 2 evaluation on such a device would allow a lower minimum routine sterilisation dose [7]. Conversely, if the ambient bioburden organisms would require more radiation than that indicated by the Method 1 table 5, Method 2 (**Table 7.11**) can be an alternative method for validation.

The steps in Method 2A and Method 2B are described next.

Method 2A: This determines the dose using information about the proportion of positives from the incremental dosage in order to determine an extrapolation factor:

- Select the SAL and obtain samples of the product (at least 280 samples for two independent production batches). The product samples must be representative of the products routinely sterilised.
- Conduct the incremental dose experiments - irradiate 20 pieces at incremental doses of 2 kGy beginning with the 2 kGy dose and using at least nine values. This is to be done for each of the three batches involved. Perform a sterility test on each of the products.
- Conduct verification dose experiments - irradiate 100 pieces at the verification dose and perform a sterility test on each of the products. Then examine the results.
- Establish the sterilising dose based on the results. This method is seldom used because of the large number of products and tests required to validate a sterilising dose.

Method 2B: Determines the dose using information about the proportion of positives from the incremental dosage in order to determine an extrapolation factor. This is applicable if:

- The entire product is tested (sample item portion = 1).
- After irradiation at any incremental dose, the number of positive sterility tests observed does not exceed 14.
- The first non-positive shall not exceed 5.5 kGy.
- Select the SAL and obtain samples of the product (at least 260 samples for three independent production batches).

The product samples must be representative of the products routinely sterilised:

- Conduct the incremental dose experiments - irradiate 20 pieces at incremental doses of 1 kGy beginning with the 1 kGy dose and using at least eight values. This is to be done for each of the three batches concerned. Perform a sterility test on each of the products.
- Conduct verification dose experiments - irradiate 100 pieces at the verification dose and perform a sterility test on each of the products.
- Examine the results.
- Establish the sterilising dose based on the results.

This method is seldom used because of the large number of products and tests required to validate it, however, it provides for a very low sterilising dose, which tissue allografts require. For example, the method 2B has been used for this validation following ISO Standard 11137 [3-5]. Three hundred allografts, for example would be collected from three defined production batches and would be dosed using a series of five incremental doses, starting at 1 kGy and increasing by 1 kGy until 5 kGy was reached. Following each sterilisation dosing, each allograft test article would be sterility tested to identify any viable (surviving) micro-organisms. The number(s) of positive sterility samples would be used to calculate the verification dose (e.g., 1.27 kGy), which would then verified by an additional batch of 100 allografts. The results from this validation must indicate that the SAL level of 10^{-6} on human allograft tissue using gamma ^{60}Co radiation that could be achieved when a dose, of at least 9.2 kGy is employed.

Another consideration involves identifying ways to lower the bioburden levels and revalidating using Method 1.

- *Method 3*: The VD_{max25} option (formerly AAMI TIR 27 [7], now in ISO 11137-2 [3]) is convenient when a company wants several product lines sterilised at the same minimum dose, when a product is expensive to make, or for companies with markets where a 25 kGy dose is the accepted standard. Furthermore, the validation is less expensive because fewer tests are necessary. Bioburden counts

must be 1,000 CFU or less. For 15 kGy the bioburden is equivalent or less than 1.5 CFU.

Table 7.11 Traditional validation radiation methods

Large lots and frequent production	**ISO 11137-2: Method 1 [3]**	**ISO 11137-2: Method 2 [3]**
ISO bioburden < or > 1,000 CFU	Yes	Yes
Radiation tolerance < or > 25 kGy	Yes	Yes
Lot size >500	Yes, for frequent or infrequent production	Yes
Lot size <500	No	No
Routine production	Yes	Yes
Low bioburden resistance	Yes	Yes*
High bioburden resistance	No	Yes
Initial sample test size	• 10 bioburden per lot • Total of 30 for 3 lots • 100 samples per verification dose • Total samples: 330	• 160-180 units for 8 or 9 doses • 100 units per verification dose • Total samples: 840
Audit sample test size - includes 10 for bioburden	110	110

* The method can apply to both, it does not matter if the bioburden is small or large.

In practice, the VD_{max} dose is calculated using an average of 10 product samples, from each of three consecutative lots, and the 10 product samples of these independent lots are subjected to a sterility test after exposure to a verification dose. If no more than one positive test in 10 fails, then the pre-selected sterilisation dose (e.g., 15 or 25 kGy) is substantiated.

This part of ISO 11137 specifies methods for determining the minimum dose needed to achieve a specified requirement for sterility and methods to substantiate the use of 25 kGy or 15 kGy as the sterilisation dose to achieve a SAL, of 10^{6}. This part of ISO 11137 also specifies methods of sterilisation dose audit to be used to demonstrate the continued effectiveness of the sterilisation dose. This part of ISO 11137 defines product families for sterilisation dose establishment and sterilisation dose audit. This method is used to establish a minimum sterilisation dose of 15 kGy for products with an average bioburden <1.5 CFU or 25 kGy (for products with an average

bioburden <1,000 CFU. The number of devices required to conduct a validation is the same as indicated in AAMI TIR 33 [8].

The method VD_{max} (for frequent or infrequent production batches) is used to establish a minimum sterilisation dose for products manufactured frequently or infrequently in large or small batches. Minimum sterilisation doses of 15, 17.5, 20, 22, 25, 27.7, 30, 32.5 or 35 kGy are selected based on the product's average bioburden. For validation of a single lot, 10 products are tested for bioburden and then a verification dose resistance experiment is performed on 10 products irradiated at the calculated verification dose (or 20 products if growth occurs in two of the first 10 tested). For frequently produced lots, the initial validation includes bioburden testing of 10 products from each of three separate lots and then a verification dose resistance experiment on 10 products from one lot. Revalidation consists of quarterly bioburden testing of 10 products followed by a verification dose resistance on 10 products from the same lot.

- AAMI ISO TIR 15844 [37] (historical) *Sterilisation of Health Care Products - Radiation Sterilisation - Selection of Sterilisation Dose for a Single Production Batch*.

The method describes selecting a sterilisation dose to be used for radiation sterilisation of a single production batch. It is intended to be used in conjunction with the requirements contained in AAMI ISO 11137 [10] regarding the manufacture and control of products intended for radiation sterilisation. As a result of the higher incidence of assumed false sterility test failures at a lower bioburden with historical ISO 11137 [3-5], a VD_{max} approach for 25 kGy was presented in AAMI TIR 27 [7].

The procedure for establishing a 25 kGy minimum sterilisation dose for small or infrequent production batches is not fully harmonised. Validation was previously addressed in AAMI ST 32 [40] as Method 3. In 1997, this document was superseded by an enhanced method in AAMI 13409 [6]. While AAMI/ISO 13409 was approved, it has essentially been replaced by ISO 11137-2 [3].

The VD_{max} method in AAMI TIR 27 was eventually superseded by Method 3 of ISO 11137-2 [3] (**Table 7.12**). It is used to establish and verify maximum doses, which were implemented for the original overkill approach by using a dose of 25 kGy. The VD_{max} results in fewer assumed false positive sterility tests, particularly at a lower bioburden, but one will potentially miss the presence of resistant contaminating microbes that may be present. If one doesn't want to see a microbial presence, then it's not a problem because statistical assurance ought to minimise and prevent them from ever surviving a full process. Only experience and time will determine if sterility assurance is truly maintained with this methodology because, as Mark Twain once

said: ‘*there are three kinds of lies: lies, damn lies, and statistics*’, however, the classical/traditional ISO 11137-2 [3] and AAMI ISO TIR 13409 [6], are likely to pick up the presence of resistant organisms if they exist; other methods such as VD_{max15} and VD_{max25} may not.

Table 7.12 Newer validation methods using radiation – ‘relative’ differences for small lots

Small lots and infrequent production	ISO 11137 -2 method 3 [3] VD_{max} (25 kGy)	ISO 11137-2 Method 3 [3] VD_{max} (15 kGy)	AAMI TIR 33 [8] VD_{max} (15-35 kGy)	AAMI ISO TIR 15844 [37]*
Bioburden <1,000	<1,000 CFU	<1.5 CFU	<0.1 up to 440,000 CFU	<1,000 CFU
Radiation tolerance	25 kGy	15 kGy	15-35 kGy	See ISO 11137
Lot size <500	Yes	Yes	Yes	No
Lot size - large	No	No	No	Yes >1,000
Infrequent production	Yes	yes	Yes	Single lot
Initial sample size	10	10	10	110
Audit size	10	10	10	100

*ISO 15844 is an older method, and has been withdrawn, but may still be used conservatively and for a singular batch. This method may be used to substantiate a sterilisation dose of 25 kGy for any of the following situations:

- A single batch of product units,
- Initial production of a new product while the sterilisation dose is being established by another method, and
- Routine production of small batches.

Information collected in applying the method of dose substantiation described in this technical specification may be applicable in meeting the product qualification requirements for the sterilisation dose selection of ISO 11137-2 [3].

Method 3 VD_{max} of ISO 11137-2 in **Table 7.12** may be limited by batch size or production frequency, and, like method 1, it is based on the standard distribution of resistance. The major difference between VD_{max} and the standard Method 1 is that the verification is performed at a SAL of 10^{-1} using only 10 product units for a verification dose, as opposed to the 100 product units required by Method 1.

Steps in performing Method 3 are:

- Obtain product samples.

- The product samples must be representative of routinely sterilised products.
- Determine the average microbial load of 3 batches of 10 pieces each.
- According to the table of ISO 11137-2 [3], determine the verification dose.
- Conduct verification dose experiments - irradiate 10 products at the verification dose and perform a sterility test on each of the products.
- Interpret the results: Accept the 25 kGy sterilisation dose if zero or one of the 10 pieces is positive.
- Conduct verification dose confirmation experiments if two are positive.
- Do not accept the verification if there are more than two positives.
- Forty products are required for this method. This method is only viable for validating sterilising doses of 15 or 25 kGy.

With the revision of ANSI AAMI ISO 11137 [41] and the supplementation of method 3 within ISO 11137-2 [3], it has been revised to include the VD_{max} approach of AAMI TIR 33 [8], and some older methods are either eliminated or reduced.

To initiate VD_{max}, determine the average bioburden level by randomly selecting 10 product units from each of three production batches. Using a table, of bioburden estimates and corresponding irradiation doses, the average bioburden value from the three production batches is used to establish the verification dose. Ten product units are then exposed to the indicated dose level and a test of sterility is performed. If no more than one positive response is observed, 25 kGy is substantiated as the sterilisation dose.

The incorporation of a single lot validation for Method 1, have eliminated or reduced the need to use older AAMI or AAMI/ISO documents such as AAMI TIR 33 [8], AAMI ISO TIR 15844 [37] and AAMI ISO TIR 13409 [6], unless there is a good reason for so doing. For example, ISO 11137-2 and TIR 33 are additional methods including Method 2 (incremental dosing) and VD_{max} for selected doses of 15-35 kGy (in 2.5 kGy increments). Each method has specific limitations and requirements that must be fully investigated before selection. Taking larger sample sizes increases the likelihood of finding a higher chance or probability of contamination (see **Tables 7.3** or **7.4**).

7.2.7.2 Dosimetry

ISO 11137-3, *Sterilisation of Health Care Products - Radiation - Part 3: Guidance on Dosimetric Aspects* [4], this document gives guidance on the requirements in

ISO 11137-2 Parts 1 and 2 relating to dosimetry. Dosimetry procedures related to the development, validation and routine control of a radiation sterilisation process are described. Measurement of dose is the key to the radiation sterilisation process. With an accurate and precise dose delivered to the product whose preliminary dose has been determined, release by dosimetry provides a presumptive verification that a SAL dose has been delivered. Radiation dose is typically measured throughout all stages of development, validation, and routine monitoring of the process. Dose mapping of product carriers and transportation systems is performed during validation. Dose mapping varies between electron beam, and gamma and X-ray sterilisation, but what is necessary is to determine minimum and maximum dose locations.

Dose mapping, is the first step of the PQ, to verify that every product in the sterilisation container receives a dose complying with the specifications (for example, 25-40 kGy). As the dose received by the products can depend upon the density of the products and their position in the sterilisation container, before performing the dose mapping validation, the product loaded pattern should be established. With this product loaded pattern, dosimeters will be placed to measure the dose received by the products at different points of the sterilisation container. The dose mapping verifies that the minimum dose and the maximum dose can be achieved:

- The minimum dose is that part of the product PQ, which makes it possible to validate the minimum irradiation dose required to sterilise the product (i.e., to guarantee a SAL of 10^{-6}).
- The maximum dose is that part of the validation procedure which verifies by means of various kinds of tests that the product and material characteristics are not degraded by irradiation, even at the maximum dose.

7.2.7.3 Routine Dose Audits

To conform to the standard, the performance of the sterilisation process must be monitored periodically. The standard requires dose audits to be performed quarterly to confirm that the current bioburden profile is equivalent to the one at the initial dose setting exercise. These audits provide assurance that the original manufacturing conditions have not changed and the bioburden level is under control. ANSI AAMI ISO 11137 [10] describes quarterly dose audits for products already validated by the standard. An audit must be performed at a defined and documented frequency. The audits are performed to demonstrate the continued validity of the sterilisation dose. An audit should be performed following any change that could significantly alter the level or nature of the bioburden (for example a bioburden in the stationary growth stage, may be more resistant than that in the log stage); changes in the way a

product is made (e.g., under anoxic conditions), in the materials used, or a change in the manufacturing facilities may also require a dose audit. Nevertheless, in the absence of any changes, the audit must be performed, at three month intervals to detect any changes in the bioburden that could require an augmentation in the sterilisation dose. If for any reason the dose established at the initial validation was augmented, the verification dose experiment should be performed at that augmented dose.

Dose auditing generally consists of three steps: (1) environmental monitoring review; (2) bioburden testing; and (3) verification dose experiment.

Environmental monitoring of the manufacturing facility is performed to track and investigate any changes in bioburden numbers or types. This monitoring includes air sampling, water sampling, and manufacturing surface testing. Environmental monitoring is an on-going programme to verify the production.

Bioburden testing determines the population of viable micro-organisms on a given sample (product), and not necessarily the package. In this phase of the dose audit, 10 samples are taken from a production batch (lot) to determine the bioburden count, or CFU. The results are compared with the bioburden counts that were initially demonstrated at the time of the original validation. If the bioburden count is significantly higher than the initial bioburden, then the process is unlikely to pass a sterilisation dose audit. It is recommended that a Gram-stain be performed at the time of bioburden testing. This is helpful in identifying if the micro-organisms have changed, or if there are any resistant types.

The verification dose experiment is performed to demonstrate whether or not a change in the sterilisation dose is needed. Typically, 10-100 samples are evaluated, unless initially validated by other (earlier) method(s). The verification dose experiment is performed at the dose determined at the time of validation. If after the completion of the sterility test, two or fewer positive sterility samples tests are obtained, the original sterilisation dose is acceptable and no action is required. Positive sterility samples are test samples, which exhibit detectable microbial growth after incubation. If after completion of the sterility test, three or more positive sterility samples are obtained, the original sterilisation dose is not acceptable and further action is required. Dose augmentations may be appropriate, see ANSI AAMI ISO 11137 [10].

If at anytime the verification dose is augmented, the next verification dose audit must be performed at the newly established verification dose. Ten to 100 samples are irradiated at the previously determined verification dose and tested for sterility.

An example of a routine periodic dose audits, is the VD_{max25} method, the procedure for dose audits is:

- Obtain product samples – these must be representative of routinely sterilised products.
- Determine the average microbial load of one batch of 10 pieces.
- Conduct the verification dose experiments - irradiate 10 pieces at the verification dose and perform a sterility test on each of the products.
- Interpret the results: accept the 25 kGy sterilisation dose if there is zero or one positive out of 10 pieces. Perform an audit on the verification dose to confirm if there are two positives. Do not accept the verification if there are more than two positives.

The frequency with which sterilising doses are audited must be justified and documented. According to ISO 11137 [3-5], the interval between dose audits is three months. This frequency can be increased to an interval of 12 months but only with the following justification: there should be a minimum of 4 consecutive satisfactory dose audits, and microbial load determinations should be made every three months, and the medical device should be manufactured according to ISO 13485 [45] standards. Alternative dose sample sizes may be found in AAMI TIR 35 [38].

Other audit considerations, may include an audit of the dosimetry system being applied and used, product handling, loading and dose source.

7.2.7.4 Conclusions and Future Considerations

While radiation validation methods based upon assumptions of distribution of resistances (Method 1 and Method 3 VD_{max}) choose to select sterility sample sizes of 10-100 samples, these sample sizes are hardly large enough to reliably measure a failure rate of 1/1,000,000 devices or less devices with low levels of contamination rate such as 0.1% (see **Table 7.4**). For example with only 10 samples there is a 99% chance of never picking up any contamination at a level of 0.1%

What would be more meaningful would be to take sample sizes greater than 10 for bioburden in order to see what types of microbes may initially be present, that may not be found or determined during sterility testing, (e.g., 10-100) depending upon the production size or simply any batch size.

Also knowledge of more micro-organisms (e.g., anaerobes) should be incorporated. To test sterility apply an equivalent number of samples for product sterility using two different growth media: SCDM and an anaerobic media such as FTGM, rather than just try to grow aerobic organisms. Apply a greater sample size depending

upon the potential bioburden population and type of microbial resistance, if necessary, determined from a larger bioburden sampling. Again, sterility testing on only 10-100 samples doesn't even come close to reliably measuring a failure rate of 1/1,000,000 devices with low contamination rates, or even less devices with even lower contamination rates. Since it is a bioburden method, we should know the bioburden more closely and intimately, rather than making assumptions and generalisations. We have many nosocomial infections in hospital, where their sources have not been fully determined, including viral infections. For example, there are a lot of *Acinetobacter* infections that appear in hospitals, and particularly during wars, without knowing their 'initial or original source that give sigmoid survival curves in response to irradiation, rather than linear log responses. Knowing the bioburden is important, for example, physiological age plays a role in radiation resistance, with microbes in their stationary growth stage, being more resistant than in their log phase of growth. Radiation resistant *Acinetobacter* have been isolated from cotton, for example. Irradiation has also been known to cause induction of release of bacteriophages from *Acinetobacter* and the role of these phages in the transfer of nucleic acid among organisms prior or during electron beam or gamma irradiation is not known. Resistant melanised fungi are many more times radiation resistant than non-melanised fungi. Spores are typically more resistant to heat and gaseous sterilants.

A sterilistion dose of 25 kGy currently provides no guarantee of sterility when it comes to virus contamination and inactivation. Lower doses of less than 25 kGy (e.g., 15 kGy) may further provide even less guarantee of sterility with viruses.

7.2.8 Hydrogen Peroxide, Ozone and New Sterilisation Agent or Process

Typically validation of any sterilisation method is demonstration of a 10^{-6} probability of a survivor, against a reference resistant spore challenge as per an applicable standard, however, the validation of a non-traditional method may be more encompassing than the use of an unspecified standard. The general criteria for characterisation of a non-traditional sterilising agent for development, validation and routine control of a sterilisation process requires IQ, OQ and PQ qualifications. These criteria can be specific as found in ISO 14937 [31]. It is intended to be applied by process developers, manufacturers of sterilisation equipment, manufacturers of products to be sterilised and organisations responsible for sterilising medical devices.

ISO 14937 is an international harmonised standard approved by ISO, AAMI and ANSI. Use of the ISO 14937 guidelines provides a structured approach consistent with established sterilisation validation processes that can be used whenever a specific standard is not appropriate, such as for H_2O_2, O_3 sterilisation, and any new or novel methods.

The ISO 14937 scheme may serve as a template for documentation for design and process validation, and includes cleaning of reprocessables:

- Cleaning agent characterisation
- Process and equipment characterisation
- Product definition
- Process definition
- Process validation, which includes: IQ, OQ and PQ
- Routine monitoring and control
- Product release
- Assessment of change

The ISO 14937 [31] standard describes different methods to document process efficacy/product sterility.

Initially when dealing with a new process or non-traditional method, it is necessary to determine the effectiveness and efficacy of the agent and process against existing microbes that must be inactivated by the sterilisation agent. This begins by obtaining bioburden data as well as applicable reference microbes that may be recognised as resistant to the agent or process. This microbiocidal effectiveness should establish whether a bacterial spore can be used as a representative model of high resistance during process characterisation studies.

7.2.8.1 Selection of Micro-organisms

The selection of species of micro-organisms to be used in demonstrating the microbicidal effectiveness of a sterilising agent should take account of all of the following factors:

- Known high resistance to the sterilising agent or an expectation of a high resistance from information in scientific literature or a knowledge of the mode of action of the sterilising agent.
- Known resistance to well-characterised sterilisation processes. For example 'natural' *Propionibacterium* species may be more resistant than a reference *G. stearothermophilus* under some circustances. *Propionibacterium* is a

representative of the permanent micro-organisms living on the skin surface, on it or on the *stratum corneum* or immediately under it, and may be resistant to H_2O_2.

- Representative species of aerobic and anaerobic Gram-positive and Gram-negative bacteria, bacterial spores, mycobacteria, fungi including sporulating forms and yeasts, parasites, and viruses may be included.
- Species that might be present as a result of the materials of construction of the product (mated surfaces, cellulosics) or the environment in which it is manufactured. For example cellulosic materials may increase the resistance of microbes within them to H_2O_2. Microbes within mated surfaces may be difficult for O_3 to inactivate them due to difficulties of penetration.
- Resistant species that have been isolated during estimations of bioburden undertaken on typical products to be processed. For example: typically prions show a greater resistance than spores. Typically spores are more resistant than *Mycobacterium*, with the possible exception of with glutaraldehyde. Non-lipid or small viruses have greater resistance than fungi and may be more resistant than vegetative microbes and more resistant than lipid viruses.
- Investigation of the prospective microbe selected to determine if it is inactivated in a logarithmic or non-logarithmic order of decline.
- Bioburden based method.
- Combined BI/bioburden method.
- Overkill approach.

Because of the difficulty in documenting bioburden levels in the diverse reprocessed equipment and new products, the 'overkill approach' (documented in Annex D of ISO 14937 [31] '*Approach 3 - Conservative process definition based on inactivation of reference micro-organisms*') is often selected. Further guidance on this approach is found in ISO 14161 [16] and incorporated into ISO 14937 by reference. This process utilises placement of a BI with a spore population of one million or more within the product at position(s) where sterilisation conditions are most difficult to achieve. However, the combined BI/bioburden method provides further confirmation that the BI selected is adequate, and that the BI is greater than the bioburden resistance.

When starting a new process or changing a process, the following must be considered:

- Describe the new sterilisation method or change, and its risk analysis.
- Establish the maximum acceptable process parameters or dose for the product. This should be the focus of the initial evaluations. The maximum acceptable

process parameters or dose is that which the product can be exposed to and meet its functional requirements throughout its defined lifetime or expiration date. If the maximum process parameters or dose attainable is not sufficiently above the sterilisation SAL parameters, then a specific (new) sterilisation method may not be feasible.

- Improve product performance through changes in process parameters. Recognise the differences between increases in temperature or process parameters or dose on product performance and a possible chemical reaction, and then modify those process parameters and dose to fit the proper product performance required. Reduce bioburden or bioburden resistance to improve process performance for product performance.
- Adhere to ISO 14937 [31], and any other standards that may apply.
- Make a brief description of the IQ and validation summary.
- Provide a summary of physical PQ and microbiological performance qualification(s).
- When possible use a sterilisation facility certified by a notified body such as ISO 9001 [33] or ISO 13485 [45].
- Details of the effect of the sterilisation change and verification that the device performance or efficacy is unchanged.
- The biocompatibility testing of the product will need to be considered, carried out and a packaging validation will also be needed. A sterilisation method may also require a label.
- The sterilisation method must have an appropriate SAL.

7.2.6 Choosing a Sterility Assurance Level

Selecting the SAL occurs during the dose-setting phase of radiation sterilisation validation. In many cases, the intended use of the device will dictate the need for a particular SAL. In America, there are two healthcare SAL values that are possible, 10^{-3} or 10^{-6}, However, the most commonly accepted and used SAL for invasive medical devices and internationally is 10^{-6}.

In industrial sterilisation, the first thing for ensuring medical device sterility is to determine the appropriate SAL, a measure of the probability that one unit in a batch will remain non-sterile after being exposed to the sterilant. Product lot sterility can only be expressed in terms of probability, because sterility is not absolute. For example, a

SAL of 10^{-3} means that one device in a thousand might be non-sterile. A SAL of 10^{-3} may be considered when the patient risk is negligible. Examples are products that may be topical and not invasive so are not intended to come into contact with broken skin or compromised tissue or topical products that contact intact skin or mucous membranes. Some examples of topical products are: tongue depressors, surgical drapes and gowns. Most combination devices are required to utilise a sterilisation process that achieves the higher assurance of sterility, an SAL of 10^{-6} or one non-sterile unit in 1,000,000 units or processed to a validated SAL of 10^{-6}.

What happens if there is more than one thousand or million units in a batch or lot? The worse location in the batch or within a product is used to determine the SAL. For example in electron beam treatment, if the irradiation dose is not delivered as a mono-dose, but rather as a distribution of doses, as demonstrated by dosimeters distributed during the run, the minimum portion of the dose distribution curve that receives the SAL of 10^{-6} should be applied. The portion of the product that receives the top end of the dose distribution may receive a sterility assurance level far better than one in million.

In hospital sterilisation *versus* industrial sterilisation, a sterility assurance of 10^{-6} is the standard one considered. Sterilisation of drapes and gowns would for example be sterilised to 10^{-6} of *G. stearothermophilus,* and since this thermophilic spore is more highly resistant than most mesophilic or pathogenic spores, there is a considerably greater assurance of sterility, often by several orders of magnitude.

While all parts of products within the sterile barrier packaging receive greater assurance of sterility, often by several orders of inactivating degree than the worse location to sterilise, some products and/or areas may receive less. For example, a solution of a parental solution in a container may receive a SAL 10^{-6}, the stopper may receive only a SAL of 10^{-3}, because it has a matted surface to glass, and is thus, considered to be topical and not invasive to the user.

To assess the SAL requires a closer look at the decline curve of the process. Beware of drawing a conclusion that a decline curve is 'essentially logarithmic.' If the points on the decline curve are consistently concave in nature, then the decline projects tailing, and requires longer exposure or dose than by a mere logarithmic decline. If the points on the decline curve are consistently convex in nature, then the process may have shorter exposure or dose than that typically concluded by a logarithmic decline. It is rare to obtain a logarithmic decline with a natural bioburden that may consist of multiple types of bioburden types, species and resistance. Consider selecting bioburdens that are either more predominant and/or more resistant or both, for performing D-values. According to Rahn [46], only 40% of bioburden resistances are typically logarithmic.

7.2.9 Liquid Sterilisation

The AOAC method is typically used by manufacturers to evaluate liquid chemical sterilants for which, they are seeking premarket clearance, and the FDA has officially recognised this method. *Bacillus subtilis* ATCC 19659 is the strain used in the AOAC Official Method 966.04 Sporicidal Activity of Disinfectants [47]. The FDA has officially recognised this method. *B. atrophaeus* would probably be acceptable. However, one might want to also consider *B. subtilis* ATCC 19659, the strain used in the AOAC Official Method 966.04 [47]. In either case, ate the suitability of whatever organism was chosen to use in the sterilisation process would have to be validated. The kinetics of sterilisation with liquid chemical sterilants are not as well understood and probably not as predictable as with other sterilisation methods such as EO, radiation, dry heat, or steam. For example, glutaraldehyde is one of the active ingredients most frequently found in commercial products used in the liquid sterilisation of medical devices. It may be shown that the microbiocidal activity of glutaraldehyde is affected by concentration, temperature, as well as pH, ionic strength, aging, and organic matter of serum(s).

The FDA may recommend that liquid chemical sterilants be used to process critical devices (e.g., those of animal origins) only when the device is heat sensitive and incompatible with other sterilisation methods (steam, or oxidising agents). $scCO_2$, glutaraldehyde, EO and radiation are methods used for sterilising products of animal origin. For fluid liquid sterilisation, a reliable standard to follow would be AAMI ISO 14160 [48]. Another method for non-animal origin, neutral (innate) material, would be AAMI 14937 [31]. If the device one is planning to sterilise in the process first develops something that will not require approval or premarket clearance by the FDA, or one may also want to check with the branch in the Center for Devices and Radiological Health, Office of Device Evaluation that would review the submission. They should be able to give better guidance on what would be acceptable.

It is important to recognise that the studies done by some are research and do not necessarily use the same challenge organisms or test procedures that Agencies recognise as official methods, would accept in an approval or premarket clearance application, or would use in support of regulatory action.

Most European countries, recognise only 10^{-6} SAL for a ‘sterile’ label claim. The European Pharmacopoeia Commission concurs. Therefore, the minimum SAL may be based on the regulatory requirements of the country in which the device will be sold as much as on the device’s intended use.

The radiation dose is typically set/determined, prequalified at a pre-determined bioburden estimate and then the SAL chosen.

7.2.10 Supercritical (Fluid) Sterilisation

Use of $scCO_2$ is an innovative process that may be used to sterilise products of animal origin, which eliminates the weakening effects of radiation. It also performs at a near body-temperature (32 °C) to minimise protein denaturation and yet exceeds a SAL of 1 part in 10^6. In $scCO_2$ sterilisation, the carbon dioxide (CO_2) is compressed slightly beyond its critical point, becoming a fluid with the high permeability of a gas and solvent features of a liquid. Its pressure is less than 7.6 MPa and its temperature may be less than body temperature (thermocouple = 31.1 °C); and minor changes in temperature and pressure above the critical point can yield steep variations in diffusive and solvent properties. It has no residual solvent (CO_2) outgasses at room temperature), and it provides green processing with no exposure to hazardous materials such as ethylene or radiation, and may result in reduced inflammatory reaction.

7.2.11 Validation of Aseptic Processing

Aseptic processing is used to prevent contamination of a device/drug, combination product, object or area to provide sterility of the device/drug or product, particularly where a sterile field and condition is required.

Aseptic processing is the process by which a sterile (aseptic) product (typically a drug or combination device) is packaged in a sterile container in a way that maintains sterility. Aseptic processing typically incorporates three disciplines intimately involved with it: engineering, microbiology, and statistics.

Aseptic processes must be designed and validated to minimise exposure of sterile articles to the potential contamination hazards of the manufacturing operation, before completing product enclosuring (sealing closed, stoppered opening). Limiting the duration of exposure of sterile product elements, providing the highest possible environmental control, optimising process flow, and designing equipment to prevent entrainment of lower quality air into the Class 100 (ISO 14644-1 [49]) clean area are essential to achieving a high assurance of sterility.

In aseptic processing, material flow and personnel need to be optimised to prevent unnecessary activities that could increase the potential for introducing contaminants to the exposed product, container-closures, or the surrounding environment. Equipment layout should provide for ergonomics that optimise comfort and movement of operators. The number of personnel in an aseptic processing room should be minimised. The flow of personnel should be designed to limit the frequency with which entries and exits are made to and from an aseptic processing room and, most significant, its critical area. Regarding the latter, the number of transfers into the

critical area of a traditional cleanroom, or an isolator, should be minimised. To prevent changes in air currents, introduce lower quality air, movement adjacent to the critical area(s) should be appropriately restricted.

Any intervention or stoppage during an aseptic process can increase the risk of contamination. The design of equipment used in aseptic processing should limit the number and complexity of aseptic interventions by personnel. For example, personnel intervention can be reduced by integrating an on-line weight check device, thus, eliminating a repeated manual activity within the critical area. Rather than performing an aseptic connection, sterilising the pre-assembled connection using sterilise-in-place (SIP) technology also can eliminate a significant aseptic manipulation. Automation of other process steps, including the use of technologies such as robotics, can further reduce risk to the product.

Products (and packaging) should be transferred under appropriate cleanroom conditions. For example, lyophilisation or related processes include transfer of aseptically filled product in partially sealed containers. To prevent contamination, a partially closed sterile product should be transferred only in critical areas [28]. Facility design should ensure that the area between a filling line and the lyophiliser or related equipment provide for Class 100 (ISO 14644-1 [50]) protection. Loading and transport procedures should afford the same protection.

The sterile drug or device combination product and its container-closures should be protected by equipment of a suitable design. Carefully designed barriers, curtains and rigid plastic shields are among the barriers that can be used in appropriate locations to achieve segregation of the aseptic processing line. Using an isolator system further enhances package and product protection.

Because of the interdependence of the various rooms that make up an aseptic processing facility, it is essential to carefully define and control the dynamic interactions permitted between cleanrooms. Use of a double-door or integrated steriliser helps ensure direct product flow, often from a lower to a higher classified area. Airlocks and interlocking doors can facilitate better control of air balance throughout the aseptic processing facility. Airlocks should be installed between the aseptic manufacturing area entrance and the adjoining unclassified area. Other interfaces such as personnel transitions or material staging areas are appropriate locations for air locks. It is critical to adequately control material (e.g., in-process supplies, equipment, utensils) as it transfers from lesser to higher classified clean areas to prevent the influx of contaminants. For example, written procedures should address how materials are to be introduced into the aseptic processing room to ensure that the conditions in the room remain uncompromised. In this regard, materials should be disinfected according to appropriate procedures or, when used in critical areas, rendered sterile

by a suitable method.

If stoppered vials or other containers leave an aseptic processing zone or room prior to capping or enclosure, appropriate assurances should be in place to safeguard the product, such as local protection until completion of the closure or crimping step. Use of devices for on-line detection of improperly seated stoppers can provide additional assurance.

Cleanrooms are normally designed as functional units with specific purposes. The materials of construction of cleanrooms ensure ease of cleaning and sanitising. Examples of adequate design features include seamless and rounded floor to wall junctions as well as readily accessible corners. Floors, walls, windows and ceilings should be constructed of smooth, hard surfaces that can be easily cleaned, decontaminated, disinfected or sanitised. Ceilings and associated high-efficient particulate air filter banks should be designed to protect sterile materials from contamination. Cleanrooms also should not contain unnecessary equipment, fixtures, or materials.

Processing equipment and systems should be equipped with sanitary fittings and valves. With rare exceptions, drains are considered inappropriate for classified areas of the aseptic processing facility other than Class 100,000 (ISO 14644-1 [50]) areas. It is essential that any drain installed in an aseptic processing facility be of a suitable design.

Equipment should be appropriately designed or broken down to facilitate ease of sterilisation. It is also important to ensure ease of installation to facilitate aseptic setup. The effect of equipment design on the cleanroom environment should be addressed. Horizontal surfaces or ledges that accumulate particles should be avoided. Equipment should not obstruct airflow and, in critical areas, its design should not disturb unidirectional airflow.

Deviation or change control systems should address atypical conditions posed by shutdown of air handling systems or other utilities, and the impact of construction activities on facility control. Written procedures should address returning a facility to operating conditions following a shutdown.

The following microbial tests can be used during sterile product development and scale-up:

- Bacterial challenge testing for sterilising filters.
- Aseptic processing validation using media fills.
- BET.
- Environmental microbial limits and bioburden testing.

- Antimicrobial effectiveness testing.
- Container and closure integrity testing.

7.2.11.1 Sterilisation and Statistics by Filtration Validation

Filtration is a common method of sterilising drugs or combined drug/device product solutions. A sterilising grade filter should be validated to reproducibly remove viable micro-organisms from the process stream, producing a sterile effluent. Currently, such filters usually have a rated pore size of 0.2 µm or smaller. Use of redundant (more than one) sterilising filters should be considered in many cases. Whatever filter or combination of filters is used, validation should include microbiological challenges to simulate worst-case production conditions for the material to be filtered and integrity test results of the filters used for the study.

Product bioburden should be evaluated when selecting a suitable challenge micro-organism to assess which micro-organism represents the worst-case challenge to the filter. The micro-organism *B. diminuta* (ATCC 19146) when properly grown, harvested and used, is a common challenge micro-organism for 0.2 µm rated filters because of its small size (0.3 µm mean diameter). The manufacturing process controls should be designed to minimise the bioburden of the unfiltered product. Bioburden of unsterilised bulk solutions should be determined to follow the trends of the characteristics of potentially contaminating organisms.

Micro-organisms in the challenge are important because a filter can contain a number of pores larger than the nominal rating, which has the potential to allow passage of micro-organisms. The probability of such passage is considered to increase as the number of organisms (bioburden) in the material to be filtered increases. A challenge concentration of at least 10^7 organisms per cm^2 of effective filtration area should generally be used, resulting in no passage of the challenge micro-organism. The challenge concentration used for validation is intended to provide a margin of safety well beyond that which would be expected in production.

Direct inoculation into the drug formulation is the preferred method because it provides an assessment of the effect of drug product on the filter matrix and on the challenge organism. However, directly inoculating *B. diminuta* into products with inherent bactericidal activity against this microbe, or into oil-based formulations, can lead to erroneous conclusions. When sufficiently justified, the effects of the product formulation on the membrane's integrity can be assessed using an appropriate alternative method. For example, a drug product could be filtered in a manner in which the worst-case combination of process specifications and conditions are simulated. This step could be followed by filtration of the challenge organism for a significant

period of time, under the same conditions, using an appropriately modified product (e.g., lacking an antimicrobial preservative or other antimicrobial component) as the vehicle. Any divergence from a simulation using the actual product and conditions of processing should be justified.

Factors that can affect filter performance generally include:

1. Viscosity and surface tension of the material to be filtered;
2. pH;
3. Compatibility of the material or formulation components with the filter itself;
4. Pressures;
5. Flow rates;
6. Maximum use time;
7. Temperature;
8. Osmolality; and
9. The effects of hydraulic shock.

When designing the validation protocol, it is important to address the effect of the extremes of processing factors on the filter capability to produce sterile effluent. Filter validation should be conducted using the worst-case conditions, such as maximum filter use, time and pressure. Filter validation experiments, including microbial challenges, need not be conducted in the actual manufacturing areas. However, it is essential that laboratory experiments simulate actual production conditions. The specific type of membrane filter used in commercial production should be evaluated in filter validation studies. There are advantages to using production filters in these bacterial retention validation studies. When the more complex filter validation tests go beyond the capabilities of the filter user, tests are often conducted by outside laboratories or by filter manufacturers. However, it is the responsibility of the filter user to review the validation data on the efficacy of the filter in producing a sterile effluent. The data should be applicable to the user's products and conditions of use because filter performance may differ significantly for various conditions and products.

Under simulated manufacturing conditions using bacteriological media (qualification) fill runs, some criteria are:

- When filling fewer than 5,000 units, no contaminated units should be detected.

    - A contaminated unit is considered to be a cause for revalidation, following an investigation.
- When filling from 5,000-10,000 units:
    - A contaminated unit should result in an investigation, including consideration of a repeat media fill.
    - Contaminated units are considered a cause for revalidation, following investigation.
- When filling more than 10,000 units:
    - A contaminated unit should result in an investigation.
    - Contaminated units are considered a cause for revalidation, following investigation.

For any run size, intermittent incidents of microbial contamination in media filled runs can be indicative of a persistent low-level contamination problem that should be investigated.

According to ISO 13408-1 [51], the action level for the number of positive units in media fill tests is specified as 0.1%, and the alert level is 0.05%.

When a processing line is initially qualified, individual media fills should be repeated enough times to ensure that results are consistent and meaningful. This approach is important because a single run can be inconclusive, while multiple runs with divergent results signal a process that is not in control.

Media fill studies should simulate aseptic manufacturing operations as closely as possible, incorporating a worst-case approach.

For validation, at least three consecutive, successful media fills should be performed during initial line qualification. It is recommended that a semi-annual requalification be used to evaluate the state of control of each filling line's aseptic process. All personnel who enter the aseptic processing area should participate in a media fill at least once a year. This participation should reflect their routine job responsibilities. The duration of the media fill run should adequately mimic worst-case conditions and include all manipulations without being the same run size as the production fill.

Tension exists between the concept of using worst-case conditions in a media fill and not attempting to validate unacceptable aseptic practices. Between 5,000-0,000 units should be filled during a media fill. For batch sizes of greater than 5,000 units, the number of media-filled units should be equal to the batch size. The target should be zero contaminated units.

7.2.11.1.1 Statistics

It is well known that routine compendial sterility tests have poor efficacy rates (**Table 7.13**).

For a sample size of 20 containers, the test will detect a 1% contamination rate with a <20% (18%) probability. Thus, the test will only consistently detect 'grossly' contaminated product.

Table 7.13 Probability of (falsely) passing the sterility test (98%* to 11.5%) with 20 samples**

Sample size (n)	**Percentage contaminated in lots (0.1% to 10%)**				
	0.1%	0.5%	1%	5%	10%
20**	98%*	94%	82%	35.5%	11.5%

*: percentages (%) 110.5% to 98%
**: number of samples (n) 20

Furthermore, the probability of passing a repeat sterility test after an initial failure is presumed to be higher (**Table 7.14**) because 'one or more microbial-contaminated containers' have been removed from the lot.

Table 7.14 Probability of (falsely) passing the repeat sterility test with 20 samples

Sample sizes	**Percentage contaminated in lots**				
	0.1%	**0.5%**	**1%**	**5%**	**10%**
20	0.99	0.99	0.99	0.84	0.58

The poor effectiveness of the compendial sterility test in detecting low contamination rates implies that manufacturers must adhere to validation of their aseptic processing methods, good facility design, good manufacturing practice (GMP), and training of the employees to achieve high levels of sterility assurance.

For validation, an acceptance criterion for process media fill simulations of a target

with zero contaminated containers, but with 0.1% contamination rate, has been developed. To make a statistical claim for a 95% confidence level, the number of media filled units has been specified (i.e., 4,750 filled units), and if one contaminated unit is found, the contamination is 0.1%. From a statistical point of view, if the true proportion of contaminated media filled units is $P = 0.001$, then the number of contaminated units will increase as the population size increases. When 3,000, 4,750, 6,300, 7,760, 9,160 and 10,520 units are filled, the number of turbid units is 0, 1, 2, 3, 4, and 5, respectively, at the 95% confidence level with a 0.1% contamination rate.

The ability of inspectors to consistently detect microbial growth in media-filled containers can be questioned. It has been demonstrated that as the number of units inspected, increased and fill volume decreased, the reliability of visual inspection decreased.

After a filtration process is properly validated for a given product, process, and filter, it is important to ensure that identical filters (e.g., of identical polymer construction and pore size rating) are used in production runs. Sterilising filters should be routinely discarded after processing of a single lot. However, in those cases when repeated use can be justified, the sterile filter validation should incorporate the maximum number of lots to be processed. Integrity testing of the filter(s) can be performed prior to processing, and should be routinely performed post-use. It is important that integrity testing be conducted after filtration to detect any filter leaks or perforations that might have occurred during the filtration. Forward flow and bubble point tests, when appropriately used, are two integrity tests that can be used. A production filter's integrity test specification should be consistent with the data generated during bacterial retention validation studies.

Pre-filtration bioburden should be evaluated. Manufacturing process controls should be designed to minimise the bioburden in the unfiltered product. In addition to increasing the challenge to the sterilising filter, bioburden can contribute impurities (e.g., endotoxins) to, and lead to degradation of, the drug product. A pre-filtration bioburden limit should be established.

7.2.11.2 Sterilisation with Aseptic Processing Equipment and Components

Equipment surfaces that contact sterilised drug product or its sterilised containers, packaging, or closures must be sterile so as not to alter the purity of the drug or product. Where the possibility of reasonable contamination potential exists, surfaces that are in the vicinity of the sterile product should also be rendered free of viable organisms. It is as important in aseptic processing to validate the processes used to sterilise such critical equipment as it is to validate processes used to sterilise the drug

product and its container and closure. Moist heat and dry heat sterilisation, the most widely used, are the primary processes discussed in this book. However, many of the heat sterilisation principles discussed are also applicable to other sterilisation methods.

Sterility of aseptic processing equipment should normally be maintained by sterilisation between each batch [14]. Following sterilisation, transportation and assembly of equipment, containers, closures, and packaging should be performed with strict adherence to aseptic methods in a way that protects and sustains the product's sterile state.

7.2.11.2.1 Qualification and Validation

Validation studies should be conducted to demonstrate the effectiveness of sterilisation cycle(s). Requalification studies should also be performed on a periodic basis. The specific load configurations, as well as BI and temperature sensor locations, should be documented in validation records. Batch production records should subsequently document adherence to the validated load patterns.

It is important to remove air from the autoclave or other chambers as part of a steam or other sterilisation cycle(s). The insulating properties of air interfere with the ability of steam to transfer its energy to the load, achieving lower lethality than is associated with saturated steam or gaseous processes that use steam or other penetrating agents (e.g., H_2O_2). It should also be noted that the resistance of micro-organisms can vary widely depending on the material to be sterilised. For this reason, careful consideration should be given during sterilisation validation, to the nature or type of material chosen as the carrier of the BI to ensure an appropriately representative study.

Potentially difficult to reach locations within the steriliser load or equipment train (for SIP applications) should be evaluated. For example, filter installations in piping can cause a substantial pressure differential across the filter, resulting in a significant temperature drop on the downstream side. It is recommended that BI are placed at appropriate downstream locations of the filter.

Empty chamber studies evaluate numerous locations throughout a sterilising unit (e.g., steam autoclave, dry heat oven, or other sterilising equipment) or equipment train (e.g., large tanks, immobile piping) to confirm uniformity of conditions (e.g., temperature, pressure). These uniformity or mapping studies should be conducted with calibrated measurement devices.

Heat and/or other penetration studies should be performed using the established steriliser loads. Validation of the sterilisation process with a loaded chamber

demonstrates the effects of loading on thermal input to the items being sterilised and may identify difficult to heat or penetrate items where there could be insufficient lethality to attain sterility. The placement of BI and PCD at numerous positions in the load, including the most difficult to sterilise places, is a direct means of confirming the effectiveness of any sterilisation procedure. In general, the BI or PCD should be placed adjacent to the temperature sensor so as to assess the correlation between microbial lethality and predicted lethality based on thermal input. When determining which articles are difficult to sterilise, special attention should be given to the sterilisation of filters, filling manifolds, and pumps. Some other examples include certain locations of tightly wrapped or densely packed supplies, securely fastened load articles, lengthy tubing, the sterile filter apparatus, hydrophobic filters, and stoppered opening.

Ultimately, cycle specifications for such sterilisation methods should be based on the delivery of adequate lethality to the slowest to heat locations. A SAL of 10^{-6} or better should be demonstrated for all sterilisation processes where possible. For more information or guidance, refer to the appropriate sterilisation ISO, e.g., ISO 13408-1 [51], PDA standard or FDA guidance '*Guideline for the Submission of Documentation for Sterilisation Process Validation in Applications for Human and Veterinary Drug Products*.'

The steriliser validation programme should continue to focus on the load areas identified as the most difficult to penetrate, maintain, heat, or non-absorb. The suitability of steriliser(s) should be established by qualification, maintenance, change control, and periodic verification of the cycle, including biological challenges. Change control procedures should adequately address issues such as a load configuration change, packaging or product change, or a modification of a steriliser, or associated equipment.

7.2.9.11.2 Equipment Controls and Instrument Calibration

For both validation and routine process control, the reliability of the data generated by sterilisation cycle monitoring devices should be considered to be of the utmost importance. Devices that measure cycle parameters should be routinely calibrated. Written procedures should be established to ensure that these devices are maintained in a calibrated state. For example, we recommend that procedures address the following:

- Temperature and pressure monitoring devices for heat sterilisation should be calibrated at suitable intervals. The sensing devices used for validation studies should be calibrated before and after validation runs.
- Devices used to monitor dwell time in the steriliser should be periodically calibrated.

- The microbial count of a BI should be confirmed. BI should be stored under appropriate conditions.
- If the reliability of a vendor's Certificate of Analysis is established through an appropriate qualification programme, the D-value of a BI (e.g., spore strips, glass ampules, PCD, BI) can be accepted *in lieu* of confirmatory testing of each lot. However, a determination of resistance (D-value) should be performed for any BI inoculated onto a substrate, or used in a way that is other than described by the vendor. D-value determinations can be conducted by an independent laboratory.
- Where applicable, instruments used to determine the purity of steam should be calibrated.
- For dry heat depyrogenation tunnels, devices (e.g., sensors and transmitters) are used to measure belt speed and should be routinely calibrated. Bacterial endotoxin challenges for measuring depyrogenation effectiveness can be prepared appropriately and measured by the laboratory.

To ensure reliable and robust process control, equipment should be properly designed and validated with attention to features such as accessibility to sterilant, pressure (vacuum), piping slope, and proper condensate removal (as applicable). Process and equipment control should be ensured through placement of measuring devices at those control points that are most likely to rapidly detect unexpected process variability. Where there are manual manipulations of closures (e.g., doors), valves are required for steriliser or SIP operations, these steps should be documented in the manufacturing procedures and batch records. Sterilising equipment should be properly maintained to allow for consistent, reliable and satisfactory function. Routine evaluation of steriliser performance-indicating attributes, such as equilibrium (come-up) time and cycle parameters are important in assuring that the unit continues to operate as per the validated conditions.

7.2.11.3 Environmental Monitoring

Environmental monitoring is the observation, measurement, trending, and ultimate control over of the presence of micro-organisms or particles in an aseptic processing facility. The control of aseptic processing includes an exertion of 'tight' control over the environment in which contamination and micro-organisms exist.

7.2.11.3.1 General Written Programme

In aseptic processing, one of the most important procedural controls is the environmental monitoring programme. This programme provides meaningful information on the quality of the aseptic processing environment (e.g., when a given batch is being manufactured) as well as environmental trends of ancillary clean areas. Environmental monitoring should promptly identify potential routes of contamination, allowing for implementation of corrections before product contamination occurs.

Evaluating the quality of air and surfaces in the cleanroom environment should start with a well-defined, written programme and scientifically sound methods. The monitoring programme should cover all production shifts and include air, floors, walls, and equipment surfaces, including the critical surfaces that come in contact with the product, container, and closures. Written procedures should include a list of locations to be sampled. Sample timing, frequency, and location should be carefully selected based upon their relationship to the operation performed. Samples should be taken throughout the classified areas of the aseptic processing facility (e.g., aseptic corridors, gowning rooms) using scientifically sound sampling procedures. Sample sizes should be sufficient to optimise detection of environmental contaminants at levels that might be expected in a given clean area.

Some of the most frequently isolated micro-organisms in controlled areas used for aseptic processing are bacteria from the human skin (e.g., the Gram-positive cocci *Staphylococcus epidermidis, Staphylococcus hominis, Staphylococcus simulans, Micrococcus luteus,* and *Micrococcus varians*), skin diphtheroids (e.g., *Corynebacteria spp*.), and airborne bacterial spores, (e.g., *Bacillus sphaericus, B. cereus, B. thuringiensis,* and *B. atrophaeus*); occasionally, airborne fungal spores (e.g., *Aspergillus niger, Penicillium spp.,* and so on); and most infrequently, Gram-negative bacteria (e.g., *Enterobacter cloacae, Burkholderia cepacia* and so on) are used. Given this pattern of isolation, the use of a general microbiological culture medium such as soybean casein digest agar incubated at 30–35 °C for 48–72 h is generally supported. Although the anaerobic *Propionibacterium* species (e.g., acnes) is found under anaerobic conditions, it is not isolated so much with soybean casein, but with Schaedler media with Vit K1 and blood. *P.* species are the relatively slow-growing, typically air tolerant anaerobic Gram-positive bacterium (rod) linked to the skin. It grows optimally and under most anaerobic conditions with a good anaerobic media (see ISO 11737-1 [14]).

Gram-positive cocci and/or Gram-positive rods are found in high numbers on human skin and are readily shed. Controlled areas are protected from the cocci with the use of suitable gowns, hoods, facemasks, gloves, proper gowning techniques, and good aseptic practices.

Bacterial spores that are formed during adverse conditions as a survival mechanism can be found in dirt, dust, cellulose materials, or floors. However, fungal spores reproduce asexually and are shed from actively growing fungal colonies within damp building materials such as cardboard packaging materials or vegetation surrounding the facility.

It is important that locations posing the most microbiological risk to the product be a key part of the programme. It is especially important to monitor the microbiological quality of the critical area to determine whether or not aseptic conditions are maintained during filling and closing activities. Air and surface samples should be taken at the locations where significant activity or product exposure occurs during production. Critical surfaces that come in to contact with the sterile product should remain sterile throughout an operation. When identifying critical sites to be sampled, consideration should be given to the points of contamination risk in a process, including factors such as difficulty of setup, length of processing time, and impact of interventions. Critical surface sampling should be performed at the conclusion of the aseptic processing operation to avoid direct contact with the sterile surfaces during processing. Detection of microbial contamination on a critical site would not necessarily result in batch rejection. The contaminated critical site sample should prompt an investigation of operational information and data that includes an awareness of the potential for a low incidence of false positives.

Environmental monitoring methods do not always recover micro-organisms present in the sampled area. In particular, low-level contamination can be particularly difficult to detect. Because false negatives can occur, consecutive growth results are only one type of adverse trend. Increased incidence of contamination over a given period is an equal or more significant trend to be tracked. In the absence of any adverse trend, a single result above an action level should trigger an evaluation and a determination about whether remedial measures may be appropriate. In all clean room classes for controlled environments, remedial measures should be taken in response to unfavourable trends.

All environmental monitoring locations should be described in SOP with sufficient detail to allow for reproducible sampling of a given location surveyed. Written SOP should also address elements such as:

1. Frequency of sampling;
2. When the samples are taken (i.e., during or at the conclusion of operations);
3. Duration of sampling;
4. Sample size (e.g., surface area, air volume);

5. Specific sampling equipment and techniques;
6. Alert and action levels; and
7. Appropriate response to deviations from alert or action levels.

7.2.11.3.2 Establishing Environmental Levels and a Trending Programme

Microbiological monitoring levels should be established based on the relationship of the sampled location to the operation. The levels should be based on the need to maintain adequate microbiological control throughout the entire sterile manufacturing facility. One should also consider environmental monitoring data from historical databases, media fills, cleanroom qualification, and sanitisation studies, in developing monitoring levels. Data from similar operations can also be helpful in setting action and alert levels, especially for a new operation.

Environmental monitoring data will provide information on the quality of the manufacturing environment. Each individual sample result should be evaluated for its significance by comparison to the alert or action levels. Averaging of results can mask unacceptable localised conditions. A result at the alert level urges attention to the approaching action conditions. A result at the action level should prompt a more thorough investigation. Written procedures should be established, detailing data review frequency and actions to be taken. The quality control unit should provide routine oversight of near-term (e.g., daily, weekly, monthly, quarterly) and long-term trends in environmental and personnel monitoring data.

Trend reports should include data generated by location, shift, room, operator, or other parameters. The quality control unit should be responsible for producing specialised data reports (e.g., a search on a particular isolate over a period of a year) with the goal of investigating results beyond established levels and identifying any appropriate follow-up actions. Significant changes in microbial flora should be considered in the review of the ongoing environmental monitoring data.

Written procedures should define the system whereby the most responsible managers are regularly informed and updated on trends and investigations.

7.2.11.3.3 Disinfection Effectiveness

The suitability, effectiveness, and limitations of disinfecting agents and procedures should be assessed. The effectiveness of these disinfectants and procedures should be measured by their ability to ensure that potential contaminants are adequately

removed from surfaces.

To prevent introduction of contamination, disinfectants should be sterile, appropriately handled in suitable (e.g., sterile) containers and used for no longer than the predefined period specified by written procedures. Routinely used disinfectants should be effective against the normal microbial vegetative flora recovered from the facility. Many common disinfectants are ineffective against bacterial spores. For example, 70% isopropyl alcohol is not effective against *Bacillus* and *Clostridium* species spores. Therefore, a sound disinfectant programme also includes a sporicidal agent, used according to a written schedule and when environmental or sterility data suggest the presence of spore forming organisms.

Disinfection procedures should be described in sufficient detail (e.g., preparation, work sequence, contact time) to enable reliability and reproducibility. Once the procedures are established, their adequacy should be evaluated using a routine environmental monitoring programme. If indicated, micro-organisms associated with adverse trends can be investigated as to their sensitivity to the disinfectants used in the cleanroom in which the organisms were isolated.

7.2.9.11.4 Monitoring Methods

Various acceptable methods for monitoring the microbiological quality of the environment include:

- *Surface monitoring*: Environmental monitoring involves sampling various surfaces for microbiological quality. For example, product contact surfaces, floors, walls, and equipment should be tested on a regular basis. Touch plates, swabs, and contact plates can be used for such tests.

- *Active air monitoring*: Assessing microbial quality of air should involve the use of active devices including but not limited to impaction, centrifugal, and membrane (or gelatin) samplers. Each device has certain advantages and disadvantages, although all allow testing of the number of organisms per volume of air sampled. It is recommended that such devices be used during each production shift to evaluate aseptic processing areas at carefully chosen locations. Manufacturers should be aware of a device's air monitoring capabilities, and the air sampler should be evaluated for its suitability for use in an aseptic environment based on collection efficiency, cleanability, ability to be sterilised, and disruption of unidirectional airflow. Because devices vary, the user should assess the overall suitability of a monitoring device before it is placed into service. Manufacturers should ensure that such devices are calibrated and used according to appropriate procedures. The volume of air sampled should be sufficient to yield meaningful measurements of air quality in a given environment.

- *Passive air monitoring (settling plates)*: Another method is the use of passive air samplers, such as settling plates (petri dishes containing nutrient growth medium exposed to the environment). Because only micro-organisms that settle onto the agar surface are detected, settling plates can be used as qualitative, or semi-quantitative, air monitors. Their value in critical areas will be enhanced by ensuring that plates are positioned in locations posing the greatest risk of product contamination. As part of the method's validation, the quality control laboratory should evaluate what media exposure conditions optimise recovery of low levels of environmental isolates. Exposure conditions should preclude desiccation (e.g., caused by lengthy sampling periods and/or high airflows), which inhibits recovery of micro-organisms. The data generated by passive air sampling can be useful when considered in combination with results from other types of air samples.

7.2.11.4 Microbiological Media and Identification

Characterisation of recovered micro-organisms provides vital information for the environmental monitoring programme. Environmental isolates often correlate with the contaminants found in a media fill or product sterility testing failure, and the overall environmental picture provides valuable information for an investigation. Monitoring critical and immediately surrounding clean areas as well as personnel should include routine identification of micro-organisms to the species (or, where appropriate, genus) level. In some cases, environmental trending data have revealed migration of micro-organisms into the aseptic processing room from either uncontrolled or lesser controlled areas. Establishing an adequate programme for differentiating micro-organisms in the lesser-controlled environments, such as Class 100,000 (ISO 14644-1 [50]), can often be instrumental in detecting such trends. At minimum, the programme should require species (or, where appropriate, genus) identification of micro-organisms in these ancillary environments at frequent intervals to establish a valid, current database of contaminants present in the facility during processing (and to demonstrate that cleaning and sanitisation procedures continue to be effective).

Genotypic methods have been shown to be more accurate and precise than traditional biochemical and phenotypic techniques. These methods are especially valuable for investigations into failures (e.g., sterility test, media fill contamination). However, appropriate biochemical and phenotypic methods can be used for the routine identification of isolates.

The goal of microbiological monitoring is to reproducibly detect micro-organisms for purposes of monitoring the state of environmental control. Consistent methods will yield a database that allows for sound data comparisons and interpretations. The microbiological culture media used in environmental monitoring should be validated

as being capable of detecting fungi (i.e., yeasts and moulds) as well as bacteria and incubated at appropriate conditions of time and temperature. Total aerobic bacterial count can be obtained by incubating at 30-35 °C for 48-72 h. Total combined yeast and mould count can generally be obtained by incubating at 20-25 °C for 5-7 days.

Incoming lots of environmental monitoring media should be tested for their ability to reliably recover micro-organisms. Growth promotion testing should be performed on all lots of prepared media. Where appropriate, inactivating agents should be used to prevent inhibition of growth by cleanroom disinfectants or product residuals (e.g., antibiotics).

7.2.11.5 Particle Counting

Routine particle monitoring is useful for rapidly detecting significant deviations in air cleanliness from qualified processing norms (e.g., clean area classification, aseptic processing). A result outside the established classification level at a given location should be investigated as to its cause. The extent of investigation should be consistent with the severity of the excursion and include an evaluation of trending data. Appropriate corrective action should be implemented, as necessary, to prevent future deviations.

7.2.11.6 Closure – Container - Packaging Integrity

The closure – container - packaging integrity of the packaging components should be addressed during product development using a sensitive and adequately validated test. Recommendations for various container - closure combinations from packaging suppliers are usually helpful. A physical container - closure integrity test may be selected and validated using a bacterial liquid immersion or aerosol test. In general, physical tests may be more sensitive than bacterial challenge tests. Therefore, the leakage observed during a physical test may not be indicative of sterility assurance loss. A comprehensive discussion about leak testing of pharmaceutical packaging systems has been published [52].

When selecting a test method, the container closure type should be considered. Although stoppered vials are subjected to a bacterial immersion test, prefilled syringes may be subjected to a bacterial aerosolisation test because the latter has a more torturous path for a container - closure integrity. Physical test methods described in the literature include the bubble method, helium mass spectrometry, liquid tracer (dye), headspace analysis, vacuum and pressure decay, weight loss or gain, and high voltage leak detection. There are two phases to the container - closure integrity assessment:

the initial evaluation and selection of the container - closure system and integrity testing within the premarketing stability programme. Suitable testing intervals may be 0, 3, 6, 9, 12, 18 and 24 months during the pre-marketing stability programme and annually during the post-marketing stability programme. The number of samples tested at each time interval reflects the sampling requirements found in *USP* General Chapter 71, '*Sterility Test*' [49]. Whenever possible, physical container - closure integrity tests for product monitoring should be substituted for sterility testing, unless the samples are filled with microbiological medium and sterilised.

7.2.11.7 Preservation System

Preservative systems are used to prevent or retard microbial growth, particularly under aseptic processing where contamination is a higher risk than with terminal sterilisation. The preservatives are typically antimicrobial ingredients which prevent or retard growth, thus protecting products from contamination and causing infections.

Some drug and device product ingredients may support the growth of micro-organisms, therefore, a system is needed to prevent this possibility. The system may consist of preservatives and in an environment (e.g., pH, or carrier) that optimises the prevention of growth of microbes.

Testing for antimicrobial or preservative robustness is an important part of many drug products' developmental phase, as well as validation. In general, the use of a preservative in single-use products to replace GMP is not supported by regulatory agencies. Multiple-use products that are stored in stoppered vials can be contaminated during repeated syringe needle entries. Thus, they are formulated with preservative systems that are tested for preservative efficacy during development using USP General Chapter 51, '*Antimicrobial Effectiveness Test*' [53]. The use of this test, when appropriate, can generate a developmental history of a formulation in terms of its preservative effectiveness against a range of micro-organisms. The test can also indicate whether a product is microbiologically stable even without the presence of a preservative system (i.e., self-preserving). During the development phase of the product life cycle, the lowest concentrations at which the preservative system is effective can be established.

The proposed formulation should be tested with the antimicrobial effectiveness test at 50, 75 and 100% of the target preservative concentration to establish the shelf-life specification for the product on the basis of preservative effectiveness and stability. A typical preservative specification for a pharmaceutical product may be 80–120% of the label claim.

Thus, the preservative system may be monitored in the research and development stage and in product stability programmes using a stability-indicating chemical assay in place of more time-consuming and more-variable antimicrobial effectiveness tests.

7.2.11.8 Aseptic Processing from Other Steps in Manufacturing

Some products undergo aseptic processing at some or all of the manufacturing steps preceding the final product closing step. With other products, there is a point in the process after which they can no longer be rendered sterile by filtration. In such cases, the product would be handled aseptically at all steps subsequent to sterile filtration. In other cases, the final drug product cannot be sterile-filtered and, therefore, each component in the formulation would be rendered sterile and mixed aseptically. For example, products containing aluminum adjuvant are formulated aseptically because once they are alum adsorbed, they cannot be filtered to sterilise.

When a product is processed aseptically from the early stages, the product and all components or other additions are rendered sterile prior to entering the manufacturing process. It is critical that all transfers, transports, and storage stages be carefully controlled at each step of the process to maintain the sterility of the product. In some cases, bulk drug substances or products should be tested for sterility.

Procedures (e.g., aseptic connection) that expose a product or product contact surfaces should be performed under unidirectional airflow in a Class 100 (ISO 14644-1 [50]) environment. The environment of the room surrounding the Class 100 (ISO 14644-1) environment should be Class 10,000 (ISO 14644-1 [50]) or better. Microbiological and airborne particle monitoring should be performed during operations. Microbial surface monitoring should be performed at the end of operations, but prior to cleaning. Personnel monitoring should be performed in association with operation monitoring.

Process simulation studies covering the steps preceding filling and sealing should be designed to incorporate all conditions, product manipulations, and interventions that could impact on the sterility of the product. The process simulation, from the early process steps, should demonstrate that process controls are adequate to protect the product during manufacturing. These studies should incorporate all product manipulations, additions, and procedures involving exposure of product contact surfaces to the environment. The studies should include worst-case conditions such as maximum duration of open operations and maximum number of participating operators. However, the process simulations do not need to mimic total manufacturing time if the manipulations that occur during manufacturing are adequately represented.

It is also important that process simulations incorporate storage of sterile bulk drug

substances or product and transport to other manufacturing areas. For example, there should be assurance of bulk vessel integrity for specified holding times. The transport of sterile bulk tanks or other containers should be simulated as part of the media fill. Process simulation studies for the formulation stage should be performed at least twice each year.

7.2.12 Some Revalidation, Qualification or Audit Drivers or Requirements

As long as the process operates in a state of control and no changes have been made to the process or output product, the process does not have to be revalidated, unless specified by the application standards. Whether the process is operating in a state of control is determined by analysing day-to-day process control data and any finished device testing data for conformance with specifications and for variability.

When changes or process deviations occur, the process must be reviewed and evaluated, and revalidation must be performed where appropriate. Review, evaluation, and revalidation activities must be documented.

Processes may be routinely validated on a periodic basis, however, periodic validation may not be adequate. More important is appropriate monitoring so that if problems develop or changes are made, the need for immediate revalidation is considered.

Once a validation has been performed, revalidation is performed when another verification of the validation may be required. This is required under a number of circumstances:

- If there have been any new models or products in the previous two years:
  - Modified product configurations.
  - New materials.
  - New vendors.
  - Modified packaging or loading.
  - Any changes in manufacturing environment.
  - Any changes in product bioburden.
  - Any new product family types.
- Any new regulatory additions in the previous two years:

    - That would affect label claim – sterility, expiration date.
- What additional international countries is the product sold to:
    - New product registrations.
    - Sterile products in the past two years.
- Any new load changes in the previous two years such as density, moisture and gas absorption.
- Items that may need to be evaluated from the last qualification:
    - Fractional cycle - stronger BI - different BI.
    - Placement of BI compared to another BI.
    - Change in incubation time.
- Any changes in sterilisation process in the previous two years:
    - Any resterilisations or process failures.
    - Any change in loading pattern such as density, humidity or gas absorption.
- Any items that may need to be put in the sterilisation process cycle:
    - EO concentration.
    - RH.
    - Data trace or measurement.
- New protocol and approval, environment, new product build.

Revalidation or audit is re-performance of a validation or part of it to prove that the system is still safe and effective, and it is particularly performed for sterilisation processes. Revalidation requires that any significant change in facility, equipment, process, or test method should be evaluated through the written change control programme, triggering an evaluation of the need for revalidation or requalification. Some of the steps in revalidation are to determine whether or not a full qualification is required. A typical sterilisation qualification procedure is shown in **Figure 7.2**.

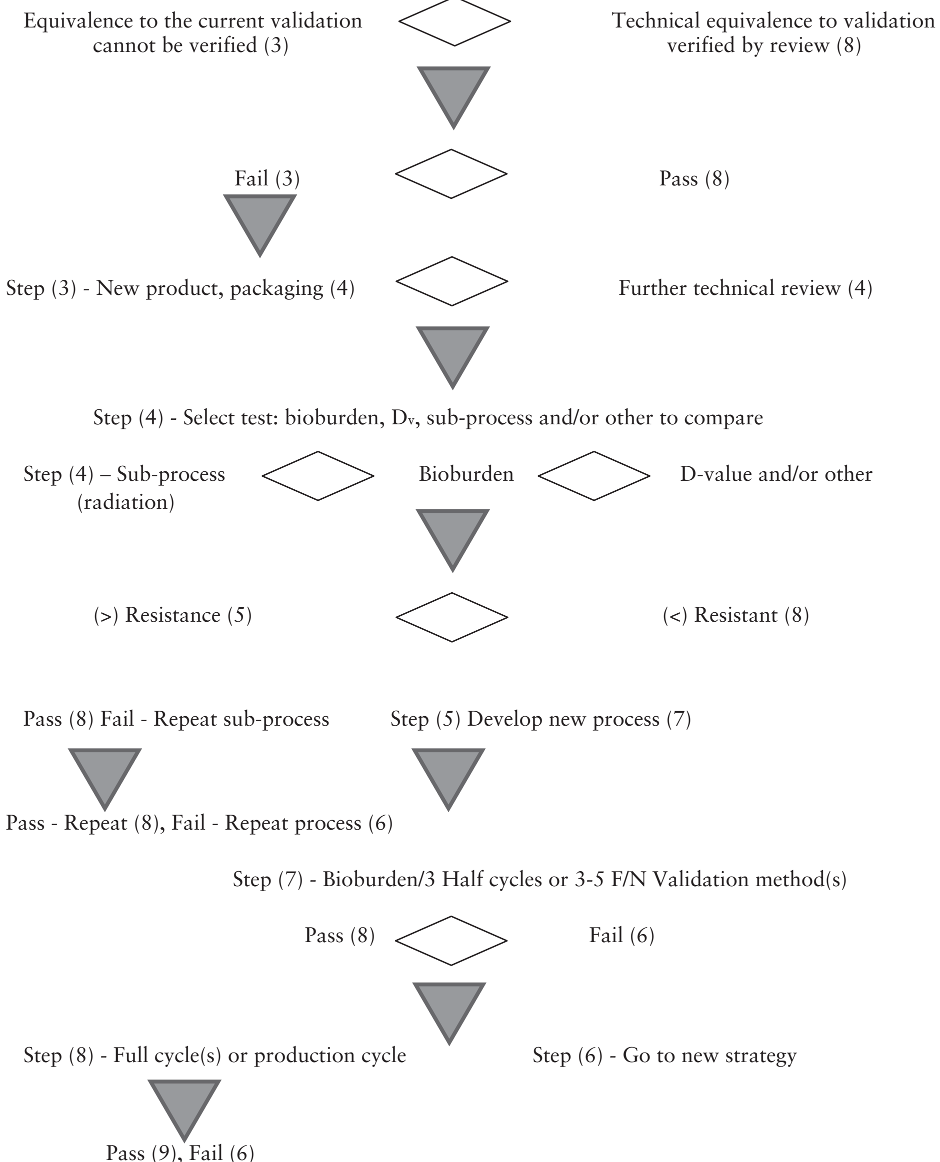

Figure 7.2 Prospective validation, revalidation or requalification flow chart

Revalidation may be required because the requalification date was reached or there may be some facility and equipment modifications, line configuration changes, significant changes in personnel, anomalies in environmental testing results, container closure system or packaging changes, extended shut downs, or end product sterility testing showing contaminated products may be cause for revalidation of the system. Written procedures should specify the frequency and reasons for revalidation.

7.3 Review and Future

Sterilisation (sterility) definition, statistics and validation together demonstrate that sterilisation processes can truly be effective, efficacious and reproducible. Labeling methods as sterilisation techniques that cannot inactivate all biological entities (e.g., viruses, prions) may lessen their effectiveness.

It is essential to validate a sterilisation process, by monitoring the process with measurement testing (e.g., temperature, % RH, exposure time, pressure, chemical concentration or dosimetry), and challenging with a BI challenge, dosimeter, and/or performing bioburden and product sterility testing under various test conditions (e.g., a fractional, half cycle, sub-process).

While enormous improvements have been made in sterilisation techniques, equipment and monitoring devices over the past 50 years, improvements in bioburden and sterility media have not. Typical recovery media have virtually remained the same, despite the fact that these media do not optimally recover the microbes that may cause disease. For example, there are media that are used which do not optimally recover microbes, but alternative media types may be appropriate where the nature of the product or method of manufacture (e.g., no face masks) may result in the presence of organisms such as the *Acinetobacter/Moraxella group of microbes, Propionibacterium s*pecies, and *Mycobacterium species*. Some *Moraxella* species can survive up to 27 days as dried secretions. Alternatively, some slow-growing microbial species (facultative anaerobes, *Mycobacterium*) can be quickly suppressed by faster-growing species on a richer culture medium. And, then there are some sterilisation methods that only use aerobic media (e.g., casein digest medium), rather than anaerobic supporting media (e.g., such as thioglycollate). Thioglycollate has antioxidants and reducer that may destroy peroxides created from such methods, also this growth supporting media may enhance radiation resistant microbes such as some *Acinetobacter-Moraxella* species, *E. faecalis*, some *Clostridium species,* and *Bacillus sphaericus*. Note these organisms (*E. faecalis* can grow anaerobically, where peroxides would not do well. And *B. sphaericus* is a facultative anaerobe and has catalase too. Anaerobes such as some *Clostridium* species are likely grow well anaerobically because they are peroxide sensitive organisms. They (*Clostridium*) do not have any catalase, an enzyme that is

capable of destroying peroxides. Some species of *E. faecalis* may grow equally well aerobically and anaerobically in peptone media with glucose because they too are peroxide sensitive.

There are some media that have inhibitory substances in them that may prevent some microbes from growing, particularly after being damaged. For example, sodium thioglycollate may be toxic to some *Clostridia* species and this antioxidant could possibly be replaced by cysteine hydrochloride. Further, chemical contamination of media may be reduced through consideration of the following types and sources of potential chemical contaminants in media:

- Metal ions, endotoxins, and other impurities in media, sera, and water.
- Plasticisers in plastic tubing and storage bottles.
- Free radicals generated in media by the photoactivation of tryptophan, riboflavin, exposed to fluorescent light.
- Deposits on glassware, pipettes, instruments and so on, left by disinfectants or detergents, anti-scaling compounds in autoclave water, residues from aluminum foil or paper.
- Residues from germicides or pesticides used to disinfect incubators, equipment, and laboratories.
- Impurities in gases used in CO_2 incubators.

Consequently a special bacteriostatic test may be recommended here. For example, spent media from a negative sterility test may be subjected to an additional growth promotion test to demonstrate its continuing nutritive properties.

There are media that have antioxidants and reducing agents that may help to recover microbes that have been damaged by sterilising agents, but these are generally not applied or used. There are general media that vary in ingredients and recovery between the agar and the liquid states, where one may recover certain microbes better than the other will.

There are general media that are incubated at temperatures and/or periods that may not optimally recover disease causing microbes. For example incubate the media at 30-35 °C and 20-25 °C for 14 days or 'greater' to pick up slow growers, in particular very dormant spores that have not been readily activated by the sterilant. For example, some *Mycobacterium* species, osmophiles and/or autotrophs and some melanised fungi will not be recovered under generalised bioburden and sterility media and or incubation temperatures of 28-32 °C and general incubation time.

General media do not contain nutrients to recover or support growth of some microbes (e.g., enriched). Nosocomial infections continue in hospitals, and some of the causes and reasons for many of them still remain in the dark awaiting some development and investigation. So while technological advances have been made in sterilising techniques and methods, equipment, facilities, and monitoring devices; bacteriological media for bioburden and sterility have not advanced. Consequently after more than 50 years it is time for technological advances to be made in the areas of bioburden and sterility recovery media. There are considerations that suggest that there are a large number of micro-organisms that are unable to replicate under standard laboratory conditions (they are viable but not cultured under current laboratory tests and conditions). For example, there may be many natural organisms (in a resting state, an altered state, a dormant state, a reversing stage, an injured state, starving, declining or a resisting state, that may only initially start to grow in a fastidious media such as with blood supplementation rather than under generalised recovery media, without such fastidious nutrients. Microbes can exist in ubiquitous physiological states in nature rather than through generalised artificial media. Sterility media can only recognise organisms able to grow under the conditions of the test and the specified media, and that the sample size is so restricted that it provides only a gross estimate of the state of 'sterility'.

Sampling sizes for sterility in many cases remain grossly futile. It is time to improve the ways that samples are best applied and used, for better effective validation of some sterilisation methods. Contamination and surviving/resistant contamination may not be totally recovered thoroughly through testing, but it may be managed better to improve both its frequency of occurrence and the seriousness of its infectious consequences. To improve general bioburden and sterility media for improved recovery of natural and damaged microbes may be a long struggle, but it is advantageous because of the number of nosocomial infections, the number of implantable and new therapies, the number of compromised patients and the number of aging patients, which will continue to increase in the future due to changes in demographics and changing therapies and technologies.

Special microbiologists and sterilisation scientists have the knowledge to design, develop, and statistically validate sterilisation processes beyond consensus standards that rely on imaginary D-values, solely on logarithmic order of death criteria, and generalised sterility media, which may lead to sterility uncertainities. It must be possible to successfully measure variables before they can be truly controlled, however, in the meantime properly trained, qualified and responsible personnel, microbiologists and/or engineers are necessary to carry out operations relating to the production of sterile items if the final product is to demonstrate a sterility level of 1 in 10^{-6} or other SAL as given in the regulations.

Every sterilisation validation must be what its own characteristics and qualities

determine. Control of sterilisation (pre-validation) must of necessity begin with its production environment and product material compatibility. The qualities of different sterilisation validations vary. Heat sterilisation validation must measure heat lethality, and not cause products or their components to distort, melt or corrode. Irradiation must measure bioburden quantities and demonstrate sterility at corresponding radiation doses to the estimated bioburden levels, without embrittlement, crosslinking, or damaging materials. H_2O_2, O_3 sterilisation must inactivate either bioburden, BI/PCD, and/or both, without damaging materials by oxidation or other energetic means, and EO must inactivate microbes without leaving EO residuals. Sterilisation validation of microbes and polymers continues to be an important challenge in hospitals and for healthcare manufacturers.

There are always trade-offs, when selecting and validating a method of sterilisation based upon acceptable environment, materials, and microbial contamination in general and locations within the product. Whichever validation technique is used, it must be able to:

- Completely sterilise both surfaces and below surfaces or penetrate difficult areas so that the product can ultimately be labelled as sterile.
- Demonstrate greater inactivation than the existing bioburden resistance on the product.
- Demonstrate and provide physical measurements of monitoring that provide correlation to microbial lethality.
- Demonstrate and provide reproducible, uniform and consistent sterilisation parameters and specifications.
- Demonstrate or provide a safety factor before a material is damaged.
- The cost of sterilisation validation, including product/material compatibility will vary. Heat validation may be the least expensive, while EO and irradiation validation may cost more.
- Demonstrate or prove that no product toxicity or process residuals exist, and that the product to be used is biocompatible.
- Demonstrate an acceptable SAL such as 10^{-6}.
- Demonstrate a margin of safety before the SAL might fail.
- Demonstrate or prove that the stability of the package, product or material being sterilised will be safe until use, after sterilisation.

- Demonstrate that the product can be resterilised, if it is to be reused or has to be resterilised.
- Demonstrate or provide adequate routine sterilisation parameters or specifications to be subsequently applied.
- Demonstrate that the process is not only effective, but also efficacious and safe.

This chapter has given the reader or student a glimpse of some of the highlights related to statistics and validation. More details can be found in references, standards, and technical information reports, and more recent and future accounts in individual treatments and research on these subjects.

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Abbreviations

AAMI	American Association for Medical Instrumentation
ABS	Acrylonitrile-butadiene-styrene
ACGIH	American Conference of Industrial Hygienists
AHP	Accelerated hydrogen peroxide
ANSI	American National Standards Institute
AOAC	Association of Official Analytical Chemists
ASME	Association for the Study of Medical Education
ASTM	American Society for Testing and Materials
ATCC	American Type Culture Collection
B/F	Bacteriostasis/fungistasis
BET	Bacterial endotoxin text
BI	Biological indicator(s)
BPL	β-Propiolactone
CAS	Chemical Abstracts Service
CCR	California Code of Regulations
CDC	Center for Disease Control and Prevention
CEN	European Committee for Standardisation/Normalisation

CERCLA	Comprehensive Environmental Responses, Compensation, and Liability Act
CFC	Chlorofluorocarbon
CFR	Code of Federal Regulations
CFU	Colony forming unit(s)
CGMP	Current Good Manufacturing Practice(s)
CIP	Clean-in-place
Cl_2	Chlorine
ClO_2	Chlorine dioxide
CO_2	Carbon dioxide
COC	Cyclic olefin co-polymer(s)
D_{121C}	D-value or D_{10} value for saturated steam
DIS	Draft International Standard
DMR	Device master record
DNA	Deoxyribonucleic acid
DPA	Dipicolinic acid
D-value (D_{10})	Decimal reduction value(s) or death value
EBI	Enzyme-based iodine
EC	European Community/Commission
ECH	Ethylene chlorohydrin
EHS	Extremely hazardous substances
EN	European Norm
EO	Ethylene oxide(s)

EPA	Environmental Protection Agency
EPDM	Ethylene-propylene diene terpolymer
ETFE	Ethylene tetrafluoroethylene
ETG	Ethylene glycol
EU	European Union
F/N	Fraction negative
F_{bio}	Notation for thermal exposure equivalent to moist heat at 121 °C
FDA	Food & Drugs Administration (US)
FEP	Fluorinated ethylene propylene
F_h	Thermal lethality
FMEA	Failure mode effect analysis
F_o	The equivalent time to sterilise at 121 °C
FPA	Flexible Packaging Association
FTGM	Fluid thioglycollate medium
GMP	Good manufacturing practice(s)
H_2O	Water
H_2O_2	Hydrogen peroxide
HCl	Hydrogen chloride
$HClO_3$	Chloric acid
$HClO_4$	Perchloric acid
HDPE	High-density polyethylene
HEPA	High efficiency particulate air

HIV	Human immunodeficiency virus
I_2	Diatomic iodine
IDLH	Immediately Dangerous to Life or Health
IPA	Isopropyl alcohol
IQ	Installation qualification
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
IV	Intravenous
LAL	*Limulus* amebocyte lysate
LC_{50}	Lethal concentration - the concentration of a chemical in air that kills 50% of a group of test animals in a given time (usually four hours)
LCG	Liquid chemical germicide(s)
LD_{50}	Lethal dose - the amount of a material, given all at once, which causes the death of 50% of a group of test animals
LDPE	Low-density polyethylene
LRV	Log reduction value
MAUDE	Manufacturer and User Facility Device Experience
MDA	4,4'-Methylenedianiline
MEC	Microbial effective concentration
MQ	Microbiological qualification
MSDS	Materials Safety Data Sheet
N_0	Initial spore population or bioburden population
NaOCl	Sodium hypochlorite

NaOH	Sodium hydroxide
NASA	National Aeronautics and Space Administration (The)
NDA	New drug application
NF	National Formulary
NFPA	National Fire Protection Association
NIOSH	National Institute for Occupational Safety and Health
NO_2	Nitrogen dioxide
O_2	Oxygen
O_3	Ozone
OPA	*Ortho*-phthalaldehyde
OQ	Operational qualification
OSHA	Occupational Safety and Health Administration
PA	Polyamide(s)
PAA	Peracetic acid
PC	Polycarbonate
PCD	Process challenge device
PCTFE	Polychlorotrifluoroethylene
PDA	Parenteral Drug Association
PE	Polyethylene(s)
PEEK	Poly(ether ether ketone)
PEI	Polyetherimide
PEK	Polyethylketone

PEL	Permissible exposure limit(s)
PET	Polyethylene terephthalate
PETG	Polyethylene terephthalate glycol
PFA	Perfluoroalkoxy
PFAc	Performic acid
PGA	Polyglycolic acid
PLA	Polylactic acid
PLGA	Poly(lactic-*co*-glycolic acid)
PMMA	Polymethyl methacrylate
PON	Peroxynitrites
PP	Polypropylene
PPCO	Polypropylene copolymer
PPG	Propylene glycol
PPO	Polypropylene oxide
PQ	Performance qualification
PS	Polystyrene
PSF	Polysulfone(s)
PTFE	Polytetrafluoroethylene
PU	Polyurethane(s)
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
PVDF	Polyvinylidene fluoride

PVF	Polyvinyl fluoride
PVP	Polyvinyl pyrrolidone
PVP-I2	Polyvinyl pyrrolidone and elemental iodine complex
Q_{10}	Temperature coefficient
QC	Quality control
Quat	Quaternary ammonium compound(s)
R&D	Research & Development
RH	Relative humidity(ies)
RNA	Ribonucleic acid
RQ	Reportable quantities
SAL	Sterility assurance level(s)
SAN	Styrene/acrylonitrile
SARA	Situational awareness and response assistant
$scCO_2$	Supercritical carbon dioxide
SCDM	Soybean casein digest medium
SIP	Sterilisation/Sterilise-in-place
SOP	Standard operating procedure(s)
SPMC	Sterilisation Packaging Manufacturers Council
STEL	Short-term exposure limit(s)
T_b	Boiling point
T_g	Glass transition temperature
TIR	Technical Information Report

TLV	Threshold limit value(s)
TLV-C	Threshold limit value - ceiling limit
TPPO	Thermoplastic polyolefin elastomer
TSA	Trypicase soy agar
TWA	Time weighted average
UHMWPE	Ultra-high molecular weight polyethylene
USP	United States Pharmacopeia
UV	Ultraviolet
VD_{max}	Verification dose maximum
Z-value	The temperature difference required to cause a 10-fold change in the D-value

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The collection of topics in this second volume of the book reflects challenges the reader to think beyond standard methods and question why certain current procedures remain static while technological advances abound in other aspects of sterilisation technology. By small means, better practices may come to pass to help answer some of the residual healthcare sterilisation and nosocomial infection queries:

- What are some of the current challenges in healthcare sterilisation, and how can they be handled?
- What are some of the acceptable current non-traditional sterilisation methods, challenging alternatives, and novel modalities?
- What are some of the packaging, validation and statistical considerations of sterilisation practices?
- How does design-of-product and packaging interrelate with sterilisation processing?
- Are the current sterility media and practices optimal for recovery of more modified and more resistant viable organism entities and product?
- Are there increased sterility and product quality needs with new types of implantables and technological advances within the three dimensional combinations of diagnostics, drug release and challenging medical devices?

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