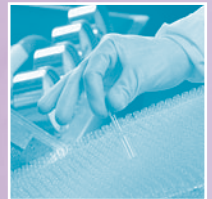


Sterilisation of Polymer Healthcare Products

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Preface

This book tries to differentiate and integrate various aspects of sterilisation to healthcare products and polymer materials. To sterilise is one thing, but to sterilise healthcare products and then polymeric materials without adversely affecting their end use with the user extends the scope of sterilisation, with a special focus on traditional considerations with new opportunities.

Common traditional terminal sterilisation methods are:

- Ethylene oxide
- Radiation - gamma and electron beam
- Steam
- Dry Heat

Other newer conventional sterilising agents are hydrogen peroxide/plasma (Sterrad), glutaraldehyde, steam formaldehyde, peracetic acid, chlorine dioxide, and ozone. These methods are not adequate for all 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 to be true to its definition.

Sterilisation is not a singular problem nor discipline, but an interfacial area of investigation of materials, biology, chemistry, product design development, manufacturing, environmental control, biocompatibility, microbiology, engineering, material/drug safety, mathematics, manufacturing, R&D, and quality and product/package design, particularly in a new era of device/drug combinations. It requires a multidisciplinary effort.

A variety of general considerations of sterilisation must be made to achieve sterility without adversely affecting product and material quality. The control of sterilisation must begin with the design of the product and the process. There must be a design quality assurance system in place to identify: when design input occurs, written specifications or procedures, personnel interfaces that design output and that design verification is documented. To achieve product and process design control, there must be adequate procedures, personnel, plans, and documentation.

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Sterilisation design encompasses an interfacial area of investigation and multiple disciplinary backgrounds. A variety of factors and functions encompass sterilisation, requiring understanding of environmental, physical, chemical, biological, engineering, manufacturing, quality control and assurance, regulatory, and marketing areas.

To effectively establish sterilisation an overview of the entire system is needed. Of necessity sterilisation begins with an understanding and control of the environment and micro-organisms (bioburden), product design under which the product is manufactured. To be effective for medical devices, a qualification of equipment upon installation or commissioning, process performance validation, and certification is required. Under contract sterilisation, the contractor is generally responsible for equipment and facilities and the manufacturers for process and product. Once a process is completely qualified and validated, the process and equipment are typically revalidated annually or periodically. A completed qualified and validated process allows for routine processing and releasing of product.

The sterilisation process cannot be considered alone from product development, rather, it is interrelated to product design, equipment, polymers, materials, bioburden to be sterilised, and product to be released. Sterilisation and product design and materials are parallel considerations.

Healthcare sterilisation focuses on the product design, compatibility of the product and the end use of the product. A comprehensive design verification and review must include many considerations. Among them are design characteristics of the product, polymers and material and product history, and compatibility with the selected or intended sterilisation process.

Sterilisation, healthcare product and polymeric materials are not without complications and solutions. There are several commercial sterilisation methods that can not inactivate all viable entities such as small target viruses, *Deinococcus*, *Pryonema domesticum*, resistant anaerobes, anthrax or a prion challenge or non viable entities such as pyrogens without adverse product or material qualities; however, presterilisation, environmental bioburden control, and statistics are applied to assure the adequacy of the process for the safety of the end product or material being treated.

There is a need to sterilise new drug/device combination products coming into to market. In producing these combinations, manufacturers may use a combination of approaches and processes from physical, chemical, radiation, and plasma agents. One company designed and validated their device/drug (prefilled syringe) with steam sterilisation - a very old method but with some new modifications to the method.

Most traditional methods continue to work but in some cases newer methods and approaches have an opportunity. One diagnostic material/device company used a liquid

sterilant with a container with terminal end filters because the diagnostic enzymic material was degraded by EO, hydrogen peroxide, radiation, and heat. The liquid sterilant in conjunction with filters was a way of sterilising and maintaining sterility of this diagnostic material/device, by flushing out the liquid sterilant and replacing it with a non sterilising solution, through the terminal filters, to prevent recontamination.

During times of conflict and war, new sterilisation methods tend to be developed. EO sterilisation was developed after WWII for germ warfare. Radiation was driven in part during the cold war as a means of using unused sources of radiation. In the terrorist anthrax challenge, x-ray irradiation appears to be a practical means of sterilising/sanitising pallets of mail against anthrax, that other current methods couldn't complete adequately or in time. For the first time in history we have x rays and the capability of sterilising bulk, dense mass and large volume of untreated (contaminated) healthcare product in a matter of minutes, just in time, without the additional handling associated with unloading and loading.

To deliver sterility, and sterile items to the soldier at the front line will not be enough, but preserving sterilised items against recontamination, and enhancing healing and repair is a further consideration of sterilisation processing in the future. Consequently sterilisation of healthcare products and polymers remains an unfinished business, and this book is only a beginning.

Finally, a preface is a place in a book where the author gets to thank all those who helped in its development and final form. I wish to acknowledge Frances Powers, Senior Commissioning Editor, Rapra Technology and her staff who have worked on this book: Claire Griffiths and Hilary Moorcroft (Editorial Assistants), Steve Barnfield (Typesetter and Graphic Designer) and John Holmes (Indexer), and for Sally Humphreys for suggesting and supporting that I write this book, after completing a Rapra Review Report on a similar subject.

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Introduction

Sterilisation of healthcare products and polymers is a special process. It is a challenged process of the highest order used to render a product free of all forms of viable micro-organisms. To demonstrate absolute freedom of micro-organisms is virtually impossible, but statistically impossible. In sterilisation, the nature of microbial death is described as a logarithmic function. Sterilisation is consequently expressed as the probability of a certain number of micro-organisms capable of surviving.

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 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 manufacturing and the release of product. In past years considerable effort has been exerted by ANSI, AAMI, FDA, PDA, USP and others to come up with harmonised standards under the International Standards Organisation (ISO) and others, to obtain harmonisation globally and universally. This task has not been easy or fruitful. Differences between countries and sterilisation methods do exist. There are several standards setting organisations involved in sterilisation: Parenteral Drug Association (PDA), Health Industry Manufacturing Association (HIMA), United States Pharmacopoeia (USP), American Association of Medical Instrumentation (AAMI), Community for European Normalisation (CEN), and ISO. In this discussion, we will discuss a few basic sterilisation guidelines, standards for dry heat, general sterilisation, radiation, steam, and ethylene oxide from AAMI, CEN, GMP (good manufacturing practices), PDA, USP (and other

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compendia) and ISO. These standards are generally recognised by pertinent regulatory agencies, such as the FDA, DHS, JMH and the International Community. For example, AAMI standards have long been used by the FDA as guidelines in assessing GMP.

The CEN standards have been established most recently for the European Community (EC) - 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. For pharmaceutical, drugs, ophthalmics, the Parenteral Drug Association and the USP have certain requirements. Their standards, in many cases, run in parallel to ISO and AAMI standards and sometimes overlap but they are in a different universe and requirements for drugs and pharmaceuticals rather than medical devices.

There are detailed requirements for various 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 many companies today deal with contract facilities and sterilisers.

Qualifications are generally not repeated unless significant changes occur. With most manufacturers new or significantly altered equipment, product or material changes are reasons for qualifications. 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-process conditions.

The backbone of all sterilisation methods is the decimal reduction value, commonly referred to as the D-value. The D-value is the time or dose that a sterilisation process takes to inactivate a microbial population one logarithm or 90%.

The approaches toward applying D-value data varies slightly with different sterilisation methods. One of the major differences is the application of D-values from bioburden, which consists of naturally occurring micro-organisms, or from biological indicators/challenges that consist of selected resistant micro-organisms to a specified sterilisation method. In radiation sterilisation, dose setting uses bioburden information from the AAMI standards. Early radiation qualifications, commonly used the KILMER method, which allowed one to qualify a 25 kGy (2.5 Mrad) with a small number of products and little bioburden information. AAMI Method 3A Dose Setting, for Infrequent Production (25 kGy); Method 3B Dose Setting for Small Lot Sizes and Infrequent Production. These have been replaced by either ISO 13409 or AAMI TIR 27. The most recent AAMI document setting has three dose setting approaches they are:

Method 1 - Dose Setting Using Bioburden Information

Method 2 - Dose Setting Using Fractional Positives, SIP of 1 Protocol

Method 3 - ISO 13490 or AAMI TIR 27.

In ethylene oxide and sometimes steam sterilisation, the BI or overkill approach is the microbiological qualification approach. Combinations are an alternative approach that facilitates 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.

One of the greatest concerns in sterilisation process qualifications/validations today is at what level of probability of survivor or sterility assurance level (SAL) will be acceptable throughout the world.

In Europe the absolute SAL is 10^{-6} . In the USA there is essentially a dual SAL standard of 10^{-3} for topical products and 10^{-6} for invasive products. Alternatively SAL is an economic necessity for radiation sterilisation, because it allows for many materials to be able to be irradiated without deleterious effect. Harmonisation of worldwide sterilisation requirements is an important issue. This harmonisation was hard. It tested the world 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.

Classical sterilisation is defined as absolute process that destroys or eliminates all micro-organisms. In a practical sense, however, sterilisation is best defined as a process capable of delivering a certain probability that an exposed or treated product or material is free from viable micro-organisms.

The term sterilisation has previously been misunderstood, abused or confused with lesser methods of eliminating viable micro-organisms, such as disinfection, decontamination, sanitisation, or antiseptic. These methods are not capable of total elimination or destruction of all types of micro-organisms.

Sterilisation, by definition, is the capability of destroying or eliminating the most resistant microbial bacterial spores that are capable of surviving most environmental conditions.

The number of agents capable of sterilising product or material without adversely or deleteriously affecting product quality or material integrity are few. Some typical methods are:

- Steam
- Ethylene oxide
- Radiation
- Dry heat
- Sterilisation by filtration

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A brief description of these sterilisation methods are presented as follows:

1 Steam Sterilisation

Steam sterilisation is a classical method and is recognised for its simplicity, efficiency, effectiveness, low cost, and speed of operation. It is currently considered more as an ideal candidate because of its compatibility with the environment and health and safety. But the number of plastic materials, chemicals, and some metals capable of tolerating its high temperature and moisture are few. In hospitals and laboratories where reusable materials are frequently used, steam sterilisation is predominantly used. It is also widely used in decontamination of infectious waste materials. Now however, with emphasis on the environment, there is renewed interest in this method of sterilisation. Unlike most other sterilisation methods, steam is compatible with most liquids. Steam can sterilise most metals, glass, and some heat resistant plastic materials. Some examples are:

- Acetals (some)
- ABS
- Aromatic polyurethanes
- Nylon
- Polyallomer
- Polycarbonate
- Polyetheretherketone (PEEK)
- Polypropylene
- Polysulfone
- PVC (some)
- Silicone
- Teflons
- Rubber

The number of plastic materials capable of being steam sterilised will vary considerably with the selected temperature of sterilisation. Standard steam sterilisation is generally carried out at 121 °C for 15 minutes. Faster or flash sterilisation is generally carried out at 134 °C. Longer sterilisation or lower steam sterilisation is carried out at 115 °C. Lower steam sterilisation can be performed at approximately 100 °C (fractional) or at 80 °C on three consecutive days (Tyndalisation), but these latter approaches are marginal and possibly questionable. Some alternative or combination approaches to classical steam sterilisation are of possible future considerations, such as with microwave, steam - ethylene oxide, steam - formaldehyde, etc.

The types of steam sterilisation processes can vary significantly. Some typical steam process types encountered are:

- Gravity (downward displacement)
- Pulsing (vacuum pulsing or pressure pulsing)
- High vacuum
- Superheat

Each type has its advantages. The selection of the particular process type is dependent upon a variety of factors such as the end use characteristic of the product.

2 Ethylene Oxide Sterilisation

Ethylene oxide sterilisation is a significant method of sterilisation used in the medical device industry, and second to steam sterilisation in hospitals. Ethylene oxide sterilisation acquired this position with the advent and popularity of plastic polymeric materials.

Ethylene oxide sterilisation is a gaseous method. It is an ideal gaseous sterilant because of its characteristically high diffusivity and permeability. This leads, however, to one of its disadvantages, toxic residuals. Other significant characteristics of this chemical is its low volatility (10.8 °C), its ring structure, its moderate chemical reactivity, and its significant compatibility with most plastic materials.

Its disadvantages are its flammability, explosivity, carcinogenicity, and reproductive toxicity. These disadvantages have been principally overcome with improved equipment control, use of non-flammable gas mixtures, deoxygenating scrubbers, facility designs, worker training and administrative controls. The benefits of the sterilant continue to outweigh its inherent risks.

To achieve sterilisation with ethylene oxide requires an understanding of its process parameters and the interrelationships between them. Ethylene oxide sterilisation consists of several cycle parameters:

- Relative humidity
- Ethylene oxide concentration
- Temperature
- Pressure changes
- Dwell/exposure

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Other conditions may include preconditioning and post cycle aeration. Preconditioning facilitates the eventual humidity conditioning of significantly dry product loads and bacterial spores, and aeration facilitates the removal of toxic residuals from materials treated with ethylene oxide. Some typical ethylene oxide process methods are:

- 100% Ethylene oxide cycle with/without nitrogen
- Standard EO/Freon cycle
- Balance pressure cycle
- Air displacement cycle
- 8.5% EO/91.5% CO₂ cycle
- 20% EO/80% CO₂ (potentially non-exposive)

A number of other ethylene oxide methods may involve humidification, preconditioning, and aeration. The selected process method varies with the end product type configuration, characteristics, and claims. One of the limiting factors for ethylene oxide is its limiting capability to diffuse into the innermost areas of a few products that require sterilisation within a reasonable time frame. Radiation is a method that can nearly always be relied upon to sterilise even the most difficult to sterilise areas of a few products.

3 Radiation Sterilisation

Radiation has been recognised as a method of sterilisation since X rays were demonstrated in 1896 to inactive micro-organisms, but its practical application followed ethylene oxide with the continuous improvement of plastic materials and medical devices.

Radiation sterilisation is a panacea for industrial sterilisation because of its excellent penetration capabilities, its fast release of treated products and simplicity of routine operation as compared to ethylene oxide

Some of its disadvantages have been its initial capital cost, incompatibility with some low cost plastic materials, fear of radiation, extended length of time for qualifying irradiated materials and its disposal of radioactive waste when gamma emitting isotopes are used.

Some typical radiation methods are:

- Cobalt 60
- Cesium 137
- Electron beam
- X-rays

Most radiation methods require only dose delivered. The method is simple, however, workers must be trained for safety. Elaborate facility designs and controls are made to minimise and eliminate the risk of irradiation of workers or surrounding environment.

In general radiation doses are extremely high in millions of rads or tens of millions of kilograys to inactivate all micro-organisms. The classical IR radiation dose has been defined as 2.5 Mrad or 20 kGy. Lower doses, however, have become common with the advent of the AAMI Gamma Radiation Process Guidelines. Further, to be able to sterilise so many products and configurations with irradiation without adversely or deleteriously affecting product quality, a dual level of probability of survivor has been accepted for 10^{-3} and 10^{-6} Sterility Assurance Level (SAL) in the USA depending upon a product's end use, however, this remains a controversial issue in other countries or regions of the world.

4 Dry Heat Sterilisation/Depyrogenation

Dry heat sterilisation is one of the oldest sterilisation methods, but it is infrequently applied in industry, except in the pharmaceutical area where it is used as part of aseptic processing. It is used in sterilising dental instruments to minimise the corrosion of sharp items. It is commonly used in laboratories for depyrogenation of glassware to be used in pyrogen testing. It has been used as method of choice for spacecraft sterilisation in the USA. The Russians used an ethylene oxide gas mixture.

Dry heat sterilisation has been generally reserved for materials and products that cannot withstand steam or for reason of depyrogenation. Some typical materials that may be dry heat resistant depending on sterilisation temperature are:

- ABS
- Acetals
- Ceramics
- Electronics (some)
- Glass
- Metals
- Nylons
- Oils
- PEEK
- Petroleum
- Polyester copolymers (some)
- Polypropylene
- Polymethylpentene
- Polysulfones
- Powders
- PU
- PVC (some)
- Silicones
- Teflons

Dry Heat Sterilisation requires extremely high temperatures/time conditions as follows:

- 170 °C - 60 minutes
- 160 °C - 120 minutes

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- 150 °C - 150 minutes
- 140 °C - 180 minutes
- 105 to 135 °C - overnight (16 hours) or greater

At high temperatures there can be deleterious affects on many products or materials, however, one can be assured of destruction of pyrogenic substances/materials. Some of the disadvantages of dry heat sterilisation are:

- Heating is slow
- Longer sterilising times compared to steam
- Very limited materials
- Limited packaging to allow for heat transfer

The transfer of heat by steam sterilisation at 121 °C is 12 times greater than with hot air.

Dry Heat Sterilisation is generally carried out by one of two ways:

- Hot air oven
- Infrared tunnel

Dry heat has been suggested as the cause of sterilisation by some atmospheric plasma process conditions because of the extreme temperatures achieved. The mechanism of inactivation of micro-organisms by dry heat is considered to be primarily an oxidative process, although the presence of moisture can cause denaturation or coagulation of protein. The following basic example of egg coagulation is an easy way to understand the influence/effect of moisture with heat:

Albumin plus 56% water - coagulates at 56 °C
Albumin plus 25% water - coagulates at 74-80 °C
Albumin plus 18% water - coagulates at 80-90 °C
Albumin plus 6% water - coagulates at 145 °C
Albumin plus 0% water - coagulates at 160-170 °C

Because of its high temperature requirements, dry heat is not likely to be the method of choice, except for special end product uses and needs.

5 Sterilisation by Filtration for Aseptic Processing

Sterilisation by filtration refers to the removal of microbes by the use of filters. Sterilisation by filtration is a practical, yet a 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 the pharmaceutical area for sterilisation of drugs that would be adversely affected by steam heat. It is also commonly used in sterilisation of air for clean rooms and other spaces. The method is also used in some devices as means of assuring against adventitious or accidental contamination during use. The method may be used in producing contact rinse solutions.

The types of filtration may be further delineated by the types of filters used:

- Porous (membrane) filters
- Depth (probability) filters)
- Charged or absorptive filters

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

Sterilisation by filtration can also be described by the filter size, its rating, or grade:

Membrane: 0.45 μm , 0.22 μm , and 0.1 μm

HEPA: 99.99%

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 being 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.

Filtration sterilisation will not be discussed in this book, because it is not the most preferred method of sterilisation. In general, it is preferred to sterilise products in their final configuration and packaging, in order to minimise the risk of microbial contamination. Products designed for aseptic processing generally consists of components that have been previously sterilised by one of the above terminal sterilisation methods.

6 Control of Sterilisation

The control of sterilisation begins with design control of product and process. There must be a design quality assurance system in place to identify when design input occurs, to ensure that there are written specifications or procedures, personnel interfaces, and that design output and design verification is documented. To achieve product and process design control, there must be adequate procedures, personnel, plans, and documentation.

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Design encompasses an interfacial area of investigation of interactive personnel and multiple disciplinary characteristics and functions. A variety of factors and functions must be considered:

- Physical
- Chemical
- Biological
- Engineering
- Material compatibility
- Manufacturing
- Microbiology
- Quality assurance
- Regulatory
- Marketing

Sterilisation design is focused primarily on the product design, and the end use of the product. A comprehensive design verification and review must include many considerations:

- Design characteristics of the product and product history
- Sterilisation possibilities
- Sterility assurance level
- Presterilisation bioburden and pyrogenic potential
- Sterilisation cycle specifications
- Sterilisation standards/guidelines
- Domestic and/or international regulatory compliance
- Issue documents for qualification and validation requirements
- Biocompatibility of materials with the sterilisation process
- Material selection/compatibility with the sterilisation process
- Environmental compatibility of the sterile product
- Environmental compatibility of the sterilisation process
- Quality of the product with the sterilisation process
- Packaging requirements for the sterilisation process
- Packaging and labelling requirements for the type of sterile claim
- Packaging integrity and shelf life
- Toxic residuals level
- Validation/verification of designed product and sterilisation process
- Document approval and subsequent document changes

When product is put into manufacturing, control of sterilisation begins with exertion of control of the manufacturing environment to control bioburden on the product through production. Other aspects of control of sterilisation is through control of the following:

- Equipment qualification
- Equipment calibration
- Equipment maintenance
- Biological indicators, positions and certification
- Dosimeters, positions and certification
- Physical/chemical monitors, positions and calibration
- Personnel qualification and training
- Product load configuration and packaging
- Process specifications and document review
- Adequate control of product movement and quarantine
- Environmental/presterilisation bioburden control
- Gas certification and isotope activity
- Post sterilisation of test
- Testing finish product

More sterilisation processes are going to product release based on process control rather than by finished product sterility testing or biological indicator testing evaluations. These product releases require tight cycle or process parameters as well as other monitors and approved validation procedures and processes. Some examples are:

Dosimetric Release for Radiation:

Dose delivered

Process Control Steam Sterilisation:

Chamber and product temperature

Heat up, exposure and cool down times

Pressure

Parametric Release for Ethylene Oxide:

Prehumidification - relative humidity

Gas concentration,

Vacuum and pressure limits and times,

Sterilisation of Polymer Healthcare Products

Exposure

Chamber and product temperature

Post sterilisation aeration

Contract sterilisation

Contract sterilisation facilities are considered to be medical device manufacturers and must meet all appropriate GMP that pertain to its operations in accordance to 21 CFR 820 since sterilisation is a special manufacturing process. Some specific GMP considerations are:

- Equipment qualification
- Calibration
- Maintenance
- In-process controls
- Segregation of sterile/non Sterile Product
- Pest control
- Record keeping
- Training
- Cycle/process validation
- Software validation
- Process change control
- Audits
- Information transfer
- Biological indicators/dosimeters
- Non compliance
- Finished product release
- Documentation and review
- Loading configurations
- Post sterilisation handling and aeration

Contract sterilisers must register with the FDA and are routinely inspected.

Special considerations are required for contract sterilisations under 21 CFR:801.150(e):

- A written agreement
- Names and addresses of firms
- Signatures

- Instruction for records
- Acknowledgement of non-sterility
- GMP requirements
- Description of the process

Manufacturers who shipped products to healthcare facilities to be sterilised or reprocessed, must design, test, and label the products for the user, and provide information for processing the product or reusable (see AAMI TIR 12 [1] and AAMI ISO ST81 [2]).

7 Labelling

Mark status of pallet/other designated unit during shipping and holding

‘Non -sterile awaiting processing’

‘Processed awaiting test results’

Manufacturers of sterile devices commonly label their devices as sterile at one facility and ship them to another facility or contract steriliser for sterilisation. Shipment of sterile devices are misbranded and adulterated if they are not properly labelled.

Sterilisation of healthcare products and polymeric materials is a specialised area of investigation and information. The primary objective of this book will be to further define and discuss sterilisation and its application to healthcare products and polymeric materials.

More methods of sterilisation of polymer products in recent years, oxidising agents and processes have been improved for sterilising applications in the healthcare industry. These agents include hydrogen peroxide, peracetic acid, ozone, sodium hypochlorite and chlorine dioxide. Combination with plasma has resulted in hydrogen peroxide/plasma. Low temperature hydrogen peroxide gas plasma (LTHPGP) sterilisation is common in hospitals *in lieu* of EO, and of great potential for point of use in manufacturing sites.

Combination with steam has resulted in steam-formaldehyde. A future combination may be steam-EO, with acceptable ethylene glycol residuals, rather than EO. Liquid sterilants include peracetic acid, glutaraldehyde and orthophthalaldehyde.

References

1. AAMI TIR 12, Designing, Testing and Labelling Reusable Medical Devices for Reprocessing in Healthcare Facilities: A Guide for Device Manufacturers, 2004.
2. AAMI ISO ST81, Sterilisation of Medical Devices – Information to be Provided by the Manufacturer for the Processing of Resterilisable Medical Devices, 2004.

1 Sterilisation Qualities and Science

1.1 Purpose

Sterilisation of healthcare products and polymeric materials is a specialised field requiring an interfacial area of investigation, discipline and information. There is much more to sterilisation than described in standards and guidelines. The primary objective of this book is to define and discuss sterilisation and its application to healthcare products and polymeric materials. Sterilisation is defined as the complete removal or destruction of viable organisms, and we need to focus on understanding the advantages and disadvantages, compatibilities and incompatibilities, capacities and capabilities of different agents. The number of agents and processes capable of achieving sterilisation without damaging, destroying or impairing healthcare products and materials are extremely few. However, in this discussion, let sterilisation not become a sterile word and let us never doubt what no consensus group is unable to completely agree about.

1.2 Definition of Sterilisation

Sterilisation for healthcare products, materials (e.g., biomaterials) is a specialised process, implying complete inactivation of all viable forms of life or reproduction.

To achieve sterilisation, a probability function (e.g., 10^{-6}) is required. It is a validated process used to render a product, polymer or material free of all forms of viable micro-organisms, including radiation resistant pathogenic spore (*Bacillus anthracis*), moist heat resistant spores (*Geobacillus stearothermophilus*) and ethylene oxide resistant mould (*Pyronema domesticatum*) and spore forming organisms (*Bacillus atrophaeus*). Some common sterilisation processes are:

- Aseptic/barrier processing
- Chemical or dry heat
- Chlorine dioxide
- Ethylene oxide

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- Hydrogen peroxide
- Hydrogen peroxide with plasma
- Ionising radiation (electron beam, gamma, x-ray)
- Liquid formulation with peracetic acid
- Liquid glutaraldehyde
- Liquid *ortho*-phthalaldehyde
- Ozone
- Saturated steam (low temperature, standard and flash)

In the classical sense, sterilisation is defined as an absolute concept meaning the complete destruction and elimination of all viable micro-organisms [1]. So it is easy to think that sterilisation is applicable to everything, including the most unimaginable bioterroristic pathogen, prions. But, there is no singular sterilisation method that is compatible with all healthcare products including drugs, polymers, devices, and materials, because of the severity of a process to meet the sterilisation criteria and definition. Some commonly sterilised polymers that require various sterilisation methods are:

- ABS, acetals, acrylics; artificial rubbers
- Co-polymers, e.g., polyallomer SAN, TPX
- Delrin, EVA, natural rubbers
- Nylon (polyamide), PC, PE, PET, PI, PVC, PP, PSF
- Polyesters, polyglycolides, polylactides
- Silicone, styrene; Teflon, TPE

This is not an easy task without deleterious effects due to one method *versus* another sterilisation method. To completely inactivate all resistant forms of microbes, they could be incinerated, but that could destroy everything else as well, and there would be no healthcare product that would be useable. Even instruments would be dulled, and metal tempered. Further, to demonstrate that something is absolutely free of viable organisms is statistically and biologically nearly impossible, but probabilistic.

In sterilisation, the nature of microbial death is typically described as organisms declining at a logarithmic or first order rate. Sterilisation is consequentially expressed as the probability of a number of micro-organisms capable of surviving.

1.3 Ideal Qualities of Sterilisation

Sterilisation is what its own qualities determine, reflect and require. Those qualities that ought to be considered, are idealised with complications and variations in the next sections.

1.3.1 Trust

Sterilisation will inactivate all viable forms of life: anaerobes spores, fungi, bacteria, and viruses. Nothing will survive to grow and multiply and this should be certifiable. No survivors, growth, contamination, or infection should be demonstrated after final processing. If the term sterilisation is broadened and defined to include prions, then the process should be capable of inactivating them and measuring their deactivation too. Prions appear to be the smallest lethal self-perpetuating biological entities in the world and they are smaller than viruses. Prion infection control yields the greatest challenge for decontamination, sterilisation and containment, including packaging [2]. Prions are extremely resistant to heat, chemicals, radiation and standard filtration. If prions can contaminate neurological instruments, then sterilisation of healthcare products should be capable of destroying them. Prions are one of the most resistant viable entities to sterilisation.

Chemicals or enzymes which degrade nucleic acids, proteolytic enzymes of the digestive tract, and usable doses of UV or ionising radiation are all ineffective in destroying the prion's infectivity. Standard heat sterilisation, domestic bleach, EO, ionisation radiation and formaldehyde sterilisation have little or no effect. Incineration may not guarantee inactivation of prions. If an evacuation army hospital in war has to control against this most unimaginable bioterroristic pathogen, then sterilisation processing must be designed and developed which will be able to destroy prions.

1.3.2 Sterilisation

Sterilisation must be compatible with as many materials, plastics, products and polymers as possible, this can include equipment and electronics. If hospital products are to be reused, then compatibility must include tensile strength, reproducible functioning and not have any adverse effects to the patient or user.

- Material improvements and advances have led to increased single use disposable devices composed of a wide variety of polymeric materials.

There are inexpensive polymers that are now alcohol and lipid resistant which helps in the healthcare field.

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- The growing market for single use disposable devices has led to polymers that could be sterilised with in place hospital steam sterilisation and with single use radiation sterilised polymers. There is a continual need for improved polymers and devices

There are trends affecting instrument processing in the healthcare field that include the increased use of minimally invasive surgical techniques, new and more stringent health and safety regulatory guidelines, the need for rapid turnaround time, and increased cost savings.

- Sterilisation of disposables can lower healthcare cost considerations attributed to single use devices in place of costly in-house reuse sterilisation devices and components that had to be laboriously decontaminated, cleaned, wrapped and then sterilised.

Currently there is a growing need to be able to sterilise products in hospital, e.g., new and used expensive devices like endoscopes, dialysers that are non sterile, but consisting of new and stable polymers that can be initially sterilised and frequently re-sterilised without adverse effects.

- It will be safe to handle and use. This includes environmental emissions, and has a low risk to personnel and patient. Typically all sterilisation agents will cause death to resistant spores and so also they may even be toxic to humans. Therefore all sterilants and processes must be engineered with safety in mind and handling with care and caution.

For example because radiation can be extremely dangerous and permeable, it requires very rigorous wall separations and sophisticated alarms and sensors to prevent access to them when the sources are available. It is not a process to be handled by simple workers.

- Sterilisation should destroy all microbes but not destroy nor significantly degrade the items it processes.

If it is a biological substance such as skin, enzymes or bones, it must maintain its activity and function, without loss of viability or activity. If it is polymer, it must not distort, melt or discolour significantly.

- Sterilisation should be easy to perform and validate, reproducible, and stable.

Many newer technologies are not completely available or qualifiable for hospitals, and in that case they ought not be considered nor used. Specialised sterilisation needs to be economical, low cost, and inexpensive enough to be useable.

Radiation sterilisation facilities and equipment are typically very expensive, so that it is not justifiable for medium-sized hospitals. However, low steam formaldehyde processes can be performed in a modified steam autoclave, and the cost of formaldehyde and steam is extremely low.

- It can sterilise small products or units as well as large numbers and volumes.

In the commercial healthcare industry, products are terminally sterilised – *already packaged*. If the product is packaged after sterilisation, the process is referred to as aseptic processing, not terminally sterilised. Terminally sterilised products are typically packaged not only in their primary packaging, but also in multiple layers of packaging as unit packs, shelf packs, and shipping cartons. Furthermore these products are made of numerous materials, (i.e., various polymers), and in many different configurations. Conventionally many health products were repeatedly sterilised and recleaned, when they were made with materials such as glass or metals that could be easily reused, but then with the advent of polymers and plastics, single use devices using polymers became less costly, easier to design and manufacture and were available much quicker. For reusable devices, radiation sterilisation is not typically recommended because many polymers and materials can not be repeatedly sterilised without degradation and destruction, except for single use disposable products that are not intended to be resterilised.

- Applicable regulatory bodies must ultimately accept the sterilisation method.

For example, in the United States, the Food and Drug Administration is responsible for approving sterilisation methods. In the past it has been the Environmental Protection Agency (EPA). In the UK, it is the Department of Health Sciences. New sterilisation methods must also meet the criteria of the International Standards Organisation (ISO) [3-10].

- Sterilisation should be fast. The militant force of sterilisation is often time and product availability. In hospitals product availability may be ‘stat’ or immediately. Consequently steam sterilisation is frequently the most common method of choice; however heat sensitive devices can not tolerate steam. To overcome this problem, many devices are presterilised, prepurchased and stocked, on shelves.
- Once sterilised, sterility must be assured.

Microbes must not be capable of repair, regeneration, or reviving, after being inactivated. There should be no visible sign of growth or reproduction. Agents that decontaminate, disinfect, kill germs, and sanitise are deemed less than sterilisation, because by definition they cannot demonstrate the certainty of killing all organisms.

The sterilisation process should be able to sterilise just-in-time (JIT), and not take a long time to sterilise or release after sterilisation

1.4 Statistics, Sterility and Sterilisation

Sterility is 100% inactivation, and freedom from all viable entities. To validate this phenomena, all products of each lot would have to be tested and be shown to be free of microbes, or else sampling only part of a load of sterilised product would lead to an erroneous conclusion with the probability of passing unsterile product as shown in Table 1.1.

So if a lot contained 3.4% contaminated product, and 20 units were sterility tested, there is a 50% chance that no growth will occur and the lot will pass; however if there is 13.9% contamination, there is only a 5% chance that no growth will occur, and the lot will pass. Increasing sample size reduces the chance of failing to find contamination. However, to be assured that the entire lot is sterile would require 100% testing of the entire lot, so that no product would be available for release and use.

To achieve sterility without testing all the product, statistics have to be designed into the exposure or dose of the sterilisation process, to give an assurance that no survivors will occur. This is more complicated than commonly thought.

Mark Twain, the American writer and humourist, once said there are three ways to lie - lies, damn lies, and statistics!

Sterilisation statistics don't lie, but the assumptions used to apply those numbers to statistics can. One common assumption is that all micro-organisms die in a logarithmic order, however, the Rahn logarithmic model potentially applies to only 40% of the curves where there is a straight logarithmic line.

Statistics of a validated sterilisation process is a very significant consideration in the sterilisation processing of diagnostics, drugs, medical devices, and healthcare items today.

Table 1.1 Relationship of probabilities of accepting product lots of varying % of contamination to sample size			
Lot sample size - Total Units Tested	Chance of not finding contaminated product		
	50%	5%	0.5%
% contamination if 10 units are tested	6.7%	25.9%	41.1%
% contamination if 20 units are tested	3.4%	13.9%	23.3%

Table 1.2 Some estimated probabilities for various sterilised items		
Steam Sterilisation	Canned Chicken Soup	10^{-11}
Steam Sterilisation	Large Volume Parenteral (LVP)	10^{-9}
Ethylene oxide, radiation, H_2O_2	Invasive Medical Devices	10^{-6}
Aseptic Fill	Small Volume Parenteral (SVP)	10^{-x}
Liquid sterilants	Lap instruments	10^x
<i>where x is variable based upon barriers, environment, and sterilant</i>		

Sterilisation must be differentiated from lesser means of destroying or removing microbes. Terms and techniques such as antiseptics, disinfection, use of germicides, commercial sterilisation, sanitation, pasteurisation, decontamination and cleaning, preservation, antimicrobials, fungicides, virucides, sporicides, bacteriocides are not synonyms of 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. The actual number of techniques or methods capable of meeting this criteria fully are limited. Moist heat sterilisation meets the criteria most fully. It not only inactivates all viruses, microbes, anaerobes and spores, but also is capable of inactivating resistant mould *Pyronema domesticum*, resistant anaerobic spores, and prions. Other methods of sterilisation may be limited in their ability to inactivate all viruses, *Pyronema domesticum*, *Bacillus anthracis*, prions, or anaerobes at typical processing parameters of dose, because they either haven't been tested to optimal recovery, under all or routine conditions; due to the fact that other recovery methods have not validated inactivation of *all* viable microbes. For example, *Bacillus cereus* or *anthrax* dormant spores may germinate much better in anaerobic conditions, facultatively than under strict aerobic conditions. This section will deal with the statistics of traditional sterilisation, because statistics and sterilisation numbers don't lie, but the assumptions to obtain and apply those numbers to statistics can, when they fail to fulfill the full meaning of sterilisation. Statistics of sterilisation is based on the assumption that all micro-organisms die or are inactivated in a logarithmic of first order reaction rate (**Figure 1.1**). This assumption is reasonably true under laboratory or pure environmental conditions. However exceptions exist. Deviations from the logarithmic or first-order death kinetic model exist. For example, steam sterilisation does characteristically kill in a logarithmic way with some exceptions, (e.g., heat activation or shoulder). Radiation at times exhibits an activation hump or shoulder with *Bacillus pumilus* 601, and tailing with anaerobic *Clostridia* spores. Dry heat sterilisation frequently exhibits tailing (non

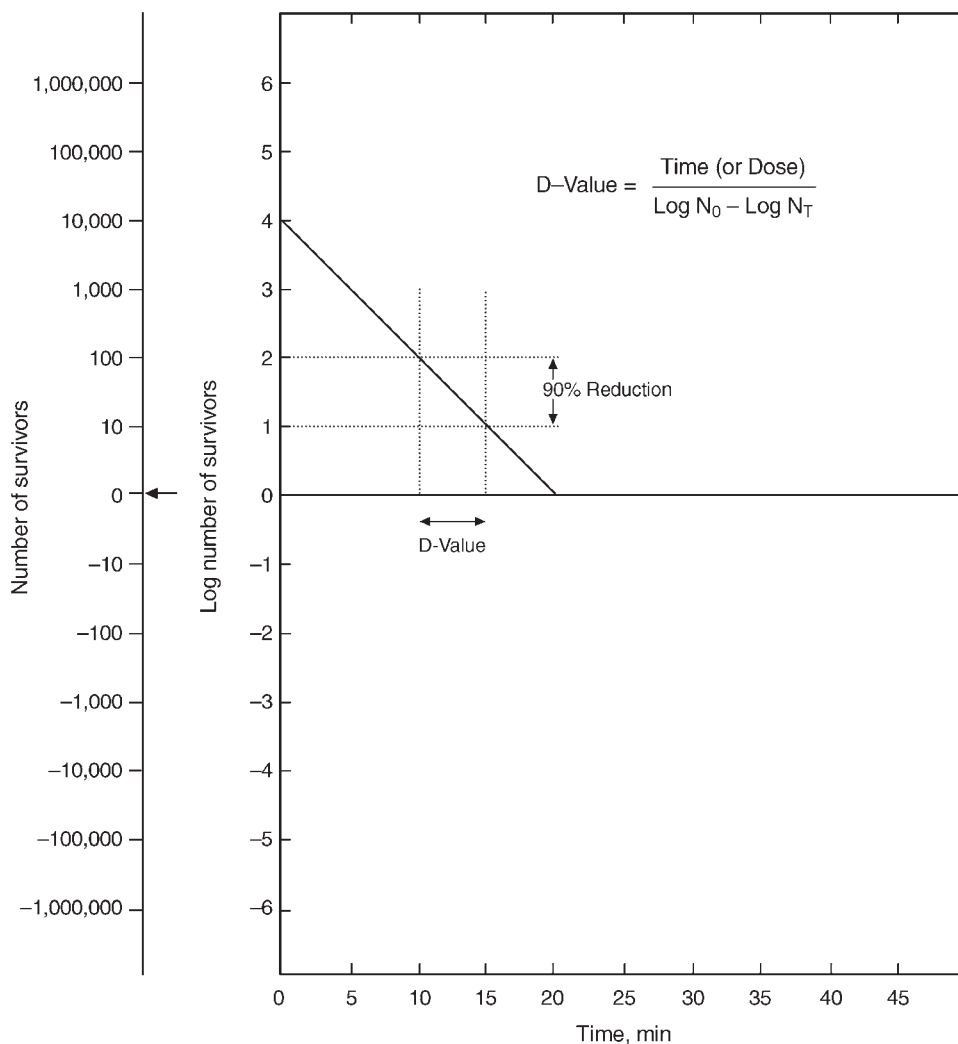


Figure 1.1 Microbial Death Rate Curve, Levels of Probability of Survivors

logarithmic order) with high populations, (e.g., greater than 10^3) [11]. A variety of mathematical models can be described to deal with these deviations, but the simplified logarithmic approach is explained as follows.

The backbone of all terminal sterilisation methods is the decimal reduction value, commonly referred to as the D-value (D_v or D). The D-value is the time, energy, or dose that a sterilisation process takes to inactivate a microbial population by one logarithm

or by 90% of its total population. A simplified equation for the D-value is the Stumbo Equation [12, 13] where:

$$D_v = \text{time} / \log N_o - \log N_b$$

where N_o is the initial microbial or spore population and

N_b is the surviving microbial or spore population after time of exposure.

Twice the D_v or D time would be characterised as 2D, 3 times as 3D, 6 times as 6D, so the time to kill 6D with a probability of 10^{-6} , would be equivalent to 12D. In sterilisation we are dealing with astronomical numbers and statistics (see **Table 1.3**).

In sterilisation we are trying to inactivate enormously resistant forms of viable reproductive entities such as bacterial spores, and prions that are difficult to assay immediately.

The approach towards applying D-value information varies with different sterilisation methods and/or approaches. One of the major differences is the application of

Table 1.3 Example of inactivation of a microbial population at incremental exposures					
Exposure time, min	Microbial population	No. of D values	No. of Log-survivors	Log killed	% Survival population
0	1,000,000	0	6 logs	0	100
2	100,000	1	5	1	10
4	10,000	2	4	2	1
6	1,000	3	3	3	0.1
8	100	4	2	4	0.01
10	10	5	1	5	0.001
12	1	6	0	6	0.0001
14	0.1	7	-1	7	0.00001
16	0.01	8	-2	8	0.000001
18	0.001	9	-3	9	0.0000001
20	0.0001	10	-4	10	0.00000001
21	0.00001	11	-5	11	0.000000001
22	0.000001	12	-6	12	

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D-values from bioburden, which consist of naturally occurring micro-organisms, or from biological indicators/challenges that consist of selected maximum resistant micro-organisms, e.g., spores, usually 1,000,000 population or 10^6 to a specified sterilisation method.

For example *Geobacillus stearothermophilus* spores are typically the most resistant spores to steam sterilisation. In radiation sterilisation, validation is determined by dose setting. Dose setting uses bioburden information, and applies known resistance of population models toward evaluating resistance, or resistance patterns. It does not use biological indicators (BI).

Biological indicators are a spore inocula or carrier of known concentration and spores which are highly resistant a specified sterilisation method, which can predict lethality to the presterilisation bioburden by use of the biological indicator system.

The biological indicator is a characterised preparation of specific micro-organism. For ethylene oxide (EO) and dry heat sterilisation, the spore of choice is typically *Bacillus atrophaeus* ATCC 9372 which is highly resistant to the EO sterilisation process. However, on remote occasions some thermotolerant aerobic spores and organically encrusted, occluded or extremely desiccated microbes may be more difficult to sterilised.

For steam sterilisation, the ‘overkill’ spore of choice is *Geobacillus stearothermophilus*, but it is not always the best spore of choice, because it is a thermophile (and most dase organisms are mesophile organisms) and most common spore organisms are extraordinarily highly resistant to it. Healthcare products sterilised by processes capable of inactivating these organisms, could be damaged destroyed or degraded, by extraordinary high and lengthy steam heat. Other spore formers have been accepted with lower heat resistance such as *Clostridium sporogenes* (an anaerobe), *Bacillus subtilis* 5230 and *Bacillus coagulans* that are not as resistant, allowing for more heat labile products including drugs to be compatible.

Not commonly found in healthcare products, but foods, *Bacillus coagulans* is a thermotolerant spore, it tolerates higher temperatures, but also grows at mesophile temperatures. For radiation, the previous spore of the choice used to be *Bacillus pumilus* E601, which is infrequently used in bioburden dose setting radiation facilities. *Geobacillus stearothermophilus* and some *Clostridium* species may be more resistant than *Bacillus pumilus*, but because the thermophile and anaerobe microbes are not commonly evaluated in dose setting experiments, these type of resistant microbes may go unnoticed. *Pyronema domesticum* is highly resistant to EO and radiation. This organism is both more resistant than biological indicators for radiation and ethylene oxide. Sterility testing conditions for dose setting experiments for radiation may not detect or even recover this organism [13].

There is no all-purpose or ubiquitous spore organism or microbe of choice for a biological indicator. The use of these resistant spores for determination of sterilisation effectiveness rather than bioburden resistance directly *per se* is referred to as an overkill approach.

In ethylene oxide, and frequently heat sterilisation the BI or overkill is a typical approach to determine if sterilisation has occurred and can occur. Combinations of BI approach and bioburden (microbes that are on the product), are used as an alternative approach that facilitates the reduction of exposure times or EO concentration or heat. The bioburden approach is the most involved and rigorous approach from an environmental control or controlled cleanroom manufacturing perspective, but the final analysis is based upon a reliable sterility test.

What constitutes a viable and reproductive organism varies. Micro-organisms that can readily grow and reproduce on their own when placed in suitable growth material are easily defined as viable. However, viruses or prions that require a living host may be more difficult to measure and prions that are potentially capable of infecting other hosts through their protein, and not their nucleic acid are very difficult to assess immediately. In some cases, we may not know they are present, (e.g., prions), until an autopsy is performed. What describes complete destruction or removal of all viable organisms or micro-organisms varies, however, sterilisation methods typically destroy or eliminate microbes in a logarithmic manner. So on this basis it is possible to measure the kill time or lethality logarithmically (D-value time to reduce a microbial population by one logarithm or 90%) and statistically and to extrapolate inactivation of sterilisation as a probability function, beyond the arbitrary biological challenge level or bioburden quantity of microbes on a product or material.

Sterilisation must of necessity be further understood beyond simple statistics.

There are many bioburden, sterility results and formulae that demonstrate variations from straight line non-logarithmic curves such as slopes with activation humps, which may be smoothed with an intercept ratio (IR). The intercept ratio is based on the ratio relationship of $\text{Log } Y_0 / \text{Log } N_0$:

Where Y_0 is the Y intercept and N_0 is the initial spore population. To reconcile the concept of non-logarithmic (shoulder) curves, it is recommended to introduce the concept of intercept ratio (IR). For example, the IR reconciles the differences between the two logarithmic curves. The IR modifies the the Rahn Model when there is not a straight line by incorporating IR as follows:

$$\text{Log } N = -\text{exposure time}/D_v + \text{Log } N_0 (\text{IR})$$

Theory and Kinetics of Destruction				
A theoretical example of the order of death of a bacteria population (applicable for either physical or chemical treatment)				
Time increment	Bacteria living at beginning of time increment	Bacteria killed during one time increment	Bacteria surviving at end of time increment	Logarithm of survivors
First	1,000,000	900,000	100,000	5
Second	100,000	90,000	10,000	4
Thirs	10,000	9,000	1,000	3
Fourth	1,000	900	100	2
Fifth	100	90	10	1
Sixth	10	9	1	0
Seventh	1	0.9	0.1	-1
Eighth	0.1	0.09	0.01	-2
Ninth	0.01	0.009	0.001	-3
Tenth	0.001	0.0009	0.0001	-4
Eleventh	0.0001	0.00009	0.00001	-5
Twelfth	0.00001	0.000009	0.000001	-6

Survival Curves

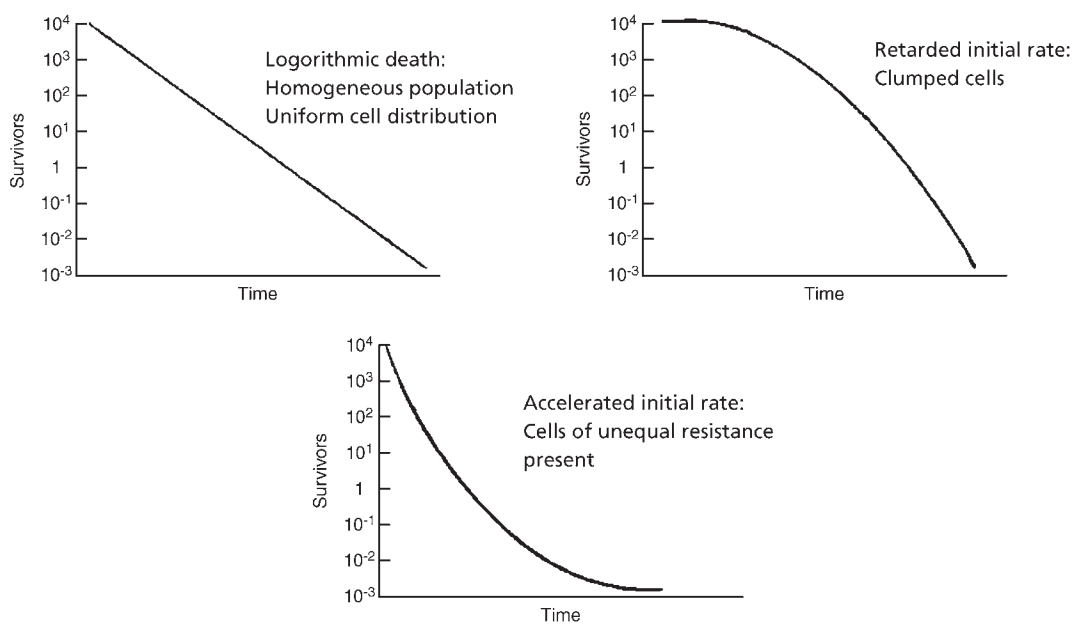


Figure 1.2 Theory and Kinetics of Destruction

Probability of a non-sterile unit (PNSU) or a sterility assurance level (SAL) = $\log^{-1} (-\text{exposure time}/D_v + \text{Log } N_o \text{ (IR)})$.

When $\text{Log } Y_o/\text{Log } N_o$ is > 1 , the curve is a downwards concave shape; when $\text{Log } Y_o/\text{Log } N_o$ is < 1 , the curve is a downwards convex shape and when $\text{Log } Y_o/\text{Log } N_o = 1$, the curve is a straight line.

There are additional statistics that can deal with exceptions such as modifying and degree of sterilisation factors of sterilisation processes. For example, if there is spore activation or increase at the beginning of the process, the IR can be applied. If the microbial population were to exceed 10^6 population, e.g., 10^7 , the time required to inactivate this excessive population plus a probability of 10^{-6} could be expressed in terms of degrees of inactivation, e.g., $10^{7+6} = 10^{13}$. In the food industry, where microbes can multiply and regenerate, some pathogens such as *Clostridium botulinum* may require inactivation factors as high as 12 D or 10^{12} inactivation. But what must be recognised is that dirty large heterogeneous populations of micro-organisms can defeat sterilisation and mess up the statistics to the point where logarithmic curves no longer work, or even apply. For example in dry heat and EO sterilisation it has often been observed that bacterial populations over 1000 colony forming units (cfu) can cause deviations from first order kinetics, and result in a failure to sterilise. Spores occluded in water insoluble crystals or within oils can prevent diffusion of steam or EO to the target bacteria, so that it survives. At times, those in the custom packing industry have found micro-organisms surviving even beyond pretreatment with ionising irradiation, followed by EO sterilisation. Large levels of anaerobic spores, (e.g., *Clostridium*), have been shown to deviate from the straight line of the logarithmic curve with what is called tailing. Biological variation may be a potential sterilisation problem.

It's time to focus from the global picture of sterilisation cycle parameters to the microscopic and mathematical response pattern of spore inactivation. The biological indicator consists of more than 1,000,000 spores. These spores are able to integrate a variety of different cycle parameters into the condition of spore inactivation or spore survival.

If the previous survivors had been saved it would have been possible to determine if the new spores were genetically or physiologically more resistant than previous, but since they were not, that assessment cannot be made. However, based on personal experience, I have never seen *Bacillus atrophaeus* develop heterogeneity genetically resistance within the same growth pool from a single starting organism. However, I have observed thermal growth tolerant spores of different *Bacillus* species or organic encrusted microbes to have greater resistance than the indicator organism, *Bacillus atrophaeus*; consequently, it is important to control and maintain low bioburden levels.

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Vegetative *Escherichia coli* organisms have been shown to be more EO resistant than the biological indicator, *Bacillus atrophaeus* spores, under organic load and low %RH conditions. It is critical to keep bioburden recovery levels low, (e.g., <100 cfu, ISO 11135 [3]). A review of preliminary pre Design of Experiments (DOE) BI data, indicates that spore populations may have heterogenic resistance. The resistance appears to occur at the lower populations, (e.g., 1-300 cfu of a spore population of > 1,000,000). What causes this potential indication of heterogenic resistance, may be mixed cultures, different populations of the same organism. Is the mathematical method, responsible for heterogenic resistance? Spores are supposed to die in a logarithmic manner. Some do, but not all. There is variation to this first order kinetics with higher spore populations it is not unusual to observe increasing resistance or tailing, among a few resistant organisms within a microbial or spore population. In reality, most bioburden consists of mixed cultures, and micro-organisms in this mixed cultures are in various stages of growth from haploid, diploid DNA, endospores, dormant spores, vegetative stage, mold, fungi, virus, aerobic, anaerobic, and microaerophilic; which would result naturally in non-logarithmic behavior; so a logarithmic demonstration of inactivation is not likely to be demonstrated. However, the logarithmic death phenomena has been exploited to predict probability of survivors.

Also, the fraction negative approach where BI are recovered in liquid media may have a certain bias when it is determined by the most probable number theorem, than to demonstrate resistance by the plate count method alone.

Liquid immersion is likely to have a better recovery efficiency than plate count recovery, and it reflects the routine way of recovering BI spore strips. Consequently, it is best to perform liquid immersion because of the simulation of routine evaluation.

However, in addition to the previous considerations, it is demonstrated that a few BI spores may be inherently resistant. The United States Pharmacopeia [14] monograph, for certifying BI resistance of manufactured lots, indicates a wide survival tolerance of up to + 4 logs, beyond the initial BI D-value and initial population. However, for no growth to occur, additional logs must be minimally applied, in order to discourage any mathematical survivors. Consequently additional variation includes +4 logs for no growth to occur and – 2 logs for complete survival response. This translates into a stacked variation of a 4 log to 6 log spread. This could quickly use up the 10^{-6} safety factor.

Under actual statistical evaluation, some typical surviving spore populations may have an excellent correlation, (e.g., $r^2 = 0.90-0.99$), for first order high resistance, unless there is tailing. But, for the entire inactivation curve to be straight from zero time to varying degrees of inactivation, the correlation may be poor to good, (e.g., $0.50-0.84$), for a first order reaction rate.

When reviewed with other assumptions that are taken into mathematical consideration, (e.g., early inactivation and/or sensitisation of dormant spores during initial sterilisation), the correlation (or curve of best fit) may be about 0.91, but it may not be as good as the residual inactivation linear correlation curve of 0.70.

However, when the exposure time is changed to the square root of time, the linear correlation may improve to 0.88-0.99. This latter consideration suggests 'additional' diffusion barrier(s)/limitations to the residual spores. One consideration would be that this additional diffusion barrier/limitation might be clumps of spores on a lot of BI carriers.

To investigate this possibility and correlation, other BI lots and their response to D-value evaluation, BI data from the design of experiments (DOE) need to be evaluated.

In summary, it is very important to know well and control the bioburden, their characteristics and resistance for healthcare sterilisation applications. For amusement and memory, think of microbes and sterilisation in a poetic manner:

The microbe is so very small
no one can hardly make him out at all
it is not the same by isotope or microscope.
Its unseen mouth lies below a hundred rows of curious teeth.
This and more have possibly been seen.
But let us not doubt
while laws of physics, chemistry, and some statistical assumptions appear to prevail
The microbiologist has found other tales and trails
The microbes ionic, enzymatic, sporulating, growth and environmental states and more.
Determine their resistance rates and behaviour
To awesome sterilising traits.

Sterilisation has been around since antiquity. Dry heat as an art and preservative has been with us from before the time of the Pharaohs. Sterilisation as a science has been around for over 125 years, since the invention by Charles Chamberland of the steam autoclave in 1879 [15]. He started using the autoclave for sterilisation of instruments. X-ray radiation was shown to kill micro-organisms as early as 1896 [16]. Other traditional sterilisation methods are EO, low steam-formaldehyde, ionising radiation, and aseptic processing. While new methods and agents have constantly been found, applied and used, traditional methods have managed to sterilise the majority of healthcare products.

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Despite attempting to discredit the use of agents such as EO because of toxicity, carcinogenicity and reproductive toxicity, its use has not diminished. It is now used to sterilise the majority of custom packaging of most medical equipment.

Since earlier times, there have been significant advances and progressive improvement in sterilisation (steam and irradiation), and development of agents and new practical processes such as EO, hydrogen peroxide, plasma, peracetic acid, ozone, hydrogen peroxide and plasma, steam-formaldehyde, and chlorine dioxide. Concurrent with sterilisation development has been the increase in compatible materials, such as polymeric plastics, and healthcare products.

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2 General Overview of Sterilisation and Related Methods for Healthcare Products and Polymers

In this book, current sterilisation agents and processes such as steam, steam-formaldehyde, EO, radiation and dry heat [1] will mainly be presented, with applications relating to hospital products, polymers, and materials. Other niche sterilisation techniques of new products, polymers and materials will also be discussed briefly.

Trillions of healthcare products, polymers and materials are sterilised annually by the healthcare facilities and industry [2]. Most of these healthcare products and materials are polymers. Sterilisation of healthcare products consists of critical processes that define and determine acceptable product and material attributes, and their dynamics and parameters.

2.1 General Considerations of Sterilisation Methods

The control of sterilisation must begin with the design of product and process. There must be a design quality assurance system in place to identify when design input occurs, that there are written specifications or procedures, personnel interfaces that design output and that design verification is documented. To achieve product and process design control, there must be adequate procedures, personnel, plans, and documentation.

Sterilisation design encompasses an interfacial area of investigation and multiple disciplinary backgrounds. A variety of factors and functions encompass sterilisation [3, 4], requiring understanding of environmental, physical, chemical, biological, engineering, manufacturing, quality control and assurance, regulatory, and marketing areas.

To effectively establish a sterilisation programme requires an overview of the entire system. Of necessity sterilisation begins with an understanding and control of the environment and micro-organisms (bioburden) under which the product is manufactured.

2.1.1 Sterilisation Encompasses a Variety of Areas [4]

This process can encompass a parallel qualification of product and package, a determination of sterilisation effectiveness on micro-organisms, effect of process on product samples,

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(i.e., irradiation), a qualification of equipment upon installation or commissioning, process performance validation, and certification.

Under contract sterilisation, the contractor is generally responsible for equipment and facilities and the manufacturers for process and product.

Once a process is completely qualified and validated, the process and equipment are typically revalidated annually or periodically. A completed qualified and validated process allows for routine processing and releasing of product.

Sterilisation matrices can describe the various interactions and relationships of sterilisation within a healthcare facility, from service and manufacturing to the release of product (see Table 2.1).

Sterilisation processes cannot be considered alone, rather, they are interrelated to design, equipment, and the product, polymers and materials to be sterilised.

2.1.2 Sterilisation and Product Design

Healthcare sterilisation is focused primarily on the product design, compatibility of the product and the end use of the product. A comprehensive design verification and review must include many considerations. Among them are design characteristics of the product, polymers and material and product history, and compatibility with the selected or intended sterilisation process. This includes biocompatibility and physico-chemical evaluation of materials with the sterilisation process and environmental compatibility of the sterilised product and process.

2.1.2.1 Sterile Packaging

Other considerations of sterilisation design are packaging requirements for the sterilisation process (see ISO 11607 [27] and EN series 868 parts 1 and 10 [5, 6]), as well as regulatory: *Labelling Requirements for the Type of Sterile Claim*, and the packaging integrity and shelf life [7].

A sterilisation process is only as good as its packaging. To maintain sterility of the product after sterilisation, microbial impermeable packaging is necessary. Typically there are no absolute self-indicating sterile packages, because positive air pressure surrounding the product will maintain and prevent microbial entry. A sterilised package that is inflated and stays inflated could indicate that the package is sterile.

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Table 2.1 Sterilisation – Steps (pre, validation, release) a global and integrated outline					
Environmental Control → ISO 14644- Parts 1, 2, 3, 4, 5, 6, 7, 8 [8-14]					
Biocontamination → ISO 14698-1, 2, 3 [5-7], PDA TR 13 [18]		Design Control, Preproduction → ISO 9000 [15], ISO 9001 [7]			
↓ ISO 13485 [20] ↓		Material Characterisation and Selection - ISO 10993 [21], PDA TR 13 [18] Material Quality AAMI TIR 17 [22], USP EN 556-1 [23], USP Equipment qualification TIR 15 [24] Sterilisation → ISO 14937 [25], AOAC Applicable GMP, USP Compendia Reusables AAMI TIR 12 [26], AAMI ST 81 [27]			
Microbial Control →					
↓					
Environmental/Bio Control PDA TR 29 [28] TR 35 [29], USP Bioburden → ISO 11737-1 [30], PDA TR 21 [31] Microbial (Compendia) PDA TR 13 [18] Sterility → ISO 1137-2 [32], USP → USP/NF & Compendia [33], PDA 19 [34]		Sterilisation Control/Validation Steam →ISO 11134 [35], EN 554 [36], ISO 17665 [37], PDA TR 1 [38], TR 30 [39], USP Radiation → ISO 11137 [40], ISO 13409 [41], TIR 27 [42], PDA TR 11 [43] TR 16 [44] Liquid/Chemical → ISO 14160 [45] EO Sterilisation → ISO 11135 [46], TIR 14 [47], TIR 28 [48] Dry Heat → AAMI ST 63 [49], PDA TR 3 [30] Sterilisation → per specific GMP, NDA, USP Compendia			
Packaging → ISO 11607 [50], TIR 22 [51] → EN 868-1 [5], EN 868-10 [6], PDA TR 5 [52], TR 27 [53], USP		BI → ISO 11138 [23]; ISO–11461 [24] Chemical Indicators → ISO 11140 [56], EN 687 [57]			
Safety/Environment → IEC 1010-2-41, IEC 1010-2-42 → ISO 14000 ↓		↓			
Laboratory Examination ISO Certification/Audits → ISO 9001 [7] → ISO 17025		Product Quarantine - until release			
Method of Release					
Record	Packaging	Dosimetric/ Parametric	Bio.Indicators	Pyrogen/Safety	EO residuals
↓	↓	↓	↓	↓	↓
ISO 9000 [58] ISO 11135 [46] ISO 11134 [47] ISO 11137 [40] ISO 14160 [45] AAMI ST 63 [49]	ISO 11607	ISO 11737-1, 2 [30, 32] ISO 11137 [40] TIR 27 [42] ISO 13409 [41] TIR 20 [59] TIR 29 [64]	ISO 11138 [54] USP/NF [33] FDA [60] or Compendia [33] ISO 11461 [55] Compendia	ISO 10993-1 [61] USP/NF FR 53:5044 [62]	ISO 10993-7 TIR 19 [63]
And for drugs, per Compendia, NDA, GMP, PMA and PDA Technical Reports					
Titles of the sterilisation ISO documents are described below in Table 2.2; Pharmaceutical compendia in many cases will be applicable in lieu of ISO standards for medical devices where drugs or other healthcare products are covered or overlap. Additionally in some cases compendia may overlap or supplement the ISO standard, because ISO standards are more consensus standards, and at times do not cover all aspects of testing or requirements, (e.g., sterility testing).					

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Other ways of demonstrating a sterile package are physical demonstrations of the seal integrity, e.g., dye penetration and Gurly Hill porosity testing.

In the pharmaceutical and drug industry, liquid product containers are filled with microbial supporting media, and the sealed containers then immersed in bacterial growth. If the container contents does not show growth, then microbes did not breach the container nor its seals.

This would not be practical with Tyvek or paper lid pouches or trays used in many healthcare devices, because moisture will diffuse and penetrate these materials. If, however, the tray or pouch is inflated with sterile air, and inflation maintained, these films would not allow microbes to cross its barrier.

The package must be compatible with the product and the sterilisation process.

Packaging integrity is also evaluated with accelerated ageing tests and real time sterility testing of the product after simulated shipping, handling, and storage.

If the sterilisation process can leave toxic residues, then safe levels of the sterilant residue must be considered (see ISO 10993-1 [61] and ISO 10993-7 [58]).

2.1.2.2 Controlled Cleanroom Areas

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

Other aspects of control of sterilisation is 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 may include IPA as well as an non-toxic residual cleaning agent. Disinfection will be periodically performed with 70% aqueous IPA, a quaternary, phenolic, oxidising agent, or other acceptable regulatory, (e.g., EPA), approved germicide. Again, disinfectants that do not leave a residue or can be subsequently cleaned up are favoured. It is important to rotate cleaning and disinfectants to minimise or prevent build up of resistant microbes. For example *Pseudomonas cepacia* has been found in iodophore solutions.

Periodically the cleanliness of the area is monitored by taking particle readings to see that high efficiency particulate air (HEPA) filters are working. Microbial surface and air monitoring is also performed.

Alert and action levels are applied. 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. For example, as per ISO 11135 [46] for validation of EO, the bioburden should not exceed 100 cfu per item, unless further inspected or investigated. In ISO 14644-1 [65] and 14644-2 [66]. For example, a 350,000 particles per m³ or other ISO classification should not be performed per its previous standard of Classes from 209 E. Alternative cleanliness levels, testing, etc., have been standardised in ISO 14644 Part 1-8 [8-13] (*Cleanroom and associated controlled environments*) and ISO 14698 [12] (*Cleanroom and associated controlled environments – Part 1-3*) without adverse effects on the products, its polymers or materials.

Some observations typically made in controlled environment or controlled cleanrooms are:

1. Revise environmental, gowning, cleaning, pre-sterilisation and related procedures:
 - To include 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.
 - Employees must communicate any possible contagious conditions, sickness and blood injuries to the supervisor of the area.
2. Filter and/or rinse alcohol or any solvent containers between uses to minimise build up of contamination or residues:
 - Do not reuse alcohol or any solvent containers without rinsing them first.
 - Do not use alcohol or solvent container after a certain period of time (manufacturing department to decide).
3. Apply part numbers to items to be used, and specify items that will reduce contamination, (e.g., sterile gloves *versus* non-sterile gloves):
 - Apply part numbers for gowns, covers, gloves, hair nets, cleaning materials, equipment.

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4. Analyse trend data and assess differences:
 - Perform a running average on all data points.
 - Look for differences.
 - Assess significant differences.
 - Periodically perform microbial identification or characterisation on predominant colonial type microbe(s), and when extremely high levels occur.
 - Trend analysis can be related to time, season, shift, facility area, and so on.
5. Establish validation of the controlled environmental area, and action and alert levels:
 - Calculate average and standard deviations on the first three or six data points
 - Determine alert levels as average + 2 standard deviations, or by other statistical means.
 - Determine action level as average + 3 standard deviations, or by other means, as appropriate.
 - Another useful level is the target level, which is the average + 1 standard deviation
 - The microbiological levels should be compared to values indicated in USP or ISO standards for applicability.
6. Ensure timely approval of environmental certifications and reports:
 - Approve certifications and reports within a predetermined period (or as established by management, microbiologist), because delays can lead to high bioburden or microbial levels and uncleanliness.
7. Evaluate particulates in finished devices. Designate work areas as semi-critical where parts or surfaces can come in contact with circulatory or compromised tissues of the users. Perform particulate matter testing on any blood contacting device.
8. Improve cleaning:
 - Meet with cleaning representatives. Periodically perform a cleaning inspection, even qualify cleaning. Periodically review area and practices.

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- Update procedures to indicate appropriate use and handling of the agents.
 - Store all primary cleaning agents, materials, and equipment within a storage cabinet.
 - Primary agents, material, and equipment part numbers must match cleaning procedure. There is always a tendency to use another agent without approval.
 - Periodically, (e.g., monthly or quarterly), inspect the area for gross signs of contamination.
 - Review results with the cleaning representative.
 - Add addendum to procedure for selection and preparation of cleaning agents.
9. Periodically perform cleaning of the controlled area, to remove non-work and non-critical items, out of the work area zones.
10. Put gowning and other instructional messages within average view:
- Place written placards at about 1.7 m above the floor, or easily accessible to the onlooker.
11. Revise gowning procedure for those working in a semi-critical area who 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.
12. Observe with a professional mental eye, actual device assembly and manufacturing in the controlled environmental area for potential contamination and infection sources:
- Observe different device assemblies and manufacturing of semi critical operations to critical areas.
13. Observations on controlled environmental areas:
- Handling of alcohol and containers often requires more control. Unfiltered alcohol 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.

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- Part numbers should be included for purchased useables, (e.g., gloves).
- 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*, shouldn't enter controlled environmental area.
- What is a suitable disinfectant? This should be defined, e.g., regulatory approved, etc.
- Assessing six or more environmental monitoring data points can provide for a validation report of the facility's controlled environment. Less than six can lead to excessive variation in determination of variance.
- Performing accumulative averages and assessments with a PC is useful to watch trends for 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 or other applicable standards, as necessary.
- Evaluation of particulates on devices as well as in the air should be considered, for areas of the device that enter the circulatory system. USP, ISO as well as other compendia have recommended limits.
- Written requirements for the gowning area, on a door or wall should be placed high enough so that all may see them.
- Outward airflow should be apparent when opening the gowning room to the outside, or the gowning room to the inner controlled environment.
- There should be a control 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 the necessity. 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.

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- 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 the glove is clean (very little bioburden, such as less than 10 cfu).
- Have all 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 maintain extra containers of disinfectants and germicides.
- The procedure may not tell the cleaning workers which disinfectant or germicide to use and for which location. Be detailed enough 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 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 drips or water pools in the sink. 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. Microbial growth is often an aquatic phenomena.
- Magnahelic gauges were operating just above 0.05 cm of water, but less than 0.1 cm of water.
- There was dust on the top of the bulletin board, and dust 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.

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- Solvent label may state non-chlorofluorocarbon (CFC) solvent, yet it contains dichlorofluoroethane, a CFC. It is important to read labels.
- Pre-sterilisation procedure needs to be updated from old standard to new standard.
- Keep tape and QC supplies, (e.g., pens), away from work areas. Time should be set aside for just cleaning the area by QC and production personnel.
- Periodically relook at all 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.
- 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.

Other means of controlling sterilisation outcomes are through equipment qualification, instrument and dosimeter calibration and equipment maintenance: BI, monitors or dosimeters, positions and certification, process control through parameters, gas certification, steam quality and isotope activity control also includes personnel qualification and training; product load configuration and packaging; process specifications, document review, and finished product testing.

2.1.3 Release of Sterilised Products

More and more sterilisation processes are going to product release based on dosimetric release, parametric or process control rather than by finished product sterility testing or BI testing evaluations. These early product releases require tight cycle or process parameters as well as other monitoring and approved validation procedures and processes.

Validations for sterilisation are performed, and then periodically repeated whenever significant changes occur. With most manufacturers new or significant altered equipment, product or material changes are the reasons for requalification. Once a process has been qualified, it will undergo requalification periodically or annually.

Some examples of routine release after validation are:

- **Dosimetric Release Radiation:** allows for release of product based purely on dose delivered. This is one of the optimal and fastest means of product release. It is however validated under strict standards, (e.g., ISO 11137 [40], ISO TS 13409 [41], AAMI TIR 27 [42]).
- **Process Control Steam Sterilisation:** allows for release of product based upon cycle performance:

Chamber and product temperature readings,

Heat up, exposure and cool down cycle phase times, and

Pressure.
- **Parametric Release for EO:** allows for release of product based upon cycle performance [59]. The parameters used include:

Preconditioning-cycle and/or prehumidification, relative humidity and temperature readings,

Vacuum and pressure limits and times,

Exposure, and dwell time(s),

Gas concentration monitoring,

Chamber and product temperature readings,

Post sterilisation aeration.
- **Reduced Biological Indicator Incubation Time or Enzyme Testing:** which allows for earlier release of product based upon shortened sterile test results from established spore population and resistance, or certified enzymes, and performance of selected process parameter conditions.

2.1.4 From In-House to Outside Sterilisation

More and more industrial manufacturers are going to outside contract sterilisation. Contract sterilisation facilities for healthcare products are considered to be medical device manufacturers and must meet all appropriate regulations that pertain to their operations

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in accordance to regulations since sterilisation is a special manufacturing process. Some specific considerations for contract sterilisation are:

- Segregation of sterile/non sterile product.
- Customer/client record keeping; rigorous personnel training; separate cycle/process and software validation; process change control; audits, and particularly adequate information transfer between customer and steriliser or processor, and issuance of non-compliance when it occurs.
- Contract sterilisers must register with the regulatory bodies and be routinely audited and inspected.
- Specific transfer of information for contract steriliser and customer are a written agreement including names and addresses of firms and signatures.
- Also there needs to be instruction for records, acknowledgement of non-sterility when it is suspected, and meeting good manufacturing practices.
- A description of the process must be included and specific labelling, e.g., the status of the load during shipping to a steriliser, and after sterilisation must be marked as 'Non -Sterile - Awaiting Processing' or 'Processed Awaiting Test Results'
- Understanding standards in sterilisation is critical.

2.2 Standards

Standards are provided by non-governmental international organisations, (e.g., ISO), pharmaceutical compendiums, and private organisations, (e.g., Association for the Advancement of Medical Instrumentation (AAMI)), as well as individual regulatory bodies of governments (e.g., FDA, DHSS) [67].

These standards include sterilisation and validation.

In the following section, the harmonisation of standards for sterilisation will be discussed.

2.2.1 Harmonisation of Sterilisation Criteria

The principle standard setting organisations for sterilisation are:

- American National Standards Institute (ANSI)

- Association for the Advancement of Medical Instrumentation (AAMI)
- European Community for Normalisation (CEN)
- The International Standards Organisation (ISO)

ISO standards are generally recognised by the FDA, DHSS and the international community. AAMI standards have long been used as guidelines by the FDA, in their compliance efforts. The CEN standards are documents that have been prepared for the European Community (EC) harmonisation of 1992, in *lieu* of individual European country compendia and regulations.

In the past few years considerable effort has been exerted by AAMI, FDA, European and other international countries to determine international standards to obtain harmonisation with the CEN, and regulations of other individual countries.

This task has not been easy. Differences between individual countries and other sterilisation standard setting groups existed. Other standards setting organisations involved in sterilisation are ASTM, Parenteral Drug Association (PDA), Health Industry Manufacturing Association (HIMA), and other sterilisation guidance organisations.

The United States strategy was to set new sterilisation standards through ANSI/AAMI that influence the ISO standards to bring about harmonisation of requirements throughout the world.

ISO is a non governmental organisation founded in 1947. ISO has over 170 technical committees and over 2,300 subcommittees and working groups. These standards are generally recognised by pertinent regulatory agency, such as the FDA, DHSS, and the international bodies such as BSI (UK), TUV Rheinland Group.

AAMI was appointed by ISO to be the secretariat for the *Sterilisation of Healthcare Products* technical committee, designated as ISO/Technical Committee 198. The AAMI was further responsible for administering the US Technical Advisory Groups (TAG) that establish the US viewpoint. For example, AAMI standards have long been used by the FDA as guidelines in assessing good manufacturing practices (GMP).

In Europe, the CEN standards have been established since 1992 for the European Community (EC).

2.2.2 Harmonisation of Standards (ISO)

The attempt to harmonise some of the AAMI and EN Standards have resulted in numerous ISO standards. A few examples are:

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1. EO sterilisation, became ISO 11135 [46] with EN 550 [68]
2. Radiation sterilisation, became ISO 11137 [40] with EN 552 [69]
3. Industrial moist heat sterilisation, became ISO 11134 [35] with EN 554 [36]

However, definition or assurance of sterilisation EN 556-1 [23] remains unharmonised, because the USA accepts two levels of sterility rather than a singular absolute sterility probability.

ISO 10993-7 [58] is partially unharmonised with the FDA's added EO residue recommendation.

In order to understand ISO standards, one has to realise that they have normative (mandatory sections and informative (non-mandatory-guidance) sections. It is critical to understand the differences.

The first normative sections are mandatory criteria that specify required or directive information. For example, the classes of air cleanliness are normative. How classes are determined is specifically spelled out by a clearly defined mathematical formula. This is also normative.

The basic document, includes scope, definitions, normative sections, and then informative sections. Indexes may be mandatory if they are specified in the normative section or non-mandatory if they are specified in the informative section.

2.2.3 Some Biological Standards

The resulting sterilisation standards rely heavily on the control or destruction or removal of micro-organisms. Consequently the control of sterilisation must of necessity begin with exertion of control over the environment(s) that the components and product will be manufactured under.

2.2.3.1 Control of Micro-organisms

Some of the ISO documents for controlled environment are:

- **ISO 14644-1: Cleanrooms and Associated Controlled Environments. Part 1: Classes of Air Cleanliness** [65].

SCOPE: 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 (see Table 1 of the Standard).

There are nine major classes of air cleanliness, 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. 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 [8] and ISO 14644-2 [66].

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 μm and 0.5 μm .

There are further provisions for defining air cleanliness based upon particles larger than 5.0 μm . These are called macroparticles or M descriptors. Macroparticles 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 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.

In addition, two of the six annexes in this document are normative. They are:

Annex B: Determination of particle cleanliness classification using a discrete-particle-counting, light scattering instrument.

Annex C: Statistical treatment of particle concentration data.

Non Mandatory: The other four annexes are informative and are provided for user guidance. They provide a relative graphical illustration of the air cleanliness classes, examples of classification calculations, consideration for counting and sizing both macroparticles and ultrafine particles as well as a procedure for sequential sampling.

- **ISO 14644-2 [66]:** *Cleanrooms and Associated Controlled Environments—Part 2: Specification for Testing and Monitoring to Prove Compliance with ISO 14644-1 [65].*

SCOPE: 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 strength from ISO 14644-1, which was published first. ISO 14644-2 spells out the mandatory and non-mandatory tests that must be performed to prove compliance with ISO 14644-1. This short document, only eight pages long, is extremely important.

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

- a) Classification of air cleanliness
- b) Pressure difference
- c) 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 DIN EN ISO-14644-3, *Metrology and Test Methods* [70].

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:

- a) Installed filter leakage
- b) Airflow visualisation
- c) Recovery time
- d) Containment leakage

Generally, ISO 14644-1 and ISO 14644-2 require fewer sample locations for air cleanliness classification than is the case with US Federal Standard 209E [71], thereby providing cost savings at no sacrifice to air cleanliness quality.

The Final Draft International Standard (FDIS) version of ISO 14644-2 is significantly different from the draft international standard (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.

- **DIN EN ISO 14644-3:** *Cleanrooms and Associated Controlled Environments—Part 3: Metrology and Test Methods* [70].

SCOPE: 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:

- 1) Airborne particle count for classification
- 2) Airborne particle count for ultrafine particles
- 3) Airborne particle count for macroparticles
- 4) Airflow
- 5) Air pressure difference
- 6) Installed filter system leakage
- 7) Flow visualisation
- 8) Air flow direction
- 9) Temperature
- 10) Humidity
- 11) Electrostatic and ion generation
- 12) Particle deposition
- 13) Recovery
- 14) 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 level 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. DIN 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.

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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, etc.

- **ISO 14644-4: *Cleanrooms and Associated Controlled Environments—Part 4: Design and Construction*** [13].

SCOPE: 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.

The scope of requirements section necessitates that the purpose of the cleanroom and the operations to be carried out within it are clearly defined. Parameters such as utility needs, process support, dimensions, overall layout, entry and exit of materials and personnel must be defined. Measurement control and monitoring parameters and the influence of external environmental factors are all part of this specification process.

The scope of requirements section details the assignment of tasks for the preparation, approval, execution, supervision, documentation, statement of criteria, basis of design, detailed design, construction, testing, commissioning, qualification and the performance and witnessing of tests. More succinctly, it states who is responsible for what.

Second is the planning and design section, which provides an overview of the details necessary for proper cleanroom design. How does the design, address the specification

of requirements described previously? For example, a contamination control concept should be developed for each zone of a cleanroom installation. Cost factors, timescale factors, design options, constructability of design and project flexibility are all covered in this section.

The third section is on construction and start-up. Specific contamination control requirements apply to construction activities whether performed on the job site or in a remote location. A cleanroom construction protocol and clean-up procedure should be established for all locations as part of an overall quality programme. Security and access control should be part of a continuous clean-up plan. Thorough cleaning is required before start-up.

After completion of construction comes the critical phase of testing and approval of the cleanroom. This section requires that all personnel in charge of the new cleanroom be properly trained in its operation. Such training is to include cleanroom operations, maintenance and in-process control.

Testing and approval comes in three distinct stages:

- Construction approval - does the cleanroom comply with the design requirements?
- Functional approval - do all parts of the cleanroom operate together to achieve the required 'as-built' or 'at rest' state?
- Operational approval - does the cleanroom operate properly in the 'operational' state?

It is important that proper documentation be created and maintained. There is an entire section of ISO 14644-4 devoted to suggestions for appropriate documentation. Included are 'as built' drawings, test and certification data, operational and maintenance manuals, spare parts lists and training records.

There are eight comprehensive Annexes to ISO 14644-4, which are helpful in detailing suggested design criteria, materials of construction, approval stages, installation layout, construction procedures, environmental control requirements and air cleanliness control.

- **ISO 14644-5: *Cleanrooms and Associated Controlled Environments—Part 5: Cleanroom Operations*** [11].

SCOPE: Specifies the basic requirements for operating a cleanroom.

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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 six 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?

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? How aggressive are your cleaning compounds? How do you avoid adding contamination by your own cleaning procedures?

- **ISO 14644-7: *Cleanrooms and Associated Controlled Environments—Part 7: Separative Enclosures (Clean Air Hoods, Glove Boxes, Isolators and Mini Environments)*** [9].

SCOPE: Specifies the minimum requirements for the design, construction, installation, testing and approval of separative enclosures in those respects 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.

In writing the ISO-14644-7 standard, all factors of a clean environment had to be considered in miniature. Such issues of material ingress and egress, personnel interface, installation and maintenance, support services, testing and certification had to be considered for a very different style of clean enclosure than that which is required for a typical cleanroom.

ISO 14644-7 is recommended for those who manufacture or use clean-air hoods, glove boxes, isolators, mini-environments or like-minded enclosures. Such enclosures may be

no-wall, soft-wall or hard-wall, but they share a unifying concept—that a continuum of separation exists between the operator and the operation.

The Standard covers such issues as design and construction, risk analysis, contamination control concept, assessment of external influences, access devices, transfer devices, installation, and testing and approval procedures, including glove breach test, leak test, pressure differential test and routine alarm requirements.

There are many other aspects of these separative enclosures for which guidance is provided in the detailed annexes.

Probably the most valuable is Annex A, which spells out the newly created '*Separation Continuum Concept*.' This is the key for defining a particular separative enclosure. It weighs the separation means (from aerodynamic to physical) against the assurance of maintaining separation (from low to high). In simple terms, anything from an airflow curtain to a stainless steel wall can be used, recognising that the more physical the barrier, the higher the assurance of separation.

Additional annexes address such issues as air handling and gas systems, access device options in detail, transfer device options in detail, leak detection and testing methods, pressure integrity of enclosures and support devices, as well as design and construction parameters.

- **ISO 14644-8: *Cleanrooms and Associated Controlled Environments—Part 8: Classification of Airborne Molecular Contamination*** [14].

SCOPE: 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 and 10^{-12} g/cm³.

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 (AMC) 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 (SMC) 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 gaseous products are released from a material under specified conditions of temperature and pressure.

ISO 14644-8 provides a formal classification system of AMC. This system has an ISO descriptor which is as follows:

AMC. ISO Class N:a;b; (c); (d)

Where:

N = the logarithmic index of concentration expressed in g/cm^3

a = the type of compound (acid, base, organic, inorganic)

b = the specified measuring method (sampling and analytical)

c = the optical extension for a particular species

d = the optional extension to include elapsed time

A few examples of this code are:

- 1) 'AMC. ISO Class-6:A:IMP-IC; (HCl); (-)'

Translated this is an airborne concentration of HCl (hydrochloric acid) of $10^{-6} \text{ g}/\text{cm}^3$, sampled with an impinger (IMP) and analysed with ion chromatography (IC) and no elapsed time period (-).

- 2) AMC. ISO Class-5:O:SOR-GC-MS; (DOP); (2016 post operational)

Again translated this expresses an airborne concentration of DOP which is an organic (o) species at $10^{-5} \text{ g}/\text{cm}^3$ sampled with a sorbent tube (SOR), analysed by gas chromatography mass spectroscopy (GC-MS) at an elapsed time of 12 weeks expressed as 2016 hours post operational, i.e., after 12 weeks of cleanroom operation.

There is also a provision for measuring surface molecular contamination and this is expressed in a similar fashion in the next example:

- 3) SMC. ISO Class-8:0:DIFF-GC-MS; (DOP); (24)

The translation here is surface concentration of DOP (an organic) of $10^{-8} \text{ g}/\text{cm}^3$ after 24 hours exposure as sampled with passive diffusive sampling (DIFF) analysed using GC-MS.

ISO 14644-8 as currently under development has been well organised to give guidance for developing a sound procedure for assessing the parameters affecting airborne and surface molecular contamination in a cleanroom or other controlled environment.

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The AMC and SMC classifications for molecular contamination are entirely separate from the classification of air cleanliness found in ISO 14644-1 for particulate air cleanliness.

Sources of molecular contamination can be from outdoor air, construction materials, cross-contamination within a facility, and from daily cleanroom operation and maintenance, including garments, cleaning fluids, packaging materials and portable equipment.

ISO 14644-8 provides a detailed checklist of potential cleanroom-related molecular contamination sources. In addition, it lists typical contaminating chemicals and substances. There is also a listing of typical methods for the measurement and analysis of molecular contamination both passive and dynamic. Five different sampling instruments and 16 different analysis methods are shown, and these are by no means all the options available. However, the instruments and methods must be measurable, verifiable and repeatable.

The idiosyncrasies of barrier technology, as found in isolators, mini-environments, glove boxes, clean hoods and the impact of molecular contamination therein, is addressed in ISO 14644-8.

The last 18 pages of this document clearly spell out standard evaluation methods for acids, bases, organics (condensables) and inorganics (dopants). This section provides a valuable guide for measuring the concentration of molecular contamination.

The control of molecular contamination is a field still in its infancy but growing and changing rapidly. It is important to our future. ISO 14644-8 does not recommend any specific control programme or device. Rather it provides the means for identifying and assessing the amount of molecular contamination present. How it is best controlled must be determined by the facility involved through determining the source, the carrier, and the resultant interactions on product, process and yield, etc. Elimination at the source is the best place to start. Establishing levels of acceptance is another key parameter.

Molecular contamination can also be controlled by chemical filtration systems, dilute chemistry, alternate chemistries, event control, that is, spills or product handling and common sense when sources are identified and quantified.

- **ISO 14698-1: *Cleanrooms and Associated Controlled Environments—Biocontamination Control Part 1: General Principles and Methods*** [15].

SCOPE: 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 (HACCP), Fault Tree Analysis (FTA), Failure Mode and Effect Analysis (FMEA) 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. Biocontamination can be airborne, on surfaces, on clothing, in liquids, even in the laundering of cleanroom textiles such as garments and wipers.

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 biocontamination sampling programme must be established for cleanroom air, walls, floors, ceilings, 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.

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.

- **ISO 14698-2:** *Cleanrooms and Associated Controlled Environments—Biocontamination Control Part 2: Evaluation and Interpretation of Biocontamination Data* [16].

SCOPE: 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. 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.

- **ISO 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* [17].

SCOPE: Describes guidance for a laboratory method for measuring the efficiency of cleaning an inert surface.

STATUS: ISO/TC209, the ISO Technical Committee on ‘Cleanrooms and Associated Controlled Environments,’ draws on over 1,000 volunteers from 37 countries to create realistic standards for practical use in the global cleanroom community.

ISO14644-1 is the key to the full series of ISO global cleanroom standards. The use of this document became mandatory in the European Union on November 1, 1999. Other parts of the world have also adopted 14644-1 as their baseline cleanroom (clean space) classification document. ISO 9000-certified organisations are required to utilise ISO 14644-1 in defining their clean space.

2.2.3.2 Biocompatibility Standards

ISO/FDA standards have been adopted for medical devices and provide additional information and guidance. The FDA's standard provides a flow chart and matrix that is useful in determining the types, numbers, and kinds of tests that are necessary. The FDA's standard includes more tests than ISO criteria, but slightly less than the previous Tripartite Biocompatibility Standard.

Reference List

AAMI/ANSI/ISO 10993, Biological Evaluation of Medical Devices, 1992:

- Part 1 Evaluation and Testing
- Part 2 Animal Welfare Requirements
- Part 3 Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicity
- Part 4 Selection of Tests for Interactions with Blood
- Part 5 Tests for Cytotoxicity: *In Vitro* Methods
- Part 6 Tests for Local Effects after Implantation
- Part 7 Ethylene Oxide Sterilisation Residuals
- Part 8 Clinical Investigation
- Part 9 Degradation of Materials Related to Biological Testing
- Part 10 Tests for Irritation and Sensitisation
- Part 11 Tests for Systemic Toxicity
- Part 12 Sample Preparation and Reference Material
- Part 13 Degradation Products from Polymers
- Part 14 Degradation Products from Ceramics
- Part 15 Degradation Products from Coated and Uncoated Metals and Alloys
- Part 16 Toxicokinetic Study Design for Degradation Products and Leachables from Medical Devices
- Part 17 Methods for the establishment of allowable limits for leachable substances using health based risk assessment
- Part 18 Chemical Characterisation of Materials
- Part 19 Physico-chemical, Mechanical, Morphological and Topographical Characterisation of Materials

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And the list is longer than this.

In 1995, FDA and CDRH started using ISO 10993, *Biological Evaluation of Medical Devices Part 1: Evaluation and Testing*, General Programme Memorandum G95-1, Rockville, MD, USA.

2.2.4 ISO Sterilisation Standards

Some of the ISO sterilisation standards for healthcare products are given in **Table 2.2**.

Table 2.2 Some ISO sterilisation standards	
Standard Number	Title
ISO 11134:1994	Sterilisation of healthcare products—Requirements for validation and routine control—Industrial moist heat sterilisation.
ISO 11135:1994 Corr 1:1994	Medical devices—Validation and routine control of ethylene oxide sterilisation.
ISO 11137:1995 Amd 1:2001	Sterilisation of healthcare products—Requirements for validation and routine control—Radiation sterilisation.
ISO 11138-1:1994	Sterilisation of healthcare products—Biological indicators—Part 1: General.
ISO 11138-2:1994	Sterilisation of healthcare products—Biological indicators—Part 2: Biological indicators for ethylene oxide sterilisation.
ISO 11138-3:1995	Sterilisation of healthcare products—Biological indicators—Part 3: Biological indicators for moist heat sterilisation.
ISO TS 11139:2001	Sterilisation of healthcare products—Vocabulary.
ISO 11140-1:1995 Amd 1:1998	Sterilisation of healthcare products—Chemical indicators—Part 1: General requirements.
ISO 11140-2:1998	Sterilisation of healthcare products—Chemical indicators—Part 2: Test equipment and methods.
ISO 11140-3:2000	Sterilisation of healthcare products—Chemical indicators—Part 3: Class 2 indicators for steam penetration test sheets.
ISO 11140-4:2001	Sterilisation of healthcare products—Chemical indicators—Part 4: Class 2 indicators for steam penetration test packs.

*General Overview of Control Sterilisation and Related Methods for
Healthcare Products and Polymers*

ISO 11140-5:2000	Sterilisation of healthcare products—Chemical indicators—Part 5: Class 2 indicators for air removal test sheets and packs.
ISO 11607:2003	Packaging for terminally sterilised medical devices.
ISO 11737-1:1995	Sterilisation of medical devices—Microbiological methods—Part 1: Estimation of population of micro-organisms on products.
ISO 11737-2:1998	Sterilisation of medical devices—Microbiological methods—Part 2: Tests for sterility performed in the validation of a sterilisation process.
ISO 13408-1:1998	Aseptic processing of healthcare products—Part 1: General requirements.
ISO 13408-2:2003	Aseptic processing of healthcare products—Part 2: Filtration.
ISO TS 13409:2002	Sterilisation of healthcare products—Radiation sterilisation—Substantiation of 25 kGy as a sterilisation dose for small or infrequent production batches.
ISO 13683:1997	Sterilisation of healthcare products—Requirements for validation and routine control of moist heat sterilisation in healthcare facilities.
ISO 14160:1998	Sterilisation of single-use medical devices incorporating materials of animal origin—Validation and routine control of sterilisation by liquid sterilants.
ISO 14161:2000	Sterilisation of healthcare products—Biological indicators—Guidance for the selection, use and interpretation of results.
ISO 14937:2000	Sterilisation of healthcare products—General requirements for characterisation of a sterilising agent and the development, validation and routine control of a sterilisation process for medical devices.
ISO/TS 15843:2000 Corr 1:2003	Sterilisation of healthcare products—Radiation sterilisation—Product families and sampling plans for verification dose experiments and sterilisation dose audits, and frequency of sterilisation dose audits.
ISO/TR 15844:1998	Sterilisation of healthcare products—Radiation sterilisation—Selection of sterilisation dose for a single production batch.
ISO 13408-2:2003	Aseptic processing of healthcare products—Part 2: General requirements.

2.2.5 CEN Sterilisation Standards

Some of the many CEN sterilisation standards are given in **Table 2.3**.

In microbiological evaluations, all sterilisation methods are concerned with the demonstration of inactivation or elimination of viable micro-organisms under sub-process conditions.

Table 2.3 Some CEN sterilisation standards	
Standard Number	Title
EN 285:1996 Corr 1998	Sterilisation - Steam sterilisers - Large sterilisers
EN 550:1994	Sterilisation of medical devices - Validation and routine control of ethylene oxide sterilisation
EN 552:1994 amd 2: 2000	Sterilisation of medical devices - Validation and routine control of sterilisation by irradiation
EN 554:1994	Sterilisation of medical devices - Validation and routine control of sterilisation by moist heat
EN 556-1:2001	Sterilisation of medical devices - Requirements for medical devices to be labelled 'Sterile' - Part 1: Requirements for terminally sterilised medical devices
EN 866-1:1997	Biological systems for testing sterilisers and sterilisation processes - Part 1: General requirements
EN 866-2:1997 Corr 1998	Biological systems for testing sterilisers and sterilisation processes - Part 2: Particular systems for use in ethylene oxide sterilisers
EN 866-3:1997	Biological systems for testing sterilisers and sterilisation processes - Part 3: Particular systems for use in moist heat sterilisers
EN 866-4:1999	Biological systems for testing sterilisers and sterilisation processes - Part 4: Particular systems for use in irradiation sterilisers
EN 866-5:1999	Biological systems for testing sterilisers and sterilisation processes - Part 5: Particular systems for use in low temperature steam and formaldehyde sterilisers
EN 866-6:1999	Biological systems for testing sterilisers and sterilisation processes - Part 6: Particular systems for use in dry heat sterilisers

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EN 866-7:1999	Biological systems for testing sterilisers and sterilisation processes - Part 7: Particular requirements for self-contained biological indicator systems for use in moist heat sterilisers
EN 866-8:1999	Biological systems for testing sterilisers and sterilisation processes - Part 8: Particular requirements for self-contained biological indicator systems for use in ethylene oxide sterilisers
EN 867-1:1997	Non-biological systems for use in sterilisers - Part 1: General requirements
EN 867-2:1997	Non-biological systems for use in sterilisers - Part 2: Process indicators (Class A)
EN 867-3:1997 Corr 1998	Non-biological systems for use in sterilisers - Part 3: Specification for Class B indicators for use in the Bowie and Dick test
EN 867-4:2000	Non-biological systems for use in sterilisers - Part 4: Specification for indicators as an alternative to the Bowie and Dick test for the detection of steam penetration
EN 867-5:2001	Non-biological systems for use in sterilisers - Part 5: Specification for indicator systems and process challenge devices for use in performance testing for small sterilisers Type B and Type S
EN 868-1:1997	Packaging materials and systems for medical devices which are to be sterilised - Part 1: General requirements and test methods
EN 868-2:1999	Packaging materials and systems for medical devices which are to be sterilised - Part 2: Sterilisation wrap - Requirements and test methods
EN 868-3:1999	Packaging materials and systems for medical devices which are to be sterilised - 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 868-4:1999	Packaging materials and systems for medical devices which are to be sterilised - Part 4: Paper bags - Requirements and test methods

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EN 868-5:1999 Corr 2001	Packaging materials and systems for medical devices which are to be sterilised - Part 5: Heat and self-sealable pouches and reels of paper and plastic film construction - Requirements and test methods
EN 868-6:1999	Packaging materials and systems for medical devices which are to be sterilised - Part 6: Paper for the manufacture of packs for medical use for sterilisation by ethylene oxide or irradiation - Requirements and test methods
EN 868-7:1999	Packaging materials and systems for medical devices which are to be sterilised - Part 7: Adhesive coated paper for the manufacture of heat sealable packs for medical use for sterilisation of ethylene oxide or radiation - Requirements and test methods
EN 868-8:1999	Packaging materials and systems for medical devices which are to be sterilised - Part 8: Re-usable sterilisation containers for steam sterilisers conforming to EN 285 - Requirements and test methods
EN 868-9:2000	Packaging materials and systems for medical devices which are to be sterilised - Part 9: Uncoated nonwoven materials of polyolefines for use in manufacture of heat sealable pouches, reels and lids - Requirements and test methods
EN 868-10:2000	Packaging materials and systems for medical devices which are to be sterilised - Part 10: Adhesive coated nonwoven materials of polyolefines for use in the manufacture of heat sealable pouches, reels and lids - Requirements and test methods
EN 980:2003	Graphical symbols for use in the labelling of medical devices
EN 1174-1:1996	Sterilisation of medical devices - Estimation of the population of micro-organisms on product - Part 1: Requirements
EN 1174-2:1996	Sterilisation of medical devices - Estimation of the population of micro-organisms on product - Part 2: Guidance
EN 1174-3:1996	Sterilisation of medical devices - Estimation of the population of micro-organisms on product - Part 3: Guide to the methods for validation of microbiological techniques

EN 1422:1997 Corr 2002	Sterilisers for medical purposes - Ethylene oxide sterilisers - Requirements and test methods
DIN EN 13060: 2004	Small steam sterilisers
ISO 14160:1998	Sterilisation of single-use medical devices incorporating materials of animal origin - Validation and routine control of sterilisation by liquid sterilants
EN ISO 14161:2000	Sterilisation of healthcare products - Biological indicators - Guidance for the selection, use and interpretation of results
<i>All of the documents and standards in this table are continually liable to change, updating, and additions.</i>	

2.3 Sterility Assurance Levels (SAL)

One of the greatest concerns in sterilisation processes and qualification/validation is assurance that the product or item being sterilised is sterile. To fully appreciate the role that statistics plays in sterility assurance, let us start from scratch and begin with the word, sterile.

Sterile is defined as the complete freedom from all living or reproducing organisms or entities. The term implies an all or 'nothing' condition. Either all micro-organisms are killed or removed or they are not. There is a tendency at times, to mistakenly use sterile in the wrong context. For example, in early medical practice sterile meant to destroy only disease organisms. In the home, baby bottles that were boiled were considered sterile.

Sterilisation must be differentiated from lesser ways of destroying or removing microbes. Terms and techniques such as disinfection, sanitisation, pasteurisation, 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. So when the House of Representative was sanitised with chlorine dioxide to eliminate *Bacillus anthracis* (anthrax), it was not intended to mean sterilisation or complete inactivation of all microbes or terrorist biological agents.

If it were sterile, it would not compromise the safety and health of inhabitants, users, or other persons passing by; it would be free not only from *Bacillus anthracis*, but freed from all micro-organisms.

To determine sterility it must be tested for, and one must know what sterile means.

Sterile is defined as 100% freedom from all viable micro-organisms during a test.

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When we test for sterility there must be no evidence of microbial growth. In general there are two basic ways to test for sterility:

- (1) Product sampling and product sterility or sub-sterility testing
- (2) The application and use of biological indicators.

In brief, product sterility testing is performed by placing a sample of a sterilised product in a suitable bacteriological recovery media and monitoring for bacteria growth. Alternatively, the lumen of a device or parts of a product may be rinsed or flushed with a recovery fluid through a bacteria retentive filter membrane or a drug filtered through a filter. These filters are then put in the recovery medium. Product sterility testing for healthcare products are described in ISO 11737-2 [72] or an official compendium such as the US Pharmacopeia/ National Formulary [33].

In the product sterility test, there is a statistical relationship between sample size and probability of passing unsterile product at different contamination levels (see **Table 2.4**).

Table 2.4 Probability of passing unsterile product at different contamination levels			
Sample Size	Probability of sample containing no non-sterile units		
Total units tested	50%	5%	0.5%
10	6.7*	25.9	41.1
20	3.4	13.9	23.3
30	2.3	9.5	16.2
40	1.7	7.2	12.4
60	1.1	4.9	8.5
*contamination rate			

So, if a lot contained 3.4% contaminated product, and only 20 units were sterility tested there is a 50% chance that growth will occur and the lot will not pass. If there was 13.9% contamination, there is only a 5% chance that no growth will occur and the lot will pass.

Another problem inherent in sterility testing is adventitious (accidental) contamination. When the sample size is increased to detect low level contamination, the chance of adventitious contamination will increase proportionally, particularly when the product is difficult to handle, manipulate or transfer aseptically. Sterility testing, depending upon

the type of product to be sterilised, typically requires careful aseptic manipulation and rigorous sterility technique.

Sterility testing of biological indicators is easier. Biological indicators typically consist of highly resistant spores which are placed on or in the product prior to sterilisation. These indicators have high microbial populations in excess of that naturally occurring on the product. The combination of high microbial population and high resistance makes these indicators a fairly reliable tool for determination and development of sterilisation and sterility.

The type of biological indicator or challenge organism is matched to the specific sterilisation method used (Table 2.5).

Table 2.5 Sterilisation methods and some appropriate biological indicator or challenge organism	
Saturated steam	<i>Geobacillus stearothermophilus</i> ATCC 7953 or ATCC 12980
	<i>Clostridium sporogenes</i> PA 3679 or ATCC 11437
	<i>Bacillus coagulans</i> FRR B666 or ATCC 51232
	<i>Bacillus subtilis</i> 5230 or ATCC 35021
Dry heat	<i>Bacillus atrophaeus</i> ATCC 9372 or NCTC 10073
Ethylene oxide	<i>Bacillus atrophaeus</i> ATCC 9372 or NCTC 10073
Radiation (when applied)	<i>Bacillus pumilus</i> E601 ATCC 27142
	<i>Deinococcus radiodurans</i> ATCC 13939
Filtration	<i>Brevundimonas diminuta</i> ATCC 19146

Similar to the product sterility test, the biological indicator or challenge is placed in a suitable bacteriological recovery medium and observed for growth. Testing of BI is easier to perform, and erroneous results can be picked up by determining if the surviving micro-organism is the indicator organism or something else. However, too much reliance on BI as proof of sterility can sometimes be misleading. For example, indigenous micro-organisms can sometimes match or exceed the resistance of the BI used, under excessive filth conditions or due to entrapped or occluded bacteria on or in the product. For example it has been observed that a cellulosic product had a number of microbes surviving after an EO process where BI had been killed. Furthermore this product had been presterilised by irradiation.

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Upon identification, some of the microbes were identified as radiation resistant microbes. EO has been referred to as providing a radiation type poison inactivation. Rarely, is a micro-organism with greater resistance to a process than the indicator organism found, but it has occurred.

Regardless of which sterility test is used, it is necessary to know what the bioburden on product is and understand the kinetics of the microbial inactivation, so that statistics can be applied in the design and validation of the product and the sterilisation process to eliminate the concern of erroneously passing a non-sterile lot.

If outliers (survivors outside statistical limits) can occur as parallel perturbations or disturbances among routine processing cycles within the same period of time and environment, then it may be by chance alone and/or by BI variation that they occur. If design of experiments (DOE) do not reveal or demonstrate any data for the direct cause of BI to survive beyond the 1 in 10^6 safety margin, then it may be due to other related causes, e.g., type of sterilisation. Note: variation may occur on the basis that EO sterilisation may not be as controllable, reproducible or predictable as steam sterilisation and dry heat, and some other traditional methodologies. There are some micro-organisms that have an unusually high resistance to radiation, and may not meet models provided without further work.

Any effort to understand outliers reveals the immensity of uncertainties that can potentially exist among spores and BI. Efforts have been made to thoroughly clean and wash spore crops and deliver spore populations and BI that will behave in a first order or logarithmic order, but in nature, bacteria face all kinds of natural barriers (occlusions, clumping, organic encrustation, etc.), to a limiting chemical sterilising agent.

If the use of DOE does not reveal any outward direct causative reasons for sterilisation cycle parameter inconsistencies or deviations, then what remains are macroscopically unknowns. These unknowns exist as some other macro environment condition, (e.g., high humidity, interacting chemicals), or something that influences the BI or microbe's microenvironment to the sterilising condition(s) and/or another intrinsic universe of diversity of the challenging spore or microbial state and population, e.g., diploid *versus* haploid DNA, microbial mutations as with *Deinococcus radiodurans*.

To discover a world of highly intrinsic bioburden resistance that could overcome the overkill kinetics, results in diverging paths, one that the sterilisation process must be responsive to and be increased; and the other that the intrinsic resistance of the BI population must be immense and should be remedied by increasing BI routinely.

This glitch in the sterilisation system halts the continual use of an in-house sterilisation approach, when the approach is both failing and behaving inconsistently compared to

cycle parameters that have been scientifically and methodically developed and validated. In bioburden and dosimetric or parametric approaches, the question of overkill high spore or microbial resistance is not generally sought, unless failures to the validation approach occur. If more sterilant or radiation is required to get spore or microbial inactivation, then new materials for devices or healthcare products may be required. The question is: 'how sterile are the lots that occurred previously to the failure, when no overkill or sterility test was being routinely performed?'

Possibly a low-level resistant heterogeneous population exists that is not commonly observed statistically. Perhaps the spore is occluded in organic matter, crystals or clumps. The design of experiments does not seek to find these answers *per se*, but rather one involving a change in cycle parameters or associated change in the macro environments to investigate or evaluate.

But use of design of experiments could attempt to make a statistical outlier determination. It has previously been suggested that BI may have a statistical outliers outcome somewhere out in the range of 1 in 10,000 or 1 in 100,000 BI spore strips. This could suggest a minimum inactivation end point somewhere out in the range of 10^{10} to 10^{11} . If the cause of the BI, spore, or microbial outlier is due to barriers, such as non-penetration of EO gas molecules, or steam water vapour, or lack of temperature diffusion, etc., then the use of DOE can change the focus on determining how to evaluate the variation. If the BI outlier phenomenon is due to intrinsically heterogeneous differences in resistance of the BI spore populations, then the DOE through sample testing may be able to determine its existence.

How many BI spore strips and how many runs would be needed to evaluate the probability and possibility of such outliers? And if they occur, do they occur randomly? What is the chance of two outliers occurring not in the same run, but closely separated by only identical parallel runs?

It has been said that we all have a virtual twin somewhere in the universe, but what is the probability of two outlier spores having such expression?

To overcome the reoccurrence of outliers, it may be deemed necessary to develop BI with additional overkill factors, with overkill resistance that ensures at least a 10^{12} inactivation factor. Also, to create sterilising environments or conditions that minimise the occurrence of outliers from expression during routine monitoring.

In Europe the absolute minimal SAL is 10^{-6} , as stated in EN 556 [73]. In the US there is essentially a dual SAL standard of 10^{-3} for topical products and 10^{-6} for invasive products. Alternative SAL is essentially an economic necessity for radiation sterilisation, because it allows for many materials to be irradiated without deleterious effect. Harmonisation of worldwide sterilisation requirements is an important issue. This harmonisation is always

going to be hard. It tests the world community, but at the end of the test, we will be better off, when there is a sense of certainty of what to anticipate and expect.

Material qualification cannot be overlooked. This has evolved from simple inspection of the finished product to design and validation of materials prior to acceptance for manufacturing.

A recent standard for material qualification is AAMI's Technical Information Report (TIR) 15, '*Material Qualification*' [24].

2.4 General Considerations of Products, Polymers, and Materials for Sterilisation

The processes capable of sterilising product, polymers or material without adversely affecting their attributes, quality or compatibility vary and are limited. There is no panacea for all polymers and materials. Some examples are: chemical (EO, plasma, oxidising agents-hydrogen peroxide, chlorine dioxide, peracetic acid, hypochlorite and iodine solutions), radiation (gamma irradiation and electron beam), and heat sterilisation (steam, dry heat). Manufacturers are very selective in the materials that they use when designing components and devices which will be exposed to sterilisation. They must be aware of how materials interact with various sterilising processes. Concern for physico-chemical and biocompatibility and stability will provide for longer life cycles and better cost-effectiveness for the user.

A variety of factors must be carefully considered in selecting a sterilisation process without adversely affecting plastics. For example, steam or dry heat sterilisation will melt and degrade some plastics, but for sterilising glass syringes, powders or dry heat may be optimal. EO has toxic residuals, and it has limited penetration, but it can sterilise almost every plastic. Radiation may destroy some plastics, biomaterials, glass, and electronics, but it has excellent penetration, and no residuals.

2.4.1 Deformation and Degradation

Sterilisation agents that predictably and repeatably kill all micro-organisms from viruses to spores, are amazing process magic bullets but not without complications and limitations. Heat can seriously deform and melt; radiation can deteriorate and damage, chemicals can leave residuals; and many alternatives may not penetrate certain plastics and mated surfaces, and even fail to demonstrate good microbiocidal kinetics.

Steam will melt many plastics; dry heat, not only melts more plastics, but distorts, decolorises, and deforms others. Radiation will discolour and degrade a few plastics,

on single exposure, but damage many plastics if repeatably sterilised with radiation. So radiation is not typical in hospital sterilisation. Chemical sterilants such as formaldehyde, glutaraldehyde, EO and chlorine dioxide are excellent at sterilising many heat sensitive polymers and materials, but they will leave residuals or by-products, and will not penetrate all designs, configurations, plastics, packaging, components, or healthcare products.

Electrons from electron beam radiation may not penetrate metals, while gamma or x-rays will penetrate many metals, but may have more adverse effects on electronics, polymers, biomaterials than electron beam. Steam may corrode certain metals, leave water marks on other materials, and be incompatible with powders, but it is excellent for sterilising a myriad of liquids, drugs, etc.

Distortion and melting of heat labile plastics is a common limitation of steam and dry heat with plastics having low temperature transition and softening points. Moist heat can melt, wet some materials; dry heat can distort, radiation may degrade, chemicals may leave toxic residuals and have limited penetration.

Deformation and degradation of polymers are common characteristics of high heat and high ionisation irradiation such as gamma or electron beams. While they both have excellent penetration of most polymers, they can attack the backbone of the plastic either by ionisation or chemical change of polymers that are sensitive to their high energy physical forces, e.g., heat and radiation.

A number of new niche sterilant methods: hypochlorite, hydrogen peroxide, gas plasma, peracetic acid and plasma have material specific effects. Among the most popular and successful of them is the STERRAD Steriliser, developed by Advanced Sterilisation Products (ASP), a Johnson & Johnson company. This steriliser applies a combination of hydrogen peroxide vapour and low-temperature gas plasma to rapidly sterilise many medical instruments and materials without leaving any toxic residues. This technology can be used to sterilise a wide range of medical devices currently sterilised in steam, EO, glutaraldehyde, or low steam formaldehyde and is particularly suited to the sterilisation of heat- and moisture-sensitive instruments since the load temperatures do not exceed 50 °C, and the sterilisation occurs in a low moisture environment. The process may only take 55 minutes.

2.4.2 Deterioration, Discoloration, Aesthetics

Deterioration, discoloration, visual damage and visual defects are negative qualities of materials. Hospital products could be totally sterilised with fire or a nuclear blast but they would not have any useful value afterwards. Good sterilisation methods do not deteriorate, discolour or create negative impact to the user. Sterile hospital products for

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the most part today are considered to be clean, wholesome, and like new. Rubber gloves that are irradiated are not acceptable if they have odour, and plastics that turn yellow after irradiation are considered to be aged and old. Teflon and acetals that turn to powder after irradiation are of no value because ionising irradiation has deteriorated them. Cellulosic products: cotton, paper, towels, muslin dressings, organic materials, water and wadding are not compatible with hydrogen peroxide or plasma. These cellulosic materials can absorb the sterilant leading to incomplete sterilisation.

Biomaterials are the result of technological advances of materials, and will replace polymers, and enhance growth and creativity of newer and futuristic methods of sterilisation.

2.4.3 Shelf Life

Shelf life is another attribute that needs to be considered when sterilising a hospital product if it is a drug or the functional material of a medical device. In the pharmaceutical industry it is common to perform acceleration ageing and shelf life testing on drugs to determine their expiration date. Expiration dates are also applied to other hospital disposable products, so that they too must be evaluated through acceleration ageing and shelf life testing. Similar testing is applied to packaging for maintenance of sterility and seal integrity, and materials that have been irradiated. Functional samples are also evaluated.

2.4.4 Residuals and Extractables

Residuals and extractables are a problem with some sterilisation methods. EO is a toxic and highly reactive gas that can leave residuals in devices (see ISO 10993-7 [58]).

Heat and irradiation may create toxic or unfavourable extractables or leachables such as low pH, particulates, and non-volatile substances.

2.4.5 Biocompatibility

A medical device must be adequately designed to be safe for its intended end use and intended patient. Simply put, biocompatibility is the ability of a device to get along with the patient. The device should not inflict harm upon its host and that host should not affect the function of the device. The device should not release any harmful substances to the patient which could lead to adverse affects. The range of biological hazards is wide. The sources of hazards can be from new materials and changes in formulation(s), process, manufacturing, sterilisation, etc. When designing a biocompatibility test outline, an aware

and knowledgeable professional or toxicologist will often have to be consulted, other applicable and available publications reviewed, information sources searched (standards and international Pharmacopoeia, Medline, Toxline, MSDS, etc.). Where the material is intended to interact with tissue for the device to perform its function, evaluation takes on dimensions not generally addressed in standards and policies to date.

Biocompatibility is a specific requirement of healthcare products, and will vary depending upon the entry or contact to the body, duration, and type of sterilisation.

ISO 10993-1 [61] EO residuals can result in irritation, haemolysis, sensitisation, or potential carcinogenicity or genotoxicity, if specific low levels are not used. Exposure directly to many sterilants can cause carcinogenicity and/or genotoxicity to the personnel using them, as well as materials being treated, including radiation, [e.g., diethylhexyl phthalate (DEHP) from irradiated plasticised polyvinyl chloride (PVC), formaldehyde from acetal]. Other examples are: steam sterilisation of certain polyurethanes may result in a toxic hydrolytic by-product referred to as dimethyl aniline. Irradiation of Teflon may result in the generation of virtual dust, significant particulate matter that can cause embolism in the circulatory system.

Irradiation of soft PVC can lead to migration and release of toxic plasticisers like DEHP.

Biocompatibility is still evolving. While there are the traditional ASTM, United Pharmacopoeia biocompatibility approaches that can still apply to drug containers, for biological safety and to defence contracts, as well as numerous international governments, newer ISO/FDA standards have been adopted for medical devices. These new standards will have an impact on medical devices, on international submissions, and FDA domestic submissions, types, and quantity of testing that needs to be performed.

Since 1987, the US FDA, and the Departments of Health in the United Kingdom, and Canada has applied the Tripartite Biocompatibility Guidance for Medical Devices to set safety standards.

In 1992 ANSI/AAMI adopted ISO 10993-1 [61], an international standard providing principles governing the biological evaluation of medical devices (not cosmetics) and materials, definition of categories, and greater selection of tests. ISO Guidance on standard requirements are similar to those of the FDA Tripartite Guidance and blue book memorandum #G95-1, but the ISO standard encompasses more defined parts which will also include a section on preparation, specific category testing, and reference materials.

In 1995, Dr. Susan Alpert, the Director of Office of Device Evaluation of the FDA announced that the FDA had replaced the Tripartite Biocompatibility Guidance by adopting Part 1 of the ISO 10993-1 standard with some modifying considerations, under a new

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blue book memorandum #G95-1, Biological Evaluation of Medical Devices. The ISO 10993-1 standard will have an impact on international submissions, and FDA domestic submissions, the types, quantities of testing required for medical devices.

Toxicological information on the potential toxicity of materials is useful; however, most classical toxicological tests were developed for a pure chemical agent and are not applicable to biocompatibility testing for medical devices.

Medical devices are an unusual test subject in toxicity testing. Often a biomaterial is a complex entity (formulation), and the materials toxicity is mediated by both physical and chemical properties. Toxicological information on the material and its chemical composition is seldom available and the possible interactions among the components in any given biological test system are seldom known.

2.4.5.1 Assessing Risks

The hazard presented by a substance with its inherent toxic potential can only be manifested when fully exposed in a patient. Therefore risk, which is actual or potential harm, is a function of toxic hazard and exposure. The safety of any leachables contained in the device or on the surface can be evaluated by determining the total amount of potential harmful substance, estimating the amount reaching the patient's tissues, assessing the risk of exposure, and performing the risk *versus* benefit analysis. When the potential harm from the use of biomaterial is identified from the biocompatibility tests this potential may be compared against the availability of an alternate material and/or test(s) for assessment of its safety and effectiveness.

2.4.5.2 Matrix

Biocompatibility cannot generally be defined by a single test. It is highly unlikely that a single parameter will be able to account for the biocompatibility. Therefore it is frequently necessary to test multiple biocompatibility parameters. It should be noted that the FDA Matrix of ISO 10993-1 has additional tests to the standard ISO 10993-1. This suggests a non-harmonised standard.

- Description of Matrix: The Matrix consists of different device categories, body contact, contact duration and tests. These are sub-divided into body contact, that includes the site of contact between the device and the body, the contact duration and the associated tests to be considered or supplemented. ISO defines in detail the type of tests to be considered and their criteria (see **Table 2.6**).

Table 2.6 Initial biocompatible evaluation tests for consideration										
Device categories			Biological effect							
Body contact		Contact duration (see Section 4.2) A-limited (24 h) B-prolonged (24 h to 30 days) C-permanent (>30 days)	Cytotoxicity	Sensitization	Irritation or intracutaneous reactivity	System toxicity (acute)	Sub-chronic toxicity (sub-acute toxicity)	Genotoxicity	Implantation	Haemocompatibility
Surface devices	Skin	A	X	X	X	.	.	.	.	.
		B	X	X	X	.	.	.	.	.
		C	X	X	X	.	.	.	.	.
	Mucosal membrane	A	X	X	X	.	.	.	.	.
		B	X	X	X	0	0	.	0	.
		C	X	X	X	0	X	X	0	.
	Breached or compromised surfaces	A	X	X	X	0	.	.	.	.
		B	X	X	X	0	0	.	0	.
		C	X	X	X	0	X	X	0	.
External communicating devices	Blood path, indirect	A	X	X	X	X	.	.	.	X
		B	X	X	X	X	0	.	.	X
		C	X	X	0	X	X	X	0	X
	Tissue/bone/dentin communicating ⁺	A	X	X	X	0	.	.	.	.
		B	X	X	0	0	0	X	X	.
		C	X	X	0	0	0	X	X	.
	Circulating blood	A	X	X	X	X	.	0	.	X
		B	X	X	X	X	0	X	0	X
		C	X	X	X	X	X	X	0	X

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Implant devices	Tissue/bone	A	X	X	X	0	.	.	.	.
		B	X	X	0	0	0	X	X	.
		C	X	X	0	0	0	X	X	.
	Blood	A	X	X	X	X	.	.	X	X
		B	X	X	X	X	0	X	X	X
		C	X	X	X	X	X	X	X	X
<i>X = ISO evaluation test for consideration</i> <i>0 = Additional tests which may be applicable</i> <i>Note + Tissue includes fluids and subcutaneous spaces</i> <i>Note ^ For all devices used in extracorporeal circuits</i> <i>* See Table 2.6A for supplementary evaluation tests</i>										

Table 2.6A Supplementary evaluation tests for consideration							
Device categories				Biological effect			
Body contact		Contact duration (see Section 4.2) A-limited (24 h) B-prolonged (24 h to 30 days) C-permanent (>30 days)		Chronic toxicity	Carcinogenicity	Reproductive development	Biodegradation
Surface devices	Skin	A		.	.	.	.
		B		.	.	.	.
		C		.	.	.	.
	Mucosal membrane	A		.	.	.	.
		B		.	.	.	.
		C		0	.	.	.
	Breached or compromised surfaces	A		.	.	.	.
		B		.	.	.	.
		C		0	.	.	.

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External communicating devices	Blood path, indirect	A	.	.	.	.
		B	.	.	.	.
		C	X	X	.	.
	Tissue/bone/dentin communicating*	A	.	.	.	.
		B	.	.	.	.
		C	0	X	.	.
	Circulating blood	A	.	.	.	.
		B	.	.	.	.
		C	X	X	.	.
Implant devices	Tissue/bone	A	.	.	.	.
		B	.	.	.	.
		C	X	X	.	.
	Blood	A	.	.	.	.
		B	.	.	.	.
		C	X	X	.	.
<i>X = ISO evaluation test for consideration</i>						
<i>0 = Additional tests which may be applicable</i>						
<i>* See Table 2.6A for supplementary evaluation tests</i>						

- **Device Categories:** are the categorisation of medical devices defined as the testing of any device that does not fall into one of the following categories should follow the general principles contained in this part of ISO 10993 [21]. Certain devices may fall into more than one category, in which case testing appropriate to each category should be considered.
- **Duration of Contact:** may be categorised as follows:
 - a) **Limited exposure (A):** devices whose single or multiple use or contact is likely to be up to 24 h
 - b) **Prolonged exposure (B):** devices whose single, multiple or long-term use or contact is likely to exceed 24 h but not 30 days.
 - c) **Permanent contact (C):** devices whose single, multiple or long-term use or contact exceeds 30 days.

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- Categorisation by nature of contact:
 - a) Non-contact devices: these are devices that do not contact the patient's body directly or indirectly such as *in vitro* diagnostic devices and are not included in ISO 10993 [21].
 - b) Surface contacting devices: these include devices in contact with the following:

Skin: devices that contact intact skin surfaces only; examples include electrodes, external prostheses, fixation tapes, compression bandages and monitors of various types.

Mucosal membranes: devices communicating with intact mucosal membranes; examples include contact lenses, urinary catheters, intravaginal and intrainestinal devices (stomach tubes, sigmoidoscopes, colonoscopes, gastroscopes), endotracheal tubes, bronchoscopes, dental prostheses, orthodontic devices and IUD.

Breached or compromised surfaces: devices that contact breached or otherwise compromised body surfaces. Examples include ulcer, burn, and granulation tissue dressings or healing devices and occlusive patches.
- External communicating devices: These include devices communicating with the following:
 - a) Blood path, indirect: devices that contact the blood path at one point and serve as a conduit for entry into the vascular system; examples include solution administration set, extension sets, transfer sets and blood administration sets.
 - b) Tissue/bone/dentine communicating: devices and materials communicating with tissue, bone and pulp/dentine systems; examples include laparoscopes, arthroscopes, draining systems, dental cements, dental filling materials and skin staples.
 - c) Circulating blood: devices that contact circulating blood; examples include intravascular catheters, temporary pacemaker electrodes, oxygenators, extra corporeal oxygenator tubing and accessories, dialysers, dialysis tubing and accessories, haemoadsorbent and immunoabsorbents.
- Implant devices: these include devices in contact with the following:
 - a) Tissue/bone: devices principally contacting with the bone; examples include orthopaedic pins, plates, replacement joints, bone prostheses, cements and intraosseous devices. Devices principally contacting tissue and tissue fluid: examples include pacemakers, drug supply devices, neuromuscular sensors and stimulators, replacement tendons, breast implants, artificial larynxes, subperiosteal implants and ligation clips.

- b) Blood: devices principally contacting blood, examples include pacemaker electrodes, artificial arteriovenous fistulae, heart valves, vascular grafts, internal drug delivery catheters and ventricular assist devices.

- Types of Biological Tests

Sensitisation assay: determines the potential of a test material and/or the extracts of a material using an animal and/or human (Sensitisation is an allergic or hypersensitive response produced by repeated exposure to a material, usually dermal exposure. For products that will contact only unbroken skin, the Buehler Patch Test is usually recommended. For most other devices, the Magnusson-Kligman Maximisation Test is preferred) [1].

Irritation Tests: Evaluates the irritation and sensitisation potential of test materials and their extracts, using appropriate site or implant tissue such as skin and mucous membrane in an animal model and/or human model.

Cytotoxicity: With the use of cell culture techniques, this test determines the lysis of cells (cell death), the inhibition of cell growth, and other toxic effects on cells caused by test materials and/or extracts from the materials.

Acute Systemic Toxicity: Determines the harmful effects of either single or multiple exposures to test materials and/or extracts, in an animal model, during a period of less than 24 hours.

Haemocompatibility: Evaluates any effects of blood-contacting materials on haemolysis, thrombosis, plasma proteins, enzymes, and the formed elements using an animal model. (*In vitro* models are available for some procedures).

Pyrogenicity – Material Mediated: Evaluates the material mediated pyrogenicity of test materials and/or extracts. (Pyrogenicity is the ability of a material to cause a fever reaction when introduced into the blood. Pyrogen tests are done in rabbits or *in vitro* using the LAL test. The LAL procedure must be validated for each device or material).

Haemolysis: Determines the degree of red blood cell lysis and the separation of haemoglobin caused by test materials and/or extracts from the materials *in vitro*.

Implantation tests: Evaluate the local toxic effects on living tissue, at both the gross level and microscopic level, to a sample material that is surgically implanted into appropriate animal implant site or tissue, e.g., muscle, bone, for 7-90 days.

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Mutagenicity (Genotoxicity): The application of mammalian or non-mammalian cell culture techniques for the determination of gene mutations, changes in chromosome structure and number, and other DNA or gene toxicities caused by test materials and/or extracts from materials. (Most materials that are mutagenic are also carcinogens. Although there are exceptions such as ethylene chlorohydrate).

Sub-chronic toxicity: The determination of harmful effects from multiple exposures to test materials and/or extracts during a period of one day to less than 10% of the total life of the test animal, e.g., up to 90 days in rats.

Chronic toxicity: The determination of harmful effects from multiple exposures to test materials and/or extracts during a period of 10% to the total life of the test animal, e.g., over 90 days in rats.

Carcinogenesis bioassay: The determination of the tumourigenic potential of test materials and/or extracts from either a single or multiple exposure, over a period of the total life, e.g., 2 years for a rat, 18 months for a mouse or 7 years for a dog.

Pharmacokinetics: To determine the metabolic processes of absorption, distribution, biotransformation, and elimination of toxic leachables and degradation products or test materials and/or extracts.

Reproductive and developmental toxicity: The evaluation of the potential effects of test materials and/or extracts on fertility, reproductive function, and prenatal and early postnatal development.

There are degrees of biocompatibility responses and types of tests to be considered. A few, for example tissue culture (cytotoxicity), are methods for toxic screening. It is very different from animal testing. It is a model and it is more sensitive than an animal test because it is isolated to just a few specific cells. It does not include the aspects of healing or long-term effects that animal testing can monitor. Cytotoxicity often responds to chemical insults(s).

An even more sensitive toxicity test than cytotoxicity is Microtox, which uses bioluminescent bacteria to measure toxicity built up in an aqueous environment, but there are some known toxins it cannot detect. This test is not included in the Matrix. System toxicity in animals most commonly affects the central nervous system. Mutagenicity is generally a precursor to carcinogenicity. Mutagenicity affects the germ cells, but can be screened by a series of short tests by bacteria (AMES). Teratogenicity is characterised by deformities in offspring.

How to Use the Matrix

First determine what the full patient/device or material interface is, and then determine the duration of exposure. From the interface, determine what contact the device or material will make on the matrix. Within the duration of exposure, select the device that best fits what you are going to use, based upon its interaction between the patient and material and then go across the matrix and consider the tests that may be necessary. If the same device duration occurs multiple times, consider what the accumulative duration effect could be. The standard does not state which tests considered must be performed for compliance, however, the tests cannot be regarded as irrelevant. For example, certain devices may fall into more than one category, in which case testing appropriate to each category should be considered. But the matrix will be the default requirement. Therefore, the relevance for all tests must be considered, and a rationale or justification for not performing it must be developed.

2.4.6 Reprocessing

Reprocessing of devices or drugs by certain sterilisation methods can result in degradation and distortion of drugs and certain polymers within healthcare products.

2.4.7 Costs

Costs will vary depending upon the selection and application of various sterilisation methods, and polymers selected for the product that is compatible with the sterilisation process. Steam can be very economical, but selection of only heat tolerant polymers are compatible. If moisture cannot penetrate, then either longer exposures and cost, radiation may be more attractive, because of short exposure time and dosimetric (early) release. Capital cost of dry heat steriliser may be the least expensive, but long exposure time and selected of heat tolerant polymers may increase costs of processing. Those processes that release product early through dosimetric release, process control, and parametric release, can influence significant cost savings through reduced product storage and immediate product availability.

2.4.8 Availability

Availability of equipment and materials is necessary for sterilisation. If regulations for EO increase, then EO will not be used predominately in certain locations, (e.g., California). If radioactive isotopes are not available, then radiation processing will decline at some locations.

2.4.9 Acceptability

Not only is the availability of the materials, polymers, parts, components and products important, but for sterilisation, to be acceptable they must have minimal bioburden, cleanliness, integrity, and traceability.

Too often, materials, parts, components, products are available for sterilisation, but they are filthy. They can have a too high bioburden or too resistant a bioburden, they may have artifacts like organic encrustation, crystals or metal parts that do not allow the sterilisation agent to reach the microbial site for inactivation, the parts or product have dead cells that can cause pyrogenic responses, or unseen grease or oil that will not allow for penetration of gases. Sometimes the parts and product are available but their integrity for sterilisation is not continuous or not intact. In such cases the parts or product are adversely affected by the subsequent sterilisation leading to deformation, deterioration, discolouration, loss of shelf life. New or different pretreatment of parts and products with cleaning agents or process should be qualified prior to sterilisation. And finally parts and products to be sterilised must have some sort of traceable identity, that communicates that the particular part or product has been evaluated for sterilisation. For example a slightly differently formulated plastic may result in a deformed plastic by heat, or lack of stabiliser may result in discoloration of a plastic by irradiation.

2.4.10 Packaging

Packaging will vary depending upon the sterilisation method. Steam, EO, and formaldehyde sterilisation require packages that are permeable to the gases and vapours. Radiation does not require permeability, except for the distortion and elution of odours that may occur. For example irradiated rubber gloves can give off tremendous malodours after irradiation. If they were sterilised in a non-breathing packaging, the off gassing would be highly recognised upon opening.

Testing and validation of packaging is critical for maintenance of sterility and handling, 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 periodic shelf life periods. These protocols will describe the parameter to be used for physical distribution of environmental stress testing such as shock, vibration, compression hazards, package strength evaluation using seal strength methods, 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 one or more of the following factors:

- 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 sterilisation process, e.g., quick pressure changes in gas or steam in the post vacuum phases.

By selecting packaged products which have been through manufacturing, sterilisation, and then subjecting them to shipping, handling, strength integrity tests, 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.

Typical packaging tests are described in ASTM, EN 868 [5] and ISO 11607 [50] standards.

Despite all this evaluation, packaging should be visually inspected at point of use for any obvious physical damage, including wetting, because Tyvek and paper packages cannot withstand water contamination, and water is an excellent source of microbes. Composite foil is available for flat-pack and deep-drawing machines along with coated or uncoated Tyvek and speciality 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. The company's QA systems are certified to EN ISO 9001 [7].

2.4.11 Process Conditions and Effects

To effectively sterilise healthcare products an understanding of parameters is necessary. **Table 2.7** gives a basic overview of process conditions. Sterilisation process time is a significant consideration, for healthcare products that need to be sterilised in a short time or just in time manufacturing.

However, process conditions as well as different sterilisation methods can adversely affect materials and product. A number of standard parameters are presented for selection of consideration of evaluation, but require further refinement depending upon products and materials being treated. Sterilisation of healthcare products consists of critical conditions that define and determine acceptable product and material attributes, beyond mere sterility but also packaging integrity and product stability. While perhaps slightly oversimplified, the following process conditions and effects are presented for an overview comparative purposes, but not necessarily for critical considerations.

Table 2.7 Abbreviated Comparison of Sterilisation Methods, Times and Effects			
Method (Type)	Standard (energy or time required)	Advantages (benefits)	Limitations (disadvantages)
Steam		Simple	No depyrogenation
Standard Autoclave	121 °C, 5-20 min Gravity	Efficient time Can sterilise liquids	No closed containers Metals may corrode
Flash Autoclave	134 °C, 3-6 min Vacuum with steam pulses	Rapid time Penetrates OK	Damages many plastics But need breathable package
Low temperature	110-115 °C 35-40 minutes	Sterilises heat sensitive drugs, blood bags, materials	Slightly longer exposure
Irradiation		Penetration	Some material damage; limited reprocessing
Gamma	11-40 kGy	Few hours to several hours (Typically hours)	Single sterilisation Some material damage Greater penetration than electron-beam
Electron beam	11-40 kGy	Seconds/minutes (Typically seconds)	Less penetration than gamma More material compatibility than gamma rays
X-rays	Varies, depends upon types of products/materials to be sterilised.	New, typically in seconds or minutes It can handle very bulk and dense materials No source radiation As with gamma Faster than gamma and more penetration than E-beam. Can sterilise bulk without additional handling	Does not start fires, as electron-beam can, on papers, mail Costly

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Dry Heat		Simplest. Can sterilise oils, grease, etc.	Many plastics melt
Standard (minutes or hours)	120 min at 160 °C	Inexpensive	Long exposure
Standard	30-60 min, 170-180 °C	No corrosion of metals	Oils, powders
Rapid	6-20 min, 190 °C	Rapid time	Pre dry instruments
*Spacecraft and validated processes for devices.	12/18+ h, 105-135 °C	Overnight + More materials are compatible, including PVC, electronics	Long exposure time
Ethylene oxide		Compatible with many heat sensitive materials and reprocessing	Long process, toxic residues, reactive with most drugs
Gas concentration	400-1500 mg/l	Sterilise most heat sensitive materials	Leave residuals in products, materials
Temperature	25-65 °C 66-85 °C	Mild Steam-EO	Rapid but forms ethylene glycol, which may or may not be a problem (See ISO 10993-7 [58] or TIR 19 [63])
Exposure	Hours of gas exposure	Long - can include millions of medical devices, polymers and materials are sterilised annually by the healthcare facilities and medical device industry. Most of these healthcare products and materials are metals and polymers sterilised without any adverse effects.	Post process testing

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Exposure (continued)		Total sterilisation and sterility requires several interfacial areas of study necessitating multilayers of tasks and control	
Humidification	Hours of pre-conditioning	Long	Humidity required
Aeration	Hours of aeration	Long	Residuals
<i>The above conditions and parameters can vary with load, items and bioburden</i>			

2.4.11.1 Typical Sterilisation Parameters

The parameters of steam and dry heat sterilisation, are temperature and time. For radiation it is dose delivered, (e.g., 25 kGy); for EO sterilisation it is much more: prehumidify dwell, gas concentration, temperature, time, pressure/vacuum, and aeration.

Table 2.8 Typical Sterilisation Parameters - conditions can vary with loads, items and micro-organism	
Time	Temperature
Steam Sterilisation-Typical Hospital and Pharmaceutical Methods	
15-30 minutes (gravity – no vacuum)	121 °C
35-45 minutes (gravity – liquids)	115 °C
3-6 minutes (flash with prevacuum)	134 °C
Conventional dry heat sterilisation set parameters	
2 hours	160 °C
1 hour	170 °C
30 minutes	180 °C
12+ hours (validated for devices)	105-135 C
Radiation	
Low dose	High dose
11 to 25 Gy	25 – 40 kGy or greater

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Ethylene oxide parameters	
Phases	Parameter Conditions
<i>Preconditioning - Outside Steriliser Chamber</i>	
Time	8-12 hours
Temperature	Ambient to 44 °C
RH	30-80%
<i>Initial Vacuum - Inside Steriliser Chamber</i>	
Vacuum	Ambient at 3,335 Pa
<i>Prehumidify Load</i>	
RH	30-80%
Dwell time	15-120 minutes
<i>Exposure time</i>	
Gas concentration	400-1,500 mg/l
Temperature	25-65 °C
Dwell time	30 minutes to 16 hours
<i>Vacuum/Air Wash</i>	
Vacuum/pressure	Ambient at 3,335 Pa
Number of washes	1-3
<i>Post Aeration</i>	
Temperature	Time
60 °C	8 hours
50 °C	12 hours
25 °C	4 days
<i>Above parameters and conditions can vary – the figures and parameters given are just examples.</i>	

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3 Steam Sterilisation of Healthcare Products and Polymers

3.1 General Considerations

In the classical sense, sterilisation has been defined as an absolute process that destroys or eliminates all viable organisms [1]. In a practical sense, however, sterilisation is better defined as processes capable of delivering a certain probability [2] that an exposed or treated product, polymer or material, is free from viable micro-organisms, including resistant microbial spores such as *Bacillus anthracis* (Anthrax), or prions in blood. The term sterilisation has previously been misunderstood, abused or confused with lesser methods of eliminating viable micro-organisms, such as commercial sterilisation, disinfection, decontamination, sanitisation or antisepsis. These methods are not capable of total elimination or destruction of all types of micro-organisms.

Sterilisation, by definition, is capable of destroying or eliminating the most resistant microbial bacterial spores that are capable of surviving most severe environmental conditions such as outer Space, Pharaoh's tombs, the Himalayas, Death Valley (USA) or the Tundra. However, more resistant forms of organism, e.g., prions, may be even more difficult to destroy or inactivate. Sterilisation methods can be categorised as physical, chemical, or physical-chemical, such as heat, chemicals or heat-chemicals, respectively. There are no biological sterilisation agents. Antibiotics would come the closest, but there is no antibiotic that will kill all micro-organisms, e.g., spores in a dormant state, etc.

The physical and chemical types of agents capable of achieving sterilisation for use with healthcare products are therefore limited. The conventional types are moist heat (autoclaving), dry heat, chemical sterilisation, (e.g., EO, formaldehyde, oxidising agents), and ionising irradiation, (e.g., electron beam and gamma irradiation). Some of the recent methods use dual entities, for example, hydrogen peroxide vapour and plasma. Some agents being considered by this author for future sterilisation are dual entities with potentiating synergists for example 8.5% EO (low EO concentration/91.5% CO₂ and formic acid vapour during prehumidification or dry heat for a short time and low temperature with dehydrating medium (i.e., vacuum).

Some lesser means of sterilisation are typically liquid chemicals such as glutaraldehyde, and aseptic assembly. Filtration is another means of sterilisation too, used heavily in the pharmaceutical and drug industries.

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Other types of methods capable of achieving inactivation of viable organisms exist, but are less effective than terminal sterilisation. These may be described as antisepsis, disinfection, use of decontaminating agents, fumigation, use of germicides, pasteurisation, and sanitisation.

Types of methods capable of sterilising product, polymer or material without adversely or deleteriously affecting polymer and material quality or packaging integrity are limited, because there are many processes like incineration, strong hydrochloric acid, bombing, nuclear blast that can kill or eliminate microbes, but in the process can destroy everything else, too. The purpose of healthcare sterilisation is to sterilise products, polymers and materials without adversely affecting their quality, function, and use.

3.1.1 Polymers and Materials

There is no single sterilisation method that will sterilise all healthcare products, polymers, or materials without some damage or destruction. Consequently, sterilisation methods must ultimately be selected carefully.

Some conventional methods are:

- Steam
- Low steam-formaldehyde
- Ethylene oxide (EO)
- Hydrogen peroxide vapour with plasma
- Radiation
- Dry heat

A brief description of these sterilisation methods are presented in the following sections.

3.2 Steam Sterilisation with Heat, Liquid and Moisture Compatible Materials

Steam sterilisation is a classical method of sterilisation [3, 4]. Steam sterilisation is recognised for its simplicity, efficiency, effectiveness, low cost, and speed of operation. It is currently considered as an ideal candidate because of its compatibility with the environment and health. But the number of plastic materials, chemicals, and some metals capable of tolerating its high temperature and moisture are limited. In hospitals and laboratories

where reusable materials are frequently used, steam sterilisation is predominantly the method of choice [1]. In Europe, India, and the UK, steam with formaldehyde is also applied [5]. Steam is also widely used in the decontamination of infectious waste materials. Recently, however, with the emphasis on the environment, there is renewed interest in this method of sterilisation. It has frequently been used in the pharmaceutical industry for sterilisation of drugs like parenteral solutions. Unlike most other sterilisation methods, steam is compatible with most liquids.

3.2.1 Common Materials Sterilised by Steam

Steam can sterilise most metals, glass, and some heat resistant plastic, polymers, or materials [4]. Some examples are:

Acetals

Acrylonitrile-butadiene-styrene (ABS) can distort if at temperatures above its heat deflection temperature

Aromatic polyurethanes

Corrosion resistant metals

Cotton

Filters (typically most filters can be autoclaved, but some materials may be heat sensitive)

Glass

Heat resistant liquids, solutions, drugs, parenterals

Laboratory equipment (typical non-electrical lab equipment can be steam sterilised, but if heat sensitive, or single use, cannot)

Muslin

Nylon

Polyallomer

Polycarbonate

Polypropylene

Polysulfone

Some PVC

Silicone (however, if the silicone is an implant, it must be permeable to the steam to allow sterilisation of internal matrices)

Teflon

Tyvec - spun polyester (below 121 °C)

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The number of plastic materials capable of being steam sterilised will vary considerably with the selected temperature of sterilisation. Standard steam sterilisation is generally carried out at 121 °C for 15 minutes. Faster or flash sterilisation is generally carried out at 134 °C. Longer sterilisation or lower steam sterilisation is carried out at 115 °C. Lower steam sterilisation can be performed at approximately 100 °C (fractional) or at 80 °C on three subsequent days (Tyndallisation), but these latter approaches are marginal, possibly questionable, and limited. Some alternative or other approaches to classical steam sterilisation are of possible, such as with microwave, steam - EO (80 °C or less), steam – formaldehyde (65-90 °C), etc.

3.2.2 The Specific Types of Steam Sterilisation Processes

Some typical steam process types are:

- Gravity (downward displacement)
- Pulsing (vacuum pulsing or pressure pulsing)
- High vacuum
- Superheat

Boiling, pasteurisation, fractional sterilisation (Tyndallisation) are not considered effective sterilising agents, because endospore destruction is not assured.

Each type has its advantages and disadvantages. The selection of the particular process type is dependent upon a variety of factors such as the end use characteristic of the product. Paper, powders, and oily materials that can get wet may not be completely compatible with moisture from steam. It may not be the method of choice for depyrogenation, or the inactivation of pyrogens (microbial cell wall fragments that can cause febrile responses in humans.)

3.2.3 Validation Procedure of Steam Sterilisation of Healthcare Products

Validation for steam sterilisation of liquid drug or non-heat sensitive healthcare products can be very complex and involved [6, 7], particularly because of the regulations and documentation of approved drug products. The following is a complex outlined procedure of a typical large industrial validation procedure for steam sterilisation of fluid drug products in the United States.

Summary

This protocol provides standard information on requirements and guidance for qualifying moist heat sterilisation of drug products at a manufacturing site.

In conjunction with this protocol, a Sterilisation Validation Document (SVD) is written and approved. The SVD specifies the requirements to be applied to applicable situation(s), conditions and/or the reason(s) for qualification(s).

Scope

This document is a policy, procedure, and also guidance. Requirements for a qualification are described in a SVD, which acts as a protocol for individual qualifications.

Terminal sterilisation must be qualified to assure continuous operation through demonstration of one or more of several of the following applicable criteria: installation qualification or commissioning, physical or operational qualification, cycle development, performance qualification with a PNSU ($\text{PNSU} \leq 10^{-6}$), special criteria, and/or verification of the appropriateness of validation approach). The minimum applicable requirements of a validation are dependent upon the reason or reasons for the qualification. Various sections in a protocol and in particular outlined sections are used for guidance and training.

Further Reading for this Section

211 CFR *Applicable Sections for Sterilisation, Sterility of Fluid Drug Products.*

AAMI ISO 11737-1, *Sterilisation of Medical Devices – Microbiological Methods - Part 1: Estimation of Population of Micro-organisms on Products*, 1995.

AAMI ISO 11737-2, *Sterilisation of Medical Devices – Microbiological Methods - Part 2: Tests of Sterility Performed in the Validation of a Sterilisation Process*, 1998.

ANSI AAMI ISO-11134, *Sterilisation of Healthcare Products-Requirements for Validation and Routine Control - Industrial Moist Heat Sterilisation*, 1994.

ANSI AAMI ISO-11138-1, *Sterilisation on Healthcare Products - Biological Indicators - Part 1: General*, 1994.

Procedure-A, *Calibration and Programming of Temperature Monitors or Probes.*

Procedure-B, *Sterility Test Using Membrane Filtration.*

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Procedure-C, *Microbiological: D and Z Determinations*.

Procedure-D *Bioburden and Relative Resistance of Micro-organisms in Solution Drug Products*.

Procedure-E, *Product Closure/Container Integrity Testing*.

Procedure-F, *Inoculated Sample Preparation, Maintenance and Processing Utilised in Steam Validation or Qualifications Runs*.

Procedure-G, *Evaluation of New Products for Sterilisation*.

Procedure-H, *Stability Study Protocol Guidelines*.

Validation of Steam Sterilisation Cycles - Technical Report No.1, PDA, Philadelphia, PA, USA, 1978.

I. Pflug, *Microbiology and Engineering of Sterilisation Processes*, Environmental Sterilisation Laboratory, Minneapolis, MN, USA, 1995.

I. Pflug and R.G. Holcomb, in *Disinfection, Sterilisation and Preservation*, 3rd Edition, Ed., S.S. Block, Philadelphia, PA, USA, Lea & Febinger; 1983.

Seminar Program - *Validation of Sterile Manufacturing Processes*, Pharmaceutical Manufacturer's Association (PMA), 1978.

C.R. Stumbo, *Thermobacteriology and Food Processing*, 2nd Edition, Academic Press, New York, NY, USA, 1973.

United State Pharmacopoeia or other Official Compendium, most recent edition.

3.3 Considerations for Qualification

Once a steriliser or process is selected it is necessary to consider a variety of factors for validation:

- Product design.
- Formulation design of liquid product.
- Particulate matter, organic matter, flocculation.
- Closure design.
- Design of container and/or components.

- Product quality that influences heating capacity sink, heat sink and diffusion such as viscosity.
- Anything that creates or causes a significant air barrier/pocket to the steam.
- Anything that changes the significant heat resistance of spores. These are only given as a guide: clumping of spores, pH, electrolytes, water activity (<100% aw), salts, crystals, chemicals, oils, physical barriers, pre-environment temperature, recovery conditions, anoxia/aerobic conditions, chemical concentration variation, desiccant conditions, organic encrustation, temperature, spore carriers, occlusion, age, cleaning, environmental (soil, water, human faeces, skin, plant surfaces, sewage, floors, food) closures, tight fitments, heterogeneous and non linear populations, high z-values).
- Changes to vessel, equipment, controllers, components.
- Instrumentation - including temperature monitors.
- Sterilisation process(es) parameters.
- Load pattern(s).
- Significant or potentially unexplainable behaviour in process parameters.
- Minimally, the least and largest fill volumes for each vessel, when using the master solution.
- Significant calibration at or after installation and commissioning.
- Significant maintenance, preventative maintenance at commissioning or since.
- Retrospective validation in cases where a process or vessel has been historically proven.
- For verification of things during prevalidation or during the initial qualification, of potential areas, places, zones of resistance in a product or package considered or to be determined during the initial qualification.

3.4 Technical Review and Design Considerations

A technical review for qualification(s) or rationale for performing qualifications needs to be made and documented on or with a SVD. The SVD will specify the requirements for qualifications, as appropriate for the purpose or objective described, and as approved by the Validation Microbiologist and the Technical Services Manager or others as specified.

3.4.1 Issue SVD

- Prior to or concurrent to starting running tests.
- Consider any recent applicable change(s) in order to documentation or regulatory submissions.
- Consider existing documentation.
- Engineering considerations.
- Facility utilities that affect sterilisation.
- Electricity, steam (thermal dynamic quality of steam), water for injection (WFI).
- Check out steriliser and related equipment, instrumentation, and/or components.
- Consider valves, filters, fans, ventilation, cooling, drains/traps, doors, boiler, water pumps, insulation.
- Any considerations to steriliser facility and process flow.
- Starting temperature, changing temperature, mix ups.
- Sterilisation process and parameters.
- Heat up, venting, exposure, cooling.
- Staging, loading, and/or production flow for sterilisation.
- Density/moisture absorption change; change from full load to partial load; small load effect; partial load position in steriliser and in relation to fan.
- Development, evolution of maintenance.
- Consideration for instruction or training programmes.
- Consideration of deviations to cycle profile.
- Consideration of engineering test, evaluation and documentation system.
- Consider sterility assurance, and considerations that affect end use to the patient, user (contamination) or third party.
- Consider reproducibility, repeatability, reliability.
- Recognise regulatory commitments.
- Recognise past work, qualifications, validations.

3.5 Metrology Requirements and Guidance

3.5.1 Calibration

All required instruments and support test equipment must be calibrated.

Calibration is a comparison of a measurement system or device of unknown accuracy to a measurement system or device of known accuracy (traceable to national standards) to detect, correlate, report, or eliminate by adjustment, any variation from the required performance limits of the unverified measurement system or device. Calibration will be performed in accordance with Procedure-A.

3.5.2 Basic Considerations of Calibration System (Temperature Measurement)

- Calibrate the measuring system before and after validation tests. Once a year carry out a dynamic calibration in the steriliser to revalidate the system.
- Accuracy and reliability: The steriliser should be accurate to within ± 0.1 °C when it is received from the vendor; accurate to within ± 0.2 °C for F_0 cycles between 8 to 12 minutes and accurate with ± 0.3 °C for F_0 cycles above 12. Must be unaffected by hostile steam/water environment. Reliability is demonstrated by consistent and reasonable data. Data values that are not consistent and meaningful during use and calibration may be considered erroneous. Erroneous or deviant data should be reason enough to discard measurements.
- Meaningfulness of units of measurements: acceptable internationally, linearity of values, comparison and possible conversion to other values: degrees Fahrenheit that can be converted to degrees Celsius. For both temperature distribution (outside containers) and heat penetration (within container). Data values must be consistent and meaningful during use and calibration. Erroneous or deviant data should trigger out of service data tracer.
- Calibration to a known standard: traceable to NIST.
- Frequency of calibration: pre and post validation tests. May be measured as a result of number of uses *versus* time limitations before and after studies, (e.g., nominal runs, minimum runs), but not greater than three days.
- Documentation of calibration: procedure, records, results, control.
- System control: know the equipment, the system, training, procedures, acceptance criteria, back up files, calibration labels, serial numbers, equipment, locations, history file.

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- Track down and correct errors in measurements during calibration studies, before beginning validation testing. Zeroing out errors may satisfy calibration requirements, but may lead to problems during testing.
- Keep a log of calibration and measurement activities as well as problems and their solutions. Enter potential measurement test problems in log that may cause measurement problems or different outcomes than normal during testing, e.g., difficulty removing air from containers, filling containers using a temporary, vessel shut down, fan off.
- Maintenance of standards.
- Maintain certificates of calibration from manufacturer.
- Interface with outside calibration agencies, representatives.
- Storage and handling conditions: store in accordance to manufacturer's instructions. Keep an inventory of all units.
- Return damaged and out of limit data tracers to the vendor.
- Status of instrument.
- Calibrated/not calibrated; out of service; out for repair.
- Location.

3.6 Know the System

- Know instrument variation: non linear quanta, hysteresis, failure limits, drift, sensitivity to extraneous noise, reliability. Know the accuracy and the range of uncertainty of the instrument:

$$\text{Uncertainty Ratio} = \frac{+/- 2 \text{ SD of the instrument accuracy}}{+/- \text{ specification limits of the process}}$$

- Write a procedure on how to operate the system, to maintain accuracy and reliability and also so that the test results do not nullify the calibration input provided.
- Vessel Instrumentation. All of the steriliser's instruments must be calibrated for use during the validation studies. These calibrations are performed under procedure. Instruments must be identified by serial numbers. Instruments labelled - calibration sticks, when applicable. Instruments must be classified as critical or semi-critical.

Instruments must be maintained regularly. The steriliser and equipment, programme, calibration will be maintained under a documented preventative maintenance program as given in the procedures.

3.7 Performance Qualification Testing and Guidance

Each vessel should be commissioned and the process(es) verified for performance qualifications. Performance consists of at least three full vessel heat distribution and three heat penetration runs, which may be performed together in single runs with biological validation. Limits of performance qualifications can be confirmed with one minimum and one maximum cycle run. However, evaluation(s) more or less depends upon the reasons and rational for qualifications (see sterilisation validation document).

3.7.1 Qualification Starts with a Sterilisation Validation Document (File)

To this document will be added further detailed documentation as needed:

- Statement of final product specifications
- Statement of equipment-installation (commissioning)
- Statement of process (empty vessel and cycle development)
- Physical evaluation of container and closure systems
- Microbiological evaluation of closure systems
- Calibration of biological indicators
- Product temperature mapping (container cold spot)
- Minimum cycle limits (heat penetration/biological)
- Maximum cycle limits (heat penetration or stability)
- Closure sterilisation (as applicable)
- Qualification specification sheet
- Bio lab information sheet for steam sterilisation challenges (reference Procedure-F)

All validation documentation (cycle charts, forms, records, etc.) must be signed and dated.

3.7.2 Equipment Qualifications (Guidance)

Each vessel must be commissioned, as needed and may be subject to the following as applicable:

3.7.2.1 Installation Qualification (Commissioning)

Installation qualification requires that any documentary evidence is obtained to show that the equipment has been provided, installed in accordance with its specification and that it functions within predetermined limits when operated with operational instructions as follows:

- *Physical specifications.* Some examples are: chamber dimensions, other materials, steam system, air system, recirculation fans, recirculation pump, heat exchanger, spray, and, carts and trays.
- *Controls.* Some types are: control system, circular chart recorder, digital data recorder, steam valves, and RTD monitor.
- *Ancillary equipment/utility systems.* Some examples are: plant air, filtered air, clean steam, distilled water, and cooling water.
- *Software.* Some examples are: validation (vendor), and, vendor supplied.
- *Statement of equipment.* Steriliser manuals, vendor information, software validation, specifications and drawings shall be included or referenced as documentation of the equipment used for the sterilising.

3.7.2.2 Operational (Physical) Qualification

Investigate process/equipment functionality (as applicable), by using the following:

- Vent blow down. Pressurise empty vessel with compressed air. Open free vent and determine elapse time between two predetermined pressures.
- Temperature step response. Manually open steam valve(s) on empty vessel. Determine elapse time to various predetermined temperatures.
- Pressure step response. Manually open air valve on empty vessel (all vents closed). Determine elapse time to various predetermined pressures.

- Process water flow rate study. Monitor process water flow rate and/or pump pressure differential.
- Vessels used for cycles employing air over pressure in dwell should, when applicable, be subjected to other tests as detailed on SVD.
- Compare results obtained in the previous tests to data collected during other qualification or other standards, as applicable.
- Review for significant deviations.
- Investigate process/parameter functionality (as applicable) considering the following:
Review process parameters for type of cycle to be used.

Empty vessel distribution study. For evaluation of vessel temperature uniformity, process parameters, pressure leak test, program reset up, for evaluation of preventative maintenance considerations made to the vessel, as well as dynamic uniformity for simulate calibration of temperature probes. Apply a minimum of 10 data tracers randomly, but geometrically distributed. BI can be applied if called for in the SVD.

3.7.2.3 Review

Each of the previous aspect(s) shall be summarised by the study leader and reviewed by the plant validation committee. Upon completion of all preperformance qualification work, a written addendum will be formalised to specify the parameters which are to be validated. The addendum shall be reviewed and accepted by the plant validation committee. All initial qualification summaries, raw data, and test notes shall be included as part of the final validation record.

3.7.3 Cycle (Process) Development

The following guidelines are to be used in cycle development studies:

3.7.3.1 Statement of Final Product

Approved Master Part Specifications and Master Parts Lists shall be used to document final product specifications. In the absence of approved MPS/MPL, a listing which specifies materials may be substituted as approved by directors of Quality Assurance, Manufacturing, and Marketing.

3.7.3.2 Microbiological Considerations

Absolute F_o criteria (overkill) indicates the probability of a contaminated unit. Validation can be performed as an overkill approach: BI with limited bioburden with minimal analysis necessarily required.

Bioburden probability approach: bioburden and limited or no BI, only probability determined, but heavy reliance on bioburden levels and resistance. A combination of overkill BI and bioburden probability approach considers relative resistivity between the two.

- Determination or Evaluation of mean F_o value and PNSU $F_o = D_{121\text{ C}} (\text{Log } N_o + \text{Log } R - \text{Log } N_s)$

where $D_{121\text{ C}}$ = the time to kill one log or 90% of the spore population of the most resistant product isolate or anticipated heat resistant micro-organism at 121 °C.

N_o = The highest acceptable initial population in the product

R = the number of product units in a steriliser load

N_s = the minimum probability of a contaminated unit

- Effectiveness of the Process

Using maximum thermal resistant bioburden or sterility isolate determine spore log reduction of calculated minimum process F_o value:

$$\text{SLR} = F_o / D_v$$

Worse case SLR can be determined with probable worse case survivor in each manufactured container per year, with probable average per unit.

$$\text{Worse Case SLR} = F_o$$

$$D_v (\text{Log No Units/ y} + \text{Log of mean bioburden/unit})$$

- Sterilisation design *versus* maximum product microbial bioburden and resistance.

$$D_v \text{ BI } (\text{Log } N_o + 1) = \text{or} > D_v \text{ isolate (maximum log bioburden} + \text{log } 10^{-6})$$

- Selection of Biological Indicator(s)

BI resistance must be sufficient to evaluate a process and the process F_o . Frequently used BI are: *Bacillus stearothermophilus* ATCC 7953, *Bacillus subtilis* 5230, *Clostridium sporogenes*

PA 3679, *Bacillus coagulans* FRR B666; *Bacillus subtilis* var. *niger* (globigii) ATCC 9372), or other(s). BI must be reproducible and of a known resistance. BI may require certain recovery conditions for NDA and/or be used to evaluate reduced BI incubation times.

3.7.4.2.1 Biological Indicator System

Outlined details of the system chosen are:

- Selection and choice of organism: may be the same as in qualification or as specified per SVD.
- History, identification, and characteristics of the spore crop of the organism.
- D- and z-values of the organism in product to be evaluated.
- Reproducibility of the organism between D-values.
- Stability (storage) of the organism: see manufacturers instruction.
- Inoculum level (verification of master solution level).
- Minimum resistance (verification) single D-value minimally.
- Certification of each spore crop: from the manufacturer.
- BI Correlation to most heat resistant product microbial ever obtained and/or most recent with last year. The inoculation, preparation, maintenance and process of BI are described in Procedure-F. A minimum of 10 solution containers of the maximum fill volume of the most significant viscous or D-value product should, when applicable, be inoculated.

The population of spores for inoculation should, when applicable, be determined as follows:

$$N_0 = \log^{-1} (F_0 / D_v \text{ value})$$

where: N_0 is the initial spore population,

F_0 is the predicted mean for the cycle run being validated, and

D_v is the D-value of the spore suspension that will be applied.

If there is a activation slope, subtract one log from the log no.

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If there is an intercept ratio associated with the spore crop, multiply by the log no.

- Calibration of biological indicator. The requirements of Procedure-C or the SVD shall be followed.
- Determine in vessel D-value(s), where there are survivors (following a minimum F_0 , subprocess, or resistant BI), using physical F_0 and SLR, in vessel D_v is determined as follows:

In vessel $D_v = F_0/\text{SLR}$

Compare in vessel D-value with laboratory D-value using biological F_0 /physical F_0 ratio.

Ratio of laboratory D_v /In Vessel D-value = ~ Ratio of Biological F_0 /physical F_0

- Container cold spot/location study. This study requires the testing of heating characteristics in a product container and selection of a container cold spot. By its very nature, the container cold spot serves as a worst case condition from which calculations of probability of a spore survivor and minimum F_0 lethality factor are made. Once this location is determined, it generally becomes the temperature probe site of future penetration studies. In certain cases, however, when it is not possible or practical to probe the cold spot, a correction factor is applied in future penetration studies to compensate for the F_0 difference between a convenient probe position and the cold spot. This study serves in a positive manner as an effective means of ensuring that the minimum F_0 delivered to the product in LVP heat sterilisation shall be measured or corrected for. The minimum standard criteria for the container cold spot shall be:

Placement of probes shall be based on physical concepts of convective heating in the container, potential insulated areas in the product, container geometry, and historical considerations.

Because of the difficulty of placing several probes in a single container and potentially introducing experimental error, the number of probes and type of probe placed in a single container shall be limited to no more than three thermocouples, unless supportive data and or devices are available.

One location in each container shall serve as a control position, which must be at the expected or assumed cold spot. In a case where this is not possible, the nearest point of convenience may be used. This study shall be performed on every container size, type, and orientation, in the most viscous or slowest to heat solution to be qualified in each size. It must be done for the maximum fill volume and for an empty container where appropriate. Interpolation between container sizes shall be allowed, if data is

available to support it. Approved container cold spot data may be interchanged for similar processes, i.e., between containers and other vessels and plant locations, after technical review.

A minimum of three different properly functioning probed containers shall be required to evaluate the F_o in each container position, relative to the control location.

Analysis of the container cold spot data shall compare F_o values from each position to the control point in the same containers. For example:

For each of the three or more containers, the F_o between each container location and the control location in the same container, must be determined as follows:

$$F_o = (F_n) - (F_c)$$

Where: $(F_n) = F_o$ from a given location

$(F_c) = F_o$ from control location in the same container.

Only data from correctly positioned, properly functioning probes and integral containers shall be used. If, in all of the three or more containers, the control location had the lowest mean F_o relative to each of the other locations within the same container, it will be designated as the 'container cold spot'. All further probing will be done at that control point. If a location other than the control location had a lower mean F_o relative to the control in all of the three or more container, the coldest location will be designated as the 'container cold spot'. All further probing will be done at that location, unless it is not practical.

If the relative cold spot of the container was not consistent in all containers tested, or if for practical reasons it is necessary to probe a position other than the coldest point, further studies must be performed in at least seven additional containers. This will yield a total of at least ten values from the location in question for comparison with the control point within the same container. The data shall be analysed as follows:

An F_o will be calculated between the suspected cold location(s) and the control point in each of the ten or more containers tested. The mean of the F_o values are also calculated.

If a majority of the F_o values are negative and the F_o is negative (thus indicating the control point of convenience is not consistently the coldest), the coldest location will be designated as the 'container cold spot', and all future probing will be done at that point. If it is not practical to routinely probe in the coldest location, a factor at least as large as the mean F_o absolute value will be used as the 'cold spot correction factor'.

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If the majority of F_0 values and the mean F_0 are positive, (thus indicating the control location was consistently the coldest), the control point will be used as the 'cold spot'. If neither of the previous conditions are met, further studies should be performed.

If no significant differences in lethality can be shown, probing will occur in a location of lower lethality.

3.7.4.3 Biological Challenge of Closures

Samples for the biological tests will be manufactured according to USP standard methods and procedures, including overwrap 101 double bags filled with tryptic soy broth. Sterilisation of the test lot will be at the nominal cycle to prevent alteration of the media. All subassemblies used in the product should be traceable. Maximum stress to the closure system should be utilised (maximum sterilisation dosage, and heat sterilisation). After autoclaving the bags will be visually inspected for leaks. Incubation of the test bags for NLT for 7 days at room temperature will verify no macroscopic evidence of microbial growth. Selected numbers of bags will be retained in the lab for positive controls. A certain number of bags will be used as bags for biological challenge positive controls. The remaining bag will have a growth promotion performed according to Procedure B.

Bags will be sent to the UPS testing lab to simulate shipping conditions. Two tests will be conducted: vibration test, and a drop test

3.7.4.3.1 Biological Challenge Conditions

- Static-ambient challenge. This test is carried out with 25 bags.

The overwrap is removed and the bags are visually inspected for any defects and microbial growth. The cone on bag is broken and the bag is filled with test media. The bags are placed in room temperature conditions making sure that all test closure systems are in contact with the growth media. Growth promotion testing is performed according to Procedure-B, using the following organisms at each interval: *Bacillus subtilis*, *Candida albicans*, and *Pseudomonas aeruginosa*.

The bags are inspected for evidence of microbial growth at the following intervals: 1 month, 3 months, 1 year, 18 months, and 2 years.

- Static immersion challenge.

This immersion challenge is performed on containers or bags using *Pseudomonas diminuta* (or *E. coli*) with a population of not less than 10^8 CFU/ml. The closure

systems are immersed in the bacterial suspension for 10 minutes. The suspension is quantitated before and after the immersion challenge. A motility test is also performed. The suspension is incubated for seven days at 30-35 °C, and then inspected for microbial growth. A positive test control is performed concurrently.

- Static immersion challenge - freeze/thaw.

The suspension is frozen for four days, then thawed for three days. Repeat, and then perform the immersion challenge test.

- Static immersion challenge - hot/cold.

The suspension is placed in a hot room for four days and then left at 25 °C for three days. The test is repeated, and then the immersion challenge is performed.

3.8 Heat Distribution

This study requires the testing of temperature characteristics of the sterilising medium in order to determine if temperature uniformity and reproducibility in the vessel and about the product can be achieved. Uniformity of the sterilising medium temperature throughout the vessel reduces the likelihood of a slow to heat zone(s) in the steriliser product load. Thus this study acts as an additional assurance of repeatable sterilisation of the product throughout the load. The minimum standards for this study are determined as stated in the SVD, but the following provides information for that determination and also what the objective/purpose of the qualification(s), requalifications are:

Successful heat distribution studies (one or more, preferably three) should, when applicable, be performed in fully loaded vessels with a minimum of ten calibrated probes or data tracers. All horizontal and vertical zones within the confines of the load must be represented. These are placed adjacent to heat penetration containers. Two additional probes are placed at the control cluster of the vessel. Typically for full qualification, three runs are required. For each cycle, the full vessel distribution study must be performed with each container size with the maximum fill volume of a solution at least as viscous as the most viscous product to be qualified for that cycle. The studies must be carried out on each steriliser, and for each applicable load configuration.

The probes or data tracers should, when applicable, be geometrically distributed throughout the vessel, adjacent to the heat penetration probes, and facing in the same direction and on the same plane. If there are more heat penetration probes than distribution probes, then place heat distribution probes adjacent to every other heat penetration probe. However, probes may be strategically placed at different times depending upon the nature of the validation. For example a repair of fan may require that probes be placed geometrically in the area or zone where it is most influential.

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For full vessel heat distribution studies, steriliser(s) and sterilisation processes should, when applicable, perform as specified in the operating procedures and specifications. Each heat distribution run should, when applicable, demonstrate a temperature uniformity within:

± 1 °C at vent closure or start of exposure (if applicable); however ± 2.5 °C may be acceptable for possible deviations during stabilisation at this stage. A temperature uniformity within ± 1 °C during stable conditions, (e.g., after a minimum 1-2 minute interval after start of exposure, due to process variation, differences in controllers, valves, recorders, and minimum lag of some probes or data tracers to PLC controller point), a mid-dwell point and air overpressure may be acceptable.

The average temperature and the maximum standard deviation in temperature observed at selected or representative time intervals throughout the sterilising medium during steady-state dwell, and at vent closure or start of exposure (where applicable) must be stated.

The heat distribution data must be comparable to the qualification data, within the limits of the variability inherent in the system, and must be within ± 1 °C at the mid-point of exposure, at the beginning of exposure, at +2 minutes and at the end of exposure. Also, temperatures from the data logger, from the PLC and adjacent data tracer(s) (if applicable) should all agree within 1 °C at the mid point of exposure.

All variations, to a steriliser or process should, when applicable, be reviewed by the validation committee, to determine if the consideration should, when applicable, require further heat distribution studies. The QC department should, when applicable, be informed of any potential effect on a steriliser or process. This requirement should, when applicable, be met unless a written deviation is granted by the validation committee.

3.8.1 Review of Outlined Elements in Heat (Temperature Distribution)

- A minimum of one or more (preferably three) runs is required, unless otherwise specified on the SVD.
- The minimum number of temperature probes or data tracers is 10 (adjacent to penetration tracers unless otherwise specified per SVD).
- The minimum number of functional temperature probes or data tracers after sterilisation are 10 or as specified.
- The steriliser loading configuration must be as described previously.

- A distribution of probes must be established - previous qualification and temperature probes are generally placed randomly, but uniformly adjacent to heat penetration probes.
- Vessel and process performance: ± 1 °C temperature distribution mid-exposure; other temperature monitoring devices should agree to within 1 °C.
- Retest requirements: as required by the validation committee.

Temperature uniformity excludes the possibility of under processing a given product or zone, not included or covered by heat penetration.

Heat distribution measurements can be performed both in empty and maximum loaded conditions. Usually empty vessel temperature distribution is run to determine if the vessel and probes are functioning properly. BI (spore strips), can be placed geometrically with the probes to verify the thermal dynamic quality of the steam or water in the vessel. The probes should be geometrically distributed so that all vertical and horizontal zones in the vessel are represented. One probe is designated at a position adjacent to the temperature controller, reference and/or recorder(s). They should all be within 1 °C of each other.

3.9 Heat Penetration Portion of the Qualification Study

This study requires the testing of the temperature characteristics of the sterilising medium to the product to determine if temperature uniformity and reproducibility in the vessel, and about the product, can be achieved.

Uniformity of the sterilising medium temperature in the product varies throughout the vessel and this reduces the likelihood of a slow to heat zone(s) or load cold location in the steriliser product load. This study serves as an additional assurance of repeatable sterilisation of the product throughout the load.

The minimum standards for this study are determined as stated in the SVD, but the following provides information for that determination and also what the objective/purpose of the validation is.

Successful heat penetration studies (one or more, typically three) should, when applicable, should be performed in fully loaded vessels with a minimum of ten calibrated probes or data tracers. Heat penetration and distribution studies may be combined. Biological validation is run with the heat penetration study to be able to compare physical F_0 and biological F_0 , where possible. Three normal heat penetration studies are required for full qualification runs with distribution. One or more runs are required for minimum or maximum cycle limit evaluations.

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For each cycle, the full vessel penetration study must be performed with each container size intended for the cycle, with the maximum fill volume of a solution at least as viscous as the most viscous product to be qualified for that cycle. The studies must be carried out on each steriliser, and for each minimum and maximum load configuration (if it exists).

The probes or data tracers should, when applicable, be randomly but geometrically distributed throughout the vessel. All horizontal and vertical zones within the confines of load must be represented.

Data required is specified in detail on the forms of the SVD for each heat distribution (full vessel) and heat penetration run, and biovalidation.

In any case where a probe or data trace fails, the number of valid temperature monitors or probes should be given on the form, and a notation made explaining the reason invalid probes were deleted, e.g., inconsistent data, loss of vacuum, out of calibration, etc.

A matrix for listing of individual F_0 values correlated with position in the vessel should be provided for each heat penetration run.

Steriliser process specifications, programmed vessel parameters (PLC), data logger, circular chart (if applicable), and types of products approved for the cycle should be provided.

For full vessel heat penetration F_0 values must be calculated. The heat penetration F_0 data obtained should, when applicable, be subjected to analysis (mean, standard deviation and range). The minimum process F_0 must be acceptable within limits of the accuracy of the system, and must meet a standard specified minimum F_0 , e.g., 8 or 12 minutes, as specified.

The standard deviation from the qualification runs must be computed so that the data may be evaluated statistically. If the previous criteria specified in the SVD are not met, or if the standard deviations are significantly increased, the data must be reviewed by the validation committee to determine if further qualification studies are required.

The previous requirements should, when applicable as stated in the SVD, be met unless a written deviation is granted by validation committee.

Some elements in a heat penetration study are:

- Number of runs: minimum of one or more (three) for each vessel, cycle.
- The typical minimum number of probes or data tracers is 10 for determination of load cold location or other considerations. Typically for minimum and maximum runs, 10 probes are required. For requalification, only 10 are required.

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- Minimum number of functional probes or data tracers allowed to fail after sterilisation are 10% when only 10 probes used, and 10% when 14 probes are used. (Must be strategically monitoring locations that are acceptable upon review.)
- Type of product: smallest container (but largest bulk load), most viscous solutions and all other solutions should be considered for worst case spore resistance.
- The steriliser loading configuration is the same as validated or as specified per the SVD.
- The pilot and vessel F_0 must be compared to an approved process (or other) as specified by the SVD or procedure.
- Consideration of slow to heat zone or load cold location, if it exists, or is significant, or determine statistically the coolest F_0 container.
- The worst case location of heat for probe placement within containers must be determined.
- Probes or data tracers are placed in the cold spot(s).
- Two additional probes or data tracers are placed next to the control cluster of the vessel.
- The operator must make sure that the cycle process specification is met.

The cycle process specification is as follows:

- Heat up time, exposure time (includes any stabilisation period/conditions), average exposure time, exposure at high and low temperatures, vessel temperature distribution, vessel cool down time (to 70 °C), pilot lethality (as applicable), and vessel lethality (as applicable).
- Calculation of F_0 , mean F_0 , standard deviation and range.
- Analysis: including both heat penetration, and heat distribution > heat should equal > heat penetration.

Compare the vessel temperature distribution and heat penetration F_0 uniformity - for the same directional results. For a cold location compare different zones to the overall average F_0 for the vessel. If the cold location is consistent among all three runs, then this can be selected as the cold location. Further analysis then can be performed.

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The reference F_0 (vessel or product lethality) can be compared to the heat penetration product F_0 from the coolest location or coolest F_0 as a ratio. The ratio from several runs, including the minimum run can be compared to determine the process specification. The calculated (average) ratio can be used to establish the minimum reference F_0 by multiplying the ratio by the minimum acceptable F_0 , e.g., 8 or 12. Experimentally, the minimum process F_0 for minimum cycles may be evaluated with the following tentative formula:

$$\text{Minimum process } F_0 = \text{Mean } F_0 - 3 \text{ SD} - t' (L \times 10^{T-T'}/10)$$

Where: the mean F_0 is the mean process F_0 value of normal run(s),

SD is standard deviation about the mean,

t' is the change of time between the normal exposure time and the minimum exposure time,

L is average lethality of mean bottle F_0 /minute for the last t' minutes of the normal exposure time,

10^T is the normal process chamber temperature during t' , and

T' is the minimum operating process temperature of intended operating specifications.

Cycle performance is comparable to the original validation or as stated in the following:

- Heat-up time - within specification or as specified.
- Exposure time - within specification or as specified.
- Average exposure temperature - within specification or as specified.
- Exposure high and low temperature - within specification or as specified.
- Vessel temperature distribution acceptable - mid-exposure within ± 1 °C.
- Vessel cool down to 70 °C time - within specification or as specified.
- Product lethality ' F_0 ' - within specification > 12 or 8 minutes or as specified per the SVD.
- Pilot lethality ' F_0 ' (if applicable) - within specification or as specified.
- Vessel lethality ' F_0 ' (if applicable)' - within specification or as specified.

3.10 Microbiological Validation

The purpose of the biovalidation portion of the qualification testing is to demonstrate that a F_{bio} of equal or greater than 8 or 12 is delivered which meets the original protocol, new drug application (NDA) or SVD and the status of an overkill cycle or other as specified. Additionally an inspection of bioburden testing and its thermal resistance is performed to verify the continued appropriateness of the overkill validation approach, and that a probability of a non sterile unit (PNSU) of $<10^{-6}$ from these results can be obtained, which will demonstrate a high degree of assurance that the processed products are sterile.

3.10.1 Bioburden and Relative Thermal Resistance

Bioburden is the population of viable micro-organisms in the finished fluid drug product that consists of raw materials, components and/packaging. The relative thermal resistance, is the moist heat resistance of this bioburden relative to a D-value of 1 minute, typically applied for the overkill approach or for referencing to lethality at 121.1 °C.

A manufacturer routinely evaluates heat resistance of the product bioburden and from any potential heat resistant sterility test isolates, since original validation and during any validation. This evaluation begins with a heat shock test of the contents of two products, in which isolates are exposed to a temperature of $90\text{ °C} \pm 2\text{ °C}$ for 10 minutes (see procedure -07-D). Also, any high bioburden level or sterility test isolate(s) are characterised and heat shocked, if they are potential heat resistant micro-organism(s). Any organism surviving a heat shock determination is identified to the genus level and their D-value in one or two media, (e.g., fluid thioglycollate medium (FTM) or tripticase soy broth (TSB) both incubated at 30-35 °C) is determined.

The z-value is determined for highly resistant organisms, (e.g., >0.2 minutes). Data from the facility representing all D-values ($>.001$ minutes) of isolates from sterility, bioburden or thermal resistance testing of isolates is used to determine the frequency of positive tests.

$$\text{Frequency of probability of positive test} = \frac{\text{Number of tests resulting in positive results}}{\text{Total of tests performed}}$$

A limit D-value is calculated based upon a design (minimum) F_0 with a maximum bioburden population, maximum production units from a full solution lot, and PNSU of 10^{-6} , using the Stumbo equation:

$$\text{Limit D-value} = \text{minimum design } F_0$$

$$\text{Log (No max + Max. Product Lot Units + 1) - Log PNSU of } 10^{-6}$$

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The probability of an isolate being recovered having a D-value meeting Limit D-value is calculated:

$$\text{Probability of recovering (limit) isolate} = \frac{\text{Number of tests resulting in Limit D-value or greater}}{\text{Total of positive tests}}$$

Multiply the probability of the isolate by the probability of a positive test. The result is the probability of recovering isolate(s) having a D-value higher than that desired:

$$\text{Probability of Recovering } D_v > \text{Isolate} = (\text{Probability of Positive Test})(\text{Probability of Isolate})$$

The PNSU of the most resistant isolate obtained during the past year is determined:

$$\text{PNSU} - \log^{-1} (\text{Log} (\text{max No} + \text{Max Product Units}^* + 1) - \text{minimum Process } F_0/\text{D-value})$$

Where * is optional, depending upon analysis and assumptions needed.

3.10.2 Biovalidation

Biovalidation is the in-vessel evaluation of moist heat lethality by means of inactivation or spore log reduction of a known biological challenge (D-value and initial inoculum level). It integrates strictly just moist heat as well as other physico-chemical, physiological resistant mechanisms.

Biovalidation of the cycle is made concurrently with confirmation of the heat penetration. Biological validation is run with the heat penetration studies to be able to compare physical F_0 and biological F_0 , where applicable. NDA solutions may, when applicable, be biologically challenged, with at least one cycle, adjacent to the master solution.

The lethality of the sterilisation process can be effectively validated with biological indicator organisms. Product containers are therefore directly inoculated with a prescribed spore population of *Bacillus stearothermophilus* ATCC 7953, *Bacillus coagulans* FRR B666, or *Clostridium sporogenes* PA 3679, of a predetermined resistance.

Note: *Clostridium sporogenes* PA 3679 and *B. coagulans* may be acceptable spore formers for evaluation of dry heat conditions, however, *B. stearothermophilus* is not. *Bacillus subtilis* var *niger* ATCC 9372 may be applied if dry heat needs to be evaluated.

3.10.3 Biological Indicator System

The selection and choice of organism - may be the same one used for qualification or as specified by the SVD. A history, identification, and characteristics of the spore crop of the organism must be maintained. Description and criteria of the organism are as follows:

- D- and z-values of the organism in the product to be evaluated.
- Reproducibility of the organism between D-values.
- Stability (storage) of the organism: see manufacturer's instructions.
- Inoculum level (verification of master solution level).
- Minimum resistance (verification): single D-value minimally.
- Certification of each spore crop: from the manufacturer.
- BI correlation to most heat resistant microbial product (ever obtained and/or most recent with last year).
- The inoculation, preparation, maintenance and process of BI are described in Procedure-F.
- A minimum of 10 for qualification or 10 solution (revalidation) containers of the maximum fill volume of the most significant viscous or D-value product should, when applicable, each be inoculated.

The population of spores for inoculation should, when applicable, be determined as follows:

$$N_o = \log^{-1} (F_o / D_v \text{ value}).$$

Where: N_o is the initial spore population,

F_o is the predicted mean F_o for the cycle run being validated,

D_v is the D-value of the spore suspension that will be applied.

If there is an activation slope, subtract one log from the log number.

If there is an intercept ratio associated with the spore crop, multiple by the log number.

The inoculated container should, when applicable, be placed adjacent to probed containers in locations representative of potential slow-to-heat zones in the vessel during the heat penetration qualification studies. However, some or all inoculated containers can be

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distributed in the vessel slow-to-heat zone. Typically, this zone is identified in the original or previous qualification runs. Because the vessels typically have excellent temperature distribution, and lethality is measured both during heat up and cool down, no significant stabilisation phase or slow-to-heat zone exists. However, for purpose of comparing physical F_0 to biological F_0 , it is recommended that 10 inoculated containers are placed adjacent to the vessel heat penetration containers for full qualification. If a load cold zone exists, only 10 inoculated containers are required, with 10 of containers in the load cold zone if possible and the remainder adjacent to the heat penetration containers. Requalification, typically requires only 10 BI inoculated containers.

Positive and negative solution controls are required, and should, when applicable, be tested at approximately the same time the test samples are tested. There are four positive controls. After samples are removed from four containers (positive controls) to verify presterilisation spore count (recovery), enumeration of the inoculated solution positive controls should, when applicable, demonstrate a sufficient concentration of viable indicator spores per container to demonstrate a biological F_0 greater than the design F_0 , (e.g., >12 or >8) for the process cycle (review mean process F_0 value):

$$N_0 - \log -1 (F_0/D\text{-value})$$

On the basis of laboratory D-value determinations, a mathematical estimation of the spore log reduction (SLR) of the indicator organisms can be determined for the actual minimum container F_0 monitored during qualification run(s). Similarly, based upon the inactivation of BI, a SLR from the inactivation and biological F_0 calculation can be performed. From the initial positive control count per container and the fraction of the total sample number which are negative (if all are negative, an assumption of one positive container is made for the purpose of this calculation), a calculation of the demonstrated SLR should, be made when applicable.

All inoculated containers should, where applicable, demonstrate no growth of the indicator organism. Any growth of contaminants must be explained. It must be demonstrated that they are not the indicator organism, or there is sufficient SLR to defend the design F_0 of the process, and in no case can more than two samples contain growth of contaminants. Concurrent with the biovalidation, product sterility is required along with positive and negative media controls.

Some details of biological validation are as follows:

Minimum number of inoculated containers - 10 per load for requalification (10 for qualification).

Inoculum level and type of organism must be established.

The process performance must meet specifications.

The master solution or applicable solution should be inoculated with an inoculum level of $>10^3$ and less than 10^6 , unless otherwise specified by the SVD.

The inoculated containers must be positioned carefully in the load, adjacent to heat penetration probed containers, or approximately half to two-thirds must be placed in the load cold location or as specified by the SVD. Procedures for inoculation location and steps must be established.

Spore log reduction (SLR) must be determined, and a minimum number of false positive growths (not greater than two) must be selected.

The acceptable minimum number of fraction negatives must be established. Five survivors should be allowed with up to ten survivors on basis of technical considerations, and inoculum or D-value or F_0 variation(s). There should be four positive and four negative controls.

The vessel D-value when survivors occur, should be noted and the D-value of the indicator organism should be determined by plate count and/or fraction negatives when counts are five or less per sample.

A z-value of 10 °C should be assumed, unless otherwise specified by the SVD.

The biological indicator should be recalibrated on a scheduled basis. A recalibration resistance (D-value no less or greater than (current) microbial isolates), e.g., 0.5 minutes must be established.

Geobacillus stearothermophilus recovery conditions must be determined in soybean casein digest or trypticase soy broth (TSB) media at 55-60 °C. Fluid thioglycollate (FTM) at 30-35 °C is used for *Clostridium sporogenes*.

Bacillus coagulans FRRR B666, another heat resistant spore, may be incubated at 50 ± 2 °C in TSB for revalidation which is typically 7 days; however, for validation or other unusual conditions, incubation should be run for up to 10 days to determine the presence of slow growers or repair of thermally damaged spores.

Analyses can be selected from or modified using appropriate mathematical and statistical considerations from the following list:

Absolute F_0 criteria (overkill) *versus* probability of contaminated unit. Validation can be performed as:

Overkill approach: no bioburden or minimal analysis is necessarily required.

Bioburden probability approach: no BI, only probability determined.

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Combination of overkill BI and bioburden probability approach.

Determination of mean F_o value and PNSU:

$$F_o = D_{121} C (\text{Log } N_o + \text{Log } R - \text{Log } N_s)$$

$$N_s = \text{Log}-1 (\text{Log } N_o + \text{Log } R + \text{mean } F_o D_{121})$$

Where: D_{121} = the time to kill one log or 90% of the spore population of the most resistant product isolate or anticipated heat resistant micro-organism at 121 °C.

N_o = the highest (initial) microbial flora population in the product.

R = the number of product units.

N_s = the probability of a contaminated unit

The effectiveness of a process can be described as follows:

Determine spore log reduction of the calculated minimum process F_o value with maximum thermal resistant bioburden or sterility isolate:

$$\text{SLR} = F_o / D_v$$

Worse Case SLR can be determined with probable worse case survivor in each manufactured container per year, with probable average per unit.

$$\text{Worse Case SLR} = F_o / D_v (\text{Log No Units/ } y + \text{Log of average bioburden/unit})$$

Compare Bio F_o to Physical F_o as a ratio: For directional results, and comparison to original qualification if available with positive BI survivors.

Sterilisation design *versus* maximum product bioburden resistance:

$$D_{\text{bio}} (\text{Log } N_o + 1) = \text{or } > D_v \text{ isolate (maximum log bioburden} + \text{log } 10^{-6})$$

Determine in vessel D-value, where there are survivors, using physical F_o and SLR:

$$\text{In vessel } D_v = F_o / \text{SLR}$$

Compare in vessel D-value with laboratory D-value using bio F_o /physical F_o ratio:

$$\text{Ratio of Lab } D_v / \text{in vessel } D\text{-value} = \sim \text{Ratio of Bio } F_o / \text{Physical } F_o$$

3.11 Final Review

Before completing the qualification, a final review is made to make sure all the items have been performed.

The SVD check list, attached forms and records, and results must be reviewed.

The issues and variables must be considered prior to completing this SVD such as:

- Are there any applicable consideration of orders to documentation?
- Are there any tests that need to be repeated?
- Are there any uncertainties that have not been addressed?

3.11.1 Documents/Organisation for Protocol

A sterilisation validation requires documentation and organisation as follows:

- Steriliser validation document.
- Statement of final product specifications (as applicable).
- Approved Master Part Specifications (MPS) and Master Parts Lists (MPL) shall be used to document final product specifications. In the absence of approved MPS/MPL, a listing that specifies materials may be substituted as approved by directors of Quality Assurance, Manufacturing, and Marketing.
- Statement of equipment.
- Steriliser specifications and drawings shall be included or referenced as documentation of the equipment used for the sterilising process.
- Calibration of BI: The requirements of Procedure-C or SVD shall be followed.
- Physical evaluation of container and closure systems (as applicable): A procedure or SVD shall be written, approved and followed.
- Microbiological evaluation of closure systems (as applicable): A procedure or SVD shall be written, approved and followed.
- Product temperature mapping (as applicable): A procedure or SVD shall be written, approved and followed.

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- Minimum cycle limits (heat penetration)(as applicable): Criteria of SVP or procedure shall be written, approved and followed.
- Maximum cycle limits (heat penetration)(as applicable): A procedure or SVD shall be written, approved and followed.
- Closure sterilisation (as applicable): A procedure or SVD shall be followed.
- Bio information should be described.
- A qualification specification sheet should be prepared.
- Summary of cycle run should be written.
- Copy of completed Record should be included.
- Copy of Steriliser Circular Chart shall be made.
- Copy of Data Logger shall be kept.
- Data Trace Records or Summaries of Records shall be composed.
- Steriliser log shall be maintained.
- Sterility Test Membrane Filtration Form to be completed.
- Bioburden and relative thermal resistance of bioburden shall be performed.
- Vessel Cart Loading (BRP) shall be described and documented.
- Raw Data on Data Tracer Disks: set points, calibration data shall be maintained.
- Qualification Probe/Bio Reports shall be made.
- Heat penetration/distribution tables shall be composed.
- F_0 calculations shall be performed.
- Summary of cycle runs shall be made.
- Validation study shall be completed.
- Bio validation study shall be completed.
- Sterilisation Process Certification be approved.
- Executive Summary Report shall be written.
- Process specification shall be prepared and documented.

Each of the appropriate study aspect(s) of a qualification shall be summarised by the study workers and reviewed by the validation specialist and plant validation committee. Upon completion of work, a written addendum will be formalised. The addendum shall be reviewed and accepted by the validation committee. All summaries, raw data, and test notes shall be included as part of the final validation record.

3.11.2 Updates

Improvements in the use of steam as a classical method of sterilisation make it an ideal candidate because of its compatibility with the environment and health and safety, and it does not have toxic residues and can be handled safely. But the number of materials capable of tolerating high temperature and moisture are more numerous than previously thought. Steam sterilisation is predominantly used in hospitals and laboratories where reusable materials and products are frequently resterilised. It is also widely used in decontamination of infectious waste materials. Now however, with the emphasis on the environment and toxicity, there is renewed interest in this method of sterilisation. Unlike most other methods, steam is compatible with liquids. Steam can sterilise most metals, glass, ceramics and a large number of heat resistant plastic materials.

Plastics transfer heat more slowly than glass or metal and may take longer to reach sterilising temperatures in the autoclave. Because of differences in heat transfer characteristics between plastics and inorganic materials, the contents of plastic containers may take longer to reach sterilisation temperature, (e.g., 121 °C). Therefore, longer autoclaving cycles are necessary for liquids in large-volume plastic containers.

3.11.3 Adequate Processing Can be Determined only by Experience with Specific Liquids or Components

Some miscellaneous considerations of steam sterilisation are:

- Some chemical additives in steam will attack transparent plastics and cause a permanently glazed surface after autoclaving.
- Some transparent plastics may absorb minute amounts of water vapour 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 below 75 °C for two hours.
- Use polypropylene copolymer (PP) bottles instead of polysulfone with Tween in the autoclave.

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- PVC must be sterilised on flat surfaces.
- Do not use polycarbonate (PC) under vacuum conditions.
- Select materials based upon their heat deflection temperature, and not necessarily their maximum use temperature. For example, ABS requires low steam temperatures.
- Non stainless steel, like carbon steel can become corroded.
- Do not overload sterilisers. Read the manual. Read gauges. Monitor sterilisation.
- Steam pressure sterilisation (autoclave): Steam must circulate and penetrate all packs for the prescribed time. Do not overload or cram packs together. Package instruments to protect from contamination during storage. Packaging must not prevent steam penetration. Leave closed containers on their sides with lids open or ajar.
- For the gravity air displacement steam autoclave: Air is displaced in the chamber by a flow of steam from a vent in the top of the chamber at 121 °C at 6.8 kg pressure for a minimum of 15 minutes for very light loads. Allow 20 to 30 minutes for a moderate load of wrapped instruments.
- Pre-vacuum or high vacuum steam autoclave: Used mainly in hospitals; a vacuum is pulled into the chamber before allowing steam to flow in. Otherwise operation time, temperature, and pressure are the same. This process is considered to be more efficient, but is not available in most portable sterilisers.
- Flash sterilisation: 134 °C at 15.6 kg pressure. Allow a minimum of 7 minutes for a light load and 10 minutes for a moderate load of wrapped instruments or 3-5 minutes for an unwrapped instrument. Consult specific times prescribed in the steriliser manufacturer's manual. Temperature cycles still must kill BI spores.
- Cautions: Time required for the steriliser to reach temperature is not included in the sterilisation times given. Begin timing after steriliser has reached its operational temperature.
- Place packs so steam can circulate and penetrate. Open the door at the end of the cycle to let the packs dry

The number of materials that can be steam sterilised will vary considerably with the temperature of sterilisation. Standard steam sterilisation is generally carried out at 121 °C for 15 minutes. Steam sterilisation can be reduced, however to as low as 105 °C, depending upon the bioburden, integrity and heat resistance of the plastic material to

steam heat. Alternative or combination approaches to steam sterilisation are possible future considerations. For example dialysers can be either steam sterilised in place (SIP) on carousels and released via process control or parametric release on a routine basis. These dialysers can also be sterilised with liquid water at high temperatures. Some sutures can be steam sterilised. There are PP films now that can be autoclaved as packaging materials. Most pharmaceutical/healthcare plastic containers with liquids such as high density polyethylene, PVC, polyallomer (copolymer of PP and polyethylene) are steam sterilised. Some of these containers with liquids are attached to devices and are classified as medical devices. Medical hospital polyester gowns and packaging may be steam sterilised at lower temperature sterilising temperatures, e.g., <120 °C.

Improvements in plastics for radiation compatibility are benefiting steam sterilisation, with the addition of heat stabilisers and copolymerisation. Use of more plastics is now feasible with reduction of steam sterilisation temperatures. The consideration of steam sterilisation in the context of recent developments of sterilisation technology may appear unusual, but improvements are being made in computer controls, microprocessors, monitoring devices, and biological and chemical indicators. In Europe, some contract facilities are building autoclaves to replace EO sterilisers, which are considered to be an acceptable alternative for environmental reasons. Consequently there is a need for more heat stable plastics as there is for radiation compatible plastics.

Acrylics, styrene, and many other heat sensitive polymers will melt and distort with steam and dry heat. Radiation and EO may be better candidates for their sterilisation. PVC may become cloudy, and typically softens significantly above 80 °C. Many metals will eventually corrode due to moisture. Electronic boards can be short circuited and damaged due to moisture.

Steam autoclaving is the most popular and prevalent means of sterilisation used in most hospitals. Some healthcare products that have been commonly sterilised with steam: Basins, bladder catheterisation trays, cotton balls, dental instruments, enema trays, eye dressing kit, gastric aspiration trays, gauze, gynaecology examination trays, gynaecology surgical pack, injection trays, instrument operating trays, irrigation trays, kidney biopsy trays, liquid vials, gowns, nasal pack trays, obstetrical packs, peritoneal dialysis trays, solutions, liquids, including numerous pharmaceutical and drugs, surgical instruments, surgical pack, suture trays, towels.

Types of trays will vary, and some trays that have heat sensitive materials may be sterilised by another method (e.g., steam-formaldehyde or EO).

3.12 Low Steam-Formaldehyde – a Hybrid Method for Heat Sensitive Products

Low steam-formaldehyde is a physico-chemical processing method combining formaldehyde gas with sub-atmospheric steam [6, 8]. Sometimes it is not considered or used as a sterilisation method, but applied as a high level disinfection process, but it is included here because of its worldwide use in hospitals. It is used in place of EO. It does not require the high temperatures that steam does at 121-134 °C, but rather can be used at slightly lower temperatures of 70-80 °C, and sometimes as low as 65 °C. This allows a lot more heat sensitive healthcare products to be sterilised.

Typical articles and polymers sterilised in low steam formaldehyde are: catheters, hospital equipment, instruments, IV infusion administration devices, mattresses, metal surgical equipment, gowns, gloves, polymers (acetal, ABS, PVC, polyethylene, PP, polystyrene, rubber - artificial and natural rubber, Teflon), materials (Formica and Tyvek), and tubing.

Types of products will vary, and some products made from heat sensitive materials may be sterilised by another method, e.g., EO, Sterrad, or another low temperature method.

Low steam-formaldehyde is not typically used in the United States, because of environmental and regulatory concerns over its carcinogenicity and toxicity in the USA. OSHA worker exposure levels are, for example, 0.75 ppm 8-hour time weighted average (TWA) or 2 ppm 15-minute short-term exposure level (STEL). A chemical with an immediately dangerous to life or health level (IDLH) of 20 ppm is considered to be a potential carcinogen. The reportable quantity (RQ) in the case of a spill is 100 pounds under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA).

Despite potential toxicological concerns, it is used frequently in Europe (UK, Germany), India and many other parts of the world, in place of EO. Sometimes it is not referred to as a sterilisation method, but as an high level disinfection process. For a number of years it was used for sanitising used mattresses in the USA. Low-temperature steam in combination with formaldehyde has continued to be improved. It is an example where synergism brings together subatmospheric steam and formaldehyde gas – neither of which is markedly sporicidal at these temperatures – to produce a highly efficient sporicidal effect. This has resulted in sterilisation at temperatures as low as 65 °C, comparable to some EO processes, which allows for many more polymers to be sterilised, (e.g., heat stabilised acrylics, low density polyethylene, polystyrene and various configurations of PVC. Much medical equipment is too sensitive for high-temperature or high-pressure sterilisation by steam.

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4 Statistics in Sterility Assurance and Sterilisation Validation of Healthcare Products

4.1 Background and Definition

Successful sterility assurance and validation is dependent on adequate application of statistics to products and the processes. To fully appreciate the role statistics plays in the sterility assurance and sterilisation validation endeavor, let us start from scratch and begin with the word, sterile.

Sterile is defined in the dictionary as the complete freedom from all living viable organisms. The term sterile implies an all or nothing condition. Either all viable organisms are killed or removed. In reality sterile is a probability function, a relative term. However, there is a tendency sometimes to mistakenly use sterile in the wrong context. For example, in early medicine sterile meant to destroy only disease organisms. In the home, baby bottles boiled in water have been implied to be sterile.

Sterile must always be differentiated from lesser means of destroying or removing microbes. Terms and techniques such as disinfected, sanitised, pasteurised, decontaminated, 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. The actual number of techniques or methods recognised as capable of meeting the criteria of sterility without adversely affecting product quality is few. Common traditional sterilisation methods used are:

Ethylene oxide (EO)

Radiation

Steam

Dry heat

Filtration

All sterilisation methods have their limitations. There are a few other sterilising agents, [e.g., Sterrad (hydrogen peroxide/plasma), glutaraldehyde, steam-formaldehyde, peracetic acid, ozone, etc.], the above methods are not adequate for all the specific applications. 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 tell if a process has completely sterilised a product without evaluating every product? We start by performing a sterility test.

4.2 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. In general there are two basic ways to test for sterility: (1) product sampling and product sterility testing, and (2) the application and use of biological indicators (BI).

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, the surface of the lumen of the product to be tested may be rinsed or flushed with a recovery fluid which is passed through a bacteria retentive membrane. The membranes are put in the recovery medium as above and evaluated. Product sterility testing methods for drugs and medical devices are generally described in the United States Pharmacopoeia, ISO AAMI standards, ISO-11737-2 [1], ISO 11137 [2], and other compendia.

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 determination of sterility. The type of BI or challenge organisms are matched to the specific sterilisation method used. For example:

Saturated steam	<i>Geobacillus stearothermophilus</i> ATCC 7953 or ATCC 12980 <i>Clostridium sporogenes</i> PA 3679 or ATCC 11437 <i>Bacillus coagulans</i> FRR B666 or ATCC 51232 <i>Bacillus subtilis</i> 5230 or ATCC 35021
Dry heat	<i>Bacillus atrophaeus</i> (<i>subtilis</i> var <i>niger</i>) ATCC 9372 or NCTC 10073, not ATCC6633
Ethylene oxide	<i>Bacillus atrophaeus</i> ATCC 9372 or NCTC 10073
Radiation	<i>Bacillus pumilus</i> E601 ATCC 27142 (when applied to NDA or other Regulatory approval).
Filtration	<i>Brevundimonas</i> (<i>Pseudomonas</i>) <i>diminuta</i> ATCC 19146

Similar to the product sterility test, the BI or challenge (inocula) is placed in a optimal bacteriological recovery medium and observed for growth.

Table 4.1 Nonsterile units that may be present in a lot, but not detected within a given sample size			
Sample size (1), Total Units Tested	Probability of sample containing no non sterile units (2)		
	50%	5%	0.5%
10	6.7% (lot contaminated)	25.9%	41.1%
20	3.4	13.9	23.3
30	2.3	9.5	16.2
40	1.7	7.2	12.4
60	1.1	4.9	8.5
(1) <i>United States Pharmacopeia, Mack Publishing Co, Easton, PA, USA [3]</i>			
(2) <i>FDA Compliance Program Evaluation Report Fiscal Year (7324.04): Percentage of non sterile units in a lot.</i>			

In product sterility testing of finished devices there is a statistical relationship between sample size and the probability of passing unsterile product, as described in **Table 4.1**.

For example, if a lot contained 3.4% contaminated product and 20 units were sterility tested 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.

Another problem, inherent in sterility testing is adventitious (accidental) contamination. When the sample size is increased to detect low level contamination, the chance of adventitious contamination will increase proportionally. Sterility testing, depending upon the type of product to be sterilised, generally requires careful aseptic manipulation and rigorous sterility techniques. Sterility testing for radiation requires only one sterility test media (aerobic), but no anaerobic media, and fewer devices to test per BS EN ISO 13409 [4], TIR 27 [5], than the USP Compendium. Consequently their chance of detecting different types of contamination, (e.g., anaerobes, fungi, and yeasts), and percentage product contamination is significantly less. 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 the BI or detection of the product sterility test used, (e.g., *Pyronema domesticum*, anaerobes, thermophiles, some thermotolerants and yeast). Some processes and product sterility tests are not able to either inactivate or detect small targeted viruses, prions or endotoxins (pyrogens).

Regardless of which sterility test is used, it is necessary to know the bioburden of microbes on products, 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.

4.3 Kinetics of Microbial Inactivation

To evaluate a sterilising agent or a product, 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 bioburden, the mixture and state of the population, BI employed, environmental conditions, product configuration, 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 when placed in favourable environmental and optimal recovery medium.

Statistics of sterilisation are based on the assumption that all micro-organisms die or are inactivated in a logarithmic first order reaction rate. This assumption is reasonably true under laboratory or pure environmental conditions. However exceptions exist. Steam sterilisation characteristically does kill logarithmically with some exceptions, (e.g., heat activation). Radiation has an activation hump with *Bacillus pumilus* 601, and tailing with *Clostridium* anaerobic spores. Dry heat sterilisation frequently exhibits tailing (non logarithmic decline) with high populations, (e.g., greater than 10^3)

In reality, most 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, mould, fungi, viruses, aerobic, anaerobic, and microaerophilic, which results naturally in non-logarithmic behaviour; so a logarithmic order of death may not be demonstrated. However, the logarithmic death approach continues to be used and exploited to predict the probability of survivors.

Whether the logarithmic phenomenon is or is not an accurate explanation is not important, only that the kinetics allow us a practical way to compute microbial inactivation values, exploit and to draw conclusions independently of complications so that statistics can tell us if we 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 D-value. The D-value is the backbone of sterilisation statistics for ethylene oxide, radiation, steam, dry heat and hydrogen peroxide/plasma sterilisation. However, a log reduction value (LRV) is used particularly for evaluating microbial filters.

The LRV can also be used for determining the inactivation factors (IF) by subtracting the log of the bioburden from the LRV.

The D-value is defined as the time or dose to reduce a bacteria 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:

$$\text{D-value} = \frac{\text{Exposure time or dose}}{\text{Log } N_o - \text{Log } N_t}$$

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 or treatment to 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, 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 on bacteria spore populations used in biological indicators because they can be prepared as a homogeneous population, with high resistance and produce 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 1000 by 90% in X minutes to 100 surviving organisms, then in a 2 X time we would expect a 99% reduction of the initial population to leave only 10 organisms and a 3 X 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 compute probabilities of survivors occurring at lengthening exposure times.

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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.

The determination and estimation of a level of probability of survivors 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 typically described:

$$N_t - \log^{-1} = \frac{(\text{Log } N_o - \text{Exposure time or dose})}{\text{D-value}}$$

Where: N_t is the probability of survivor at a given sterilising exposure or dose

N_o is the initial product bioburden or biological indicator 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 required. 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 is typically a higher standard than that which doesn't (10^{-11} *versus* 10^{-6}). However, a product that is invasive requires a higher standard than one that doesn't (10^{-6} *versus* 10^{-3}). Some examples of probability of survivors for sterilised product procedures:

Invasive medical devices	10^{-6}
Topical medical devices	10^{-6}
Canned chicken soup	10^{-11}
Large volume parenterals	10^{-9}
Small volume parenteral (1)	10^{-3}
Laparoscopic instruments (2)	10^{-2}
Limits of limited USP sterility test (3)	$10^{-1.2}$

(1) Sterile fill

(2) Sterilised with liquid chemicals, not within a terminal package or container.

(3) With 95% confidence, but with two sterilising media.

Given that one knows what level of probability of survivors may be required for a particular product and process/product, a sterilisation process can be designed (see Section 4.4)

4.4 Design of a 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 leaves residues. Radiation may damage some electronics, some plastics, and drugs, but can sterilise many polymers and very dense materials. Low dry heat may sterilise silicone prosthesis, electronics, but destroy many plastics and heat sensitive materials. It must be recognised that a variety of factors must be carefully considered for each sterilisation method selected in order to achieve reproducible and repeatable processes without adversely affecting product quality and materials' chemical and biocompatibilities.

There are several ways to design a sterilisation process statistically to improve product quality and materials' compatibilities. A simplified mathematical expression for designing a sterilisation process is:

$$\text{Exposure time or dose} = D_v (\text{Log } N_o - \text{Log } 10^{-x})$$

Where: Exposure time or dose varies depending upon D_v , N_o , and 10^{-x} .

D_v is the time or dose to destroy 1 log or 90% of a product bioburden or BI.

N_o is the initial product bioburden or BI challenge population.

10^{-x} is the probability of survivors after exposure to specified parameters.

The design of a sterilisation process may be approached statistically or 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:

Sterilised parenteral solutions (1)	8 logs
Sterilised food to prevent botulism or overkill process (2)	12 logs
Microbial challenge to filters	7 logs/cm ² of filter
Sterilised devices based upon bioburden (3)	6 logs + log of bioburden
Topical devices based upon bioburden (4)	3 logs + log of bioburden

(1) 8 logs are applied where there is an initial population of 100 organisms or 2 logs.

(2) 12 logs is required for botulism, but 12 logs is typical overkill where the BI is 6 logs and an additional probability of survivor is 6 logs.

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(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.

To apply log levels to the design of the sterilisation process, the following simplified mathematical equation is provided:

$$E = n (D_v)$$

Where: E can be the sterilising exposure time or dose at given sterilising parameters, heat temperature, process parameters, or irradiation source conditions.

N is the number of logs of inactivation required or desired.

D_v is the D-value at the specified sterilisation parameters, heat temperature, or irradiation source conditions.

Besides designing a sterilisation process on the basis of a desired level of probability of a survivor or 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 BI where spore populations may typically range between 10^{-4} to 10^{-6} and 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 *Bacillus pumilus* 601. The traditional overkill approach to steam sterilisation was 15 minutes at 121 °C which implied a >9 log reduction of *Geobacillus stearothermophilus* spores.

With the bioburden approach the design of a sterilisation process is established or verified on actual bioburden counts and resistance.

The most highly used example of the bioburden approach has been with radiation where AAMI/ISO have published guidelines to establish the radiation dose based upon computerised disburden 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 [2])

A modified approach of either the overkill or bioburden approach has been the sterilisation of parenteral solutions where an equivalent time to sterilise at 121 °C for 3.5 to 4.0 minutes has been accepted with sterilising temperatures of only 105-115 °C that are compatible

with many parenteral drug solutions. Similarly, spacecraft sterilisation has demonstrated 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 less than 121 °C, for example, but where lethality has been adjusted to this temperature through the statistical use of z-values. The z-value is defined as temperature difference to cause a 10-fold change in the D-value. The z-value may be derived from the following equations:

$$z = \frac{T_x - T_o}{\text{Log } D_o - \text{Log } D_x}$$

Where D_o is the D-value at the initial temperature, T_o

D_x is the D-value at a later temperature, T_x .

A typical z-value of *Geobacillus stearothermophilus* spores, for example, is 10 °C. The application of the z-value, to determine a F_o value is typically represented as follows:

$$F_o = \int_{t_o}^{t_f} L(dt)$$

Where F_o is the equivalent time to sterilised at 121 °C

$$L \text{ is the lethality value} = 10^{\frac{T(t) - 121^\circ\text{C}}{z}}$$

T is time variable to initial time, t_f final time

T (t) is time-dependent temperature variable.

In practice the applied sterilisation of product is based on both killing highly resistant spores of *Geobacillus stearothermophilus* or less resistant *Clostridium sporogenes*, *Bacillus coagulans* or *Bacillus subtilis* 5230, where the bioburden resistance is performed concurrently.

Once a sterilisation process has been designed, the process must be validated. In general, process validation will consist of performing verification (sub cycles) or half cycles, and a series of full validation cycles at established sterilising parameters.

4.5 Sterilisation Validation

Sterilisation validation is a formal procedure to demonstrate that a designed process can reliably sterilise a specific product. A validation program generally consists of four major steps:

1. Installation or material/product qualification(s)
2. Sterilisation cycle development
3. Sterilisation performance qualification
4. Certification

Statistics plays a primary role in steps 2 and 3. Starting with process development where D-values (desired probability of survivors) are considered, or where process parameters are checked for their compatibility with the product to be sterilised. During sterilisation, performance results of half cycle runs or sub process verification runs are performed that verify results of D-value calculations by showing complete or near complete inactivation typically out of 10-100 samples of micro-organisms on product, to indicate that the desired probability of survivor 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 survivors for the process. From this information, a sterilisation process can be reliably shown to sterilise.

For further details of performance qualification see Chapters 3 (steam), 5 (radiation) and 6 (ethylene oxide).

4.6 Summary

In review, statistics plays a significant role in the sterilisation validation and sterility assurance.

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 requires statistical considerations. Sterile therefore is not an absolute term, but a relative one, requiring the application of statistics. The kinetics of microbial sterilisation has been described as a logarithmic phenomenon with the backbone of sterilisation statistics typically described as a D-value, the time to inactivate one log or 90% of a known population.

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5 Radiation Sterilisation

Radiation has long been recognised as a means of sterilisation since X-rays were first demonstrated in 1896 to inactivate micro-organisms [1]. However, its practical application followed use of ethylene oxide (EO) because of the continuous improvement of plastic materials and medical devices, and the availability of improved electron beam accelerators and radioactive materials.

Radiation sterilisation is 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. But understanding its effect on polymers is of utmost importance [2, 3].

Many polymers have a natural tolerance for sterilising radiation doses of up to and beyond 50 kGy, with the notable exceptions of acetal, polypropylene (PP) and Teflon.

Sterilisation doses are orders of magnitude lower than the nuclear reactor environment in terms of 30-year lifetime, radiation in space, or even outdoor UV irradiation seen in automobile finishes, garden furniture, trash cans, etc. Solar incident energy is approximately 1 kW/m².

Only a few medical plastics cannot be sterilised by radiation, such as those likely to fail, which include acetal, PP (natural unstabilised) and Teflon, i.e., PTFE (others will be discussed later). Avoid these three materials and you can generally expect good results, but not always. However, many more polymers cannot be reprocessed, limiting the use of radiation sterilisation mainly for disposables but not reuseables.

Polymers react to electron bombardment by crosslinking their molecular chains to become stronger and stiffer or by scissoring (breaking) the polymer chains which become weaker with reduced impact strength and lower elongation to break. Most materials do both (crosslink and scission), making prediction of physical performance more complex to forecast. Materials can be ranked by crosslink to scissoring ratio.

Materials that crosslink more than they scissor generally do better in the radiation environment. Highly crystalline materials generally have higher resistance to molecular chain break-up by electron impact due to their strong, nested, compact, mutual reinforcement

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of polymer chains. Highly amorphous materials (non-crystalline) are generally resistant to radiation because the natural relaxed amorphous molecular configuration is capable of great ductility and elongation before break. Amorphous polymers can tolerate many scissions without breaking up.

Molecular chemical structure may contribute to radiation resistance: the benzene ring structure acts as an 'electron bank', rearranging itself to accept or lend an electron as needed without losing integrity – like a radiation shock absorber. Examples are styrenes, styrene alloys, polyester, polyurethane (PU), polycarbonate and polysulfone.

Discoloration occurs when specific chromophores are involved (not all of which have been fully defined) that appear prior to any measurable loss in physical properties. For example, polyvinyl chloride (PVC) conjugated double bonds produce yellowing before any measurable loss in physical properties occurs.

When odours occur, they are also assignable to specific chemistries. In the case of polyethylene, the use of antioxidants and lowered processing temperatures can reduce odour formation after radiation sterilisation. Other examples include PVC, rancid oil odour from oxidised soybean and linseed oils in the plasticiser. Some PU are the source of some very strong odours. Odour clearance takes place in about a week at an elevated temperature of 38-66 °C, which may be similar to the clearance time for free radicals and retained gases.

Thick polymer sections are more resistant to oxidative radiation damage than thin films or fibres; an example is Nylon 66.

Moisture content in the polymer can produce additional free oxygen and hydrogen by hydrolysis during irradiation. All polymers contain a finite amount of absorbed water. Moisture content is a variable that may need control.

5.1 Some Unexpected Radiation Results and Considerations for Evaluating Radiation

Qualification of medical devices for radiation sterilisation is generally easier to accomplish than anticipated if those few materials most likely to fail are avoided. Avoid materials having less than 25% elongation and acetals, PP (unstabilised) and Teflon.

Polymers which appear to have marginal physical properties after irradiation can be improved by moulding in hotter moulds, often increasing impact strength and elongation by ten times.

Material processing parameters can affect a part's physical performance ten times more than radiation will. This explains why early irradiation data has been so variable.

Better physical properties can be had after irradiation than you have now by running hotter moulds, accepting slower cycles, and using an engineering safety factor to resolve each customer field complaint.

Accelerated ageing using Arrhenius $Q_{10} = 2$ is valid only in a limited way. Q_{10} is a temperature coefficient that reflects a doubling in reaction rate for every 10 °C rise in temperature. Free radicals generated by electron bombardment are present in such limited amounts that they may recombine, dissipate or be quenched in a matter of hours or days after irradiation. An additional two-eight weeks may be more encompassing.

First-, second- or third-order reactions based on the presence of several types of free radicals, i.e., antioxidant, polymer main chain scissoring, terminal end groups, side chains, pigments, processing aids, etc., may complicate determination of reaction rate equations. This produces a constantly reduced reaction rate. If initial reaction rates are used to extrapolate a shelf life measured in years, an unrealistically short lifetime will be the result. That is, initial reaction rates are faster when all free radicals are present in abundance. The exception is natural unstabilised PP that has a special autocatalytic oxidation process that attacks the carbon-carbon main chain bond, producing a steady degradation with time over a two-year period in which elongation may drop from 600% to zero and molecular weight undergoes a similar reduction.

Stabilised PP has proved feasible for radiation sterilisation. For example, syringes are stable at 50 kGy. By using short- and long-term antioxidants, liberal polyethylene and a mobilising oil to facilitate free radical recombination, syringes can be successfully processed and radiation sterilised.

Elastomers as a family tolerate radiation well – natural rubber typically survives very well. An exception is butyl rubber which crosslinks to become stiffer with attendant loss of elongation which makes it more friable, tending to shed particles. Radiation of other butylene-containing polymers such as ABS and PBT are known to lose impact strength and elongation as a result of degradation of the butylene component that was originally added to improve impact strength.

Elastomers survive radiation partly due to their chemistry and partly because the polymer is self-relieving of moulding stresses. This protects the polymer from scissoring. This gives credence to the theory that it is the molecule under the greatest combined stress that is preferentially attacked first by radiation.

Silicone rubbers crosslink without scissoring to take on increased hardness and shape memory after radiation. Those materials having the lowest crosslink density before

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irradiation are affected most. Silicone greases and lubricants also stiffen measurably after radiation.

PVC vinyl tubing crosslinks to become measurably stiffer by approximately 10%, necessitating more clamp force to regulate flow in intravenous (IV) sets. PVC tubing coil shape is also locked in by crosslinking, giving the tubing a firmer memory of the coiling pattern. Because of this, tube kinking is enhanced.

Solvent joints are more likely to leak after radiation sterilisation because they lack the heat setting cycle provided by EO at 60-70 °C for four to 12 hours. The solution is to get more solvent or adhesives into the joint and use a leak tester to validate solvent joints.

Special polymer selection for toxicity, mutagenicity, haemolysis, and cytotoxicity is not required for radiation sterilisation. It has been observed that radiation sterilised polymers will show increased turbidity, oxidisable extractions, greater conductivity and lower pH after United States Pharmacopoeia/National Formulary (USP/NF) standard extraction studies than seen in other sterilisation processes, but in no case has it been shown that toxic limits are exceeded after radiation sterilisation, except for increased extractables. The source of increased extractables is attributed to the presence of free radicals at the surface having increased polarity for solubilisation.

If accelerated ageing proves to be an inaccurate or elusive prognosticator of product life, use of exaggerated dosing at 100 kGy immediately reveals marginal materials. Make polymer substitutions promptly - alternate materials exist for all materials.

Rely on real time ageing. Release a new product after limited (90 days) ageing with appropriate expiration dating while real time ageing continues to extend allowable shelf life.

Follow polymer manufacturers who have five years' satisfactory real time polymer ageing. Recognise that their results can be expected to be optimistic compared to actual moulded products.

Electron beam irradiation is typically gentler to polymers than gamma radiation, because the cycle for electron beams is so fast (measured in seconds) that oxidative effects are minimised (starved). Gamma radiation with its greater penetration and lower energy electrons (3 MeV) takes much longer cycle time (6-18 hours), giving time for more oxygen to permeate the polymers and produce oxidative degradation.

10 MeV beam electrons give useful penetration in a unit density product up to 60 cm that equates to 120 cm double-sided irradiation. This makes electron beam irradiation a viable, if not preferable, alternative to gamma radiation in a lower density product.

Comparing electron beam and gamma irradiation for sterilising a family of medical polymers at doses up to 100 kGy can fail to establish any significant difference in the physical properties of the polymers when sterilised by either method, when polymers are well stabilised with antioxidants and processed under ideal conditions of heat history.

Some of the disadvantages of radiation sterilisation have been its high initial capital cost, incompatibility with some low cost plastic materials, fear of radiation, extended length of time for qualifying irradiated materials and the disposal of radioactive waste when gamma emitting isotopes are used.

5.2 Radiation Ionising Sources

Some simple typical sources of radiation are cobalt 60, caesium 137, electron beam (3-12MeV) and X-rays. Non-ionising sources are heat and microwave.

Most radiation methods require only dose delivered and/or time of exposure. The method is simple; however workers must be trained for safety. Elaborate facility designs and controls are made to minimise and eliminate the risk of irradiation of workers or the surrounding environment.

5.3 Radiation Sterilising Doses

In general radiation doses needed to inactivate all micro-organisms are extremely high in millions of rads or tens of millions of kilograys. The classical radiation dose has been defined as 25 kGy. Lower doses, however, have become common with the advent of the AAMI/ISO Gamma Radiation Process dose setting guidelines.

Dose setting approaches vary. In radiation sterilisation, dose setting uses bioburden information from the standards. Early radiation qualifications commonly used the KILMER method, which allowed one to qualify a 25 kGy dose with a small number of products and little bioburden information. See AAMI Method 3A Dose Setting, for Infrequent Production (25 kGy); Method 3B Dose Setting for Small Lot Sizes and Infrequent Production [4], which have been combined in ISO TIR 13409 [9]. AAMI TIR 27 is similar to the Kilmer Method in regard to the number of samples and radiation dose. The reason for disallowing Kilmer and going to TIR 27 vary.

The most recent standards have three dose setting approaches:

- Method 1 Dose Setting Using Bioburden Information

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- Method 2 Dose Setting Using Fractional Positives, sample item proportion (SIP) of 1 Protocol
- Substitute for Method 3, is $VD_{\max}$ in AMMI TIR 27: 2001, 15844: 1998 or ISO 13409 (See AAMI Method 3A Dose Setting, for Infrequent Production (25 kGy); Method 3B Dose Setting for Small Lot Sizes and Infrequent Production).

Tables 5.1 and 5.2 of ISO 11137 [5] of AAMI Method 1 Dose Setting uses bioburden information, to determine sub-dose level(s) from a table to evaluate survivors. Two or fewer survivors can confirm adequacy of the method.

Table 5.1 Validation Radiation Methods – ‘Relative’ Differences for small lots			
Small Lots and Infrequent	ISO 13409	ISO 15844	TIR 27 $VD_{\max}$
Bioburden < 1000	Yes	Yes	Yes
Radiation Tolerance	25 kGy	25 kGy	25 kGy
Lot Size <500	Yes	Yes	Yes
Lot Size- Large	No	No	No
Infrequent Production	Yes	Single batch	Yes
Initial Sample Size	66-306	66-306	10
Audit Size	20-100	20-100	10

Table 5.2 Validation Radiation Methods – ‘Relative’ Differences for large lots		
Large Lots and Frequent	ISO 11137: Method 1	ISO 11137: Method 2
Bioburden < or > 1000 cfu	Yes	Yes
Radiation Tolerance < or > 25 kGy	Yes	Yes
Lot Size > 500	Yes	Yes
Lot Size < 500	No	No
Routine Production	Yes	Yes
Low Bioburden Resistance	Yes	Yes*
High Bioburden Resistance	No	Yes
Initial Sample Test Size	1346	643
Audit Sample Test Size	110	110
*when low bioburden resistance doesn't fit ISO 11137 Method 1, see also Part 2 of the eventual ISO 11137-02.		

Methods 1 and 2 of the ANSI/AAMI/ISO 11137 guideline involve establishing a sterilising dose using a bioburden resistance model. Method 1 is preferred because of its reasonable cost and study time. Because it employs model population data from Whitby and Gelda that is based on historical data received from manufacturers, it provides a greater challenge than the natural bioburden on a device. With method 2, the dose is determined experimentally based upon the resistance of the device under study.

Method 2 consists of two protocols. Each requires a greater number of samples during validation than the other method. For protocol 2A - validation for normal product bioburden distribution with a sample item portion (SIP) of 1.0 or less - the minimum number of samples used is 640; 540 are used for the incremental dose series and 100 for the verification dose experiment.

For protocol 2B - validation for product with consistent and low bioburden and an SIP of 1.0 (i.e., the entire device) - approximately 580 are generally tested. In each method - 2A and 2B - an extra 200 samples (100 from each of the lots not used for the verification dose experiment) must be available. If they are not used in the study, they will be discarded if the SIP is <1.0, or they can be returned to the manufacturer for terminal sterilisation if the SIP is 1.0.

A good reason to choose method 2 is its ability to validate a lower dose than method 1. Method 2 is based on a device micro-organisms' average resistance to radiation, whereas method 1 is based on a theoretical model population that may or may not be as similarly resistant to radiation as the organisms under study.

There may be a need in the future for a mathematical determination of verification dose other than that currently provided in AAMI and ISO 11137 guidelines that would improve or demonstrate the correlation between sterility failures at low bioburden *versus* sterility successes at higher bioburden levels. The following is a simple step-by-step derivation of a mathematical verification formulation for that purpose:

Step 1: Given a SAL dose of 10^{-6}

$$\text{SAL Dose} = D_v (\text{Log } A_n + 6)$$

Where: D_v = the imaginary decimal reduction value

A = is the average bioburden level

n = the number of test samples for sub-dose, and

n is the maximum number of products in a lot.

Step 2: Given a D_v formula

$$D_v = \text{SAL dose} / \text{Log } A_n - \text{Log } B$$

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Step 3: Determining the expression from step 2 above when only one survivor will occur, we have:

$$D_v = \frac{V \text{ sub-dose}}{\text{Log } A_n - \text{Log } B}$$

$$D_v = \frac{V}{\text{Log } A_n - \text{Log } (1)}$$

$$D_v = V/\text{Log } A_n - 0$$

$$D_v = V/\text{Log } A_n$$

Step 4: Modifying the above formula for solving for V (verification dose) we have:

$$V = D_v (\text{log} A_n)$$

Where: D_v is assumed to have an imaginary D_v of 2.1 kGy.

Step 5: Determining the modified verification dose and comparing it to the AAMI and ISO Guidelines of Table B1 of ISO 11137, we have the following correlation(s) (shown in Table 5.3).

The results indicate that there is a better fit at a higher bioburden at about 1000 cfu than at a low bioburden of less than 100 cfu. Consequently there is a higher probability of favourable sterility outcomes for ISO 11137, at a higher bioburden than at a lower bioburden,. There is a higher probability of false sterility test failures for ISO 11137, at lower bioburden levels than at higher bioburden levels, but the imaginary D-value approach is likely to be a better fit between 2 cfu and 1000 cfu, for favourable sterility test results.

Table 5.3 Comparison of ISO sub dose from Table B1 <i>versus</i> a calculated verification sub-dose, using an imaginary D-value			
B, cfu	ISO sub-dose, kGy	Calculated V sub-dose, kGy	Correlation between ISO sub-dose and calculated V
2.04	3.6	4.8	0.75
10.69	5.3	6.3	0.84
104	8	8.4	0.95
1021	11	10.5	1.05

As a result of the higher incidence of false sterility test failures at a lower bioburden, the VD_{max} approach for 25 kGy has been presented in AAMI TIR 27: 2001[6].

VD_{max} AAMI TIR 27:2001 [6] is used to establish and verify maximum doses which are implemented for the overkill approach by using a dose of 25 kGy. The VD_{max} will result in fewer false positive sterility tests, particularly at a lower bioburden, but one will potentially miss the presence of resistant microbes that may be present. If one doesn't want to see 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 D-value imaginary number is likely a better fit at lower bioburdens than VD_{max} , and will likely pick up the presence of resistant organisms if they exist, but it doesn't have the AAMI acceptance that VD_{max} or ISO 11137 has.

The use of VD_{max} may or may not be used in place of AAMI ISO TIR 13409 [7] and ISO 11137 [8].

VD_{max} has been specifically addressed in AAMI TIR 27 microbiological or sterilisation guidelines [6]. ISO 11137 (1995) will eventually be divided into three parts [9, 10, 11]. Part 2 will include dose setting for both 15 and 25 kGy and other possible doses to establish a SAL of 10^6 . Note: Requirements for pharmaceutical, drug, ophthalmic products do not follow these ISO standards. See Parenteral Drug Association's Technical Reports 11 and 16 [12, 13].

The purpose of this overview is to present a scenario in which the use of VD_{max} may lead to unknowns and to potential clinical significance.

Method 1 of ISO 11137 [5] and AAMI ISO TIR 13409 [7] use the bioburden population approach. Resistance of organisms is verified through the use of established sub-dosing, based on average populations.

To verify Method 1 (ISO 11137) without the use of VD_{max} , subsequent testing of the same lot without the use of VD_{max} demonstrated the presence of resistant organism(s), e.g., *Acinetobacter radioresistens*. *Acinetobacter* species have been observed as a noscomial infection in military hospitals where many sterile disposable devices are employed.

In a similar related case a few years previously, *Moraxella* species were found on a device that had been irradiated. With the help of a Public Health microbiologist, two species of this organism were identified. Proceeding after failure of Method 1 to Method 2 of AAMI, the problem passed, with virtually the same sub-dose, and this problem with this organism never occurred again.

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Without knowing that a resistant bioburden exists in a population, subsequent lots where neither bioburden nor resistance are evaluated could result in the release of non-sterile units, without us knowing it.

Also, the type of resistant population could go unnoticed. Without knowing what potential resistant organisms exist, a company exists in the dark waiting for potential developments and unknown risks. Radiation methodologies for dose settings are established around aerobic microbes. It has been my experience to observe many devices and components where anaerobe and/or microaerophilic microbes outnumber total aerobic microbes.

It has also been my experience to observe 'at times' that sterile microaerophilic/anaerobic media like thioglycolate recover more survivors than common aerobic casein digest media after sub dosing. It is under the microaerophilic/anaerobic sterility media that I have observed recovery of some resistant organisms. So know your Bioburden.

Also, some microbes are recovered after an initial low incubation temperature followed by a typical incubation temperature. It may be that these microbes repair or slowly adapt under lower temperature conditions. So it is vital to know how your product is used clinically and handled after irradiation.

Let us now move from the pre-clinical side of a product to the clinical side.

Let us assume for a moment that the resistant portion of the population survives within the entire population of the next lot, a lot that has the higher than average VD_{max} of the first three lots, but a fourth lot has lower bioburden and was tested. However, this lot is not tested for bioburden or survivors, and the survivors go unnoticed pre-clinically, but show up clinically in our product. Because in one device, the *Acinetobacter* organism has been shown, surprisingly, to grow and multiply in an infusate, much like gram-negative bacteria; also *Acinetobacter* has been shown to be clinically a pathogen. Unlike other survivors that have been characterised in this product, this organism grows and is infective.

What is being considered by ISO today, is not that a product be merely sterile, but that the product is also safe for use.

A product can be labelled sterile because of a convention or standard test, but is the product safe, free from infection in the way that it is used? That is the question.

Many products in use could support growth from a surviving micro-organism, for example, an infusion device could during use, after lying for several hours or days at a patient's side could become infected. It is very important that the product is sterile before use.

Microbial inactivation data must be demonstrated through an acceptable validation of sterility.

The use of VD_{max} can undermine the logarithmic and mathematical basis of Method 1, based on increased sub-doses. Bacteria are inactivated, in general, in a logarithmic nature. Just raising the sub-dose without regard to this does not demonstrate assurance that future lots will be sterile.

Acinetobacter radioresistens in low populations as well as *Moraxella* species have been previously demonstrated to be extremely resistant and survive low dose irradiation, but in a higher population it could fail the sub-dose, or the corresponding higher limit of the process sub dose dose. It is necessary to know how your bioburden fluctuates over time. While dose settings typically require only quarterly sub dose audits which is fine under normal circumstances, but when starting work on a new product or facility, perform bioburden testing more frequently than quarterly in order to know, and understand its variability, or fluctuation.

The basis of Method 1 is the bioburden average. If an average includes VD_{max} , it ignores spike variations and bacterial resistance. If one of three bioburden averages demonstrates greater than double the overall average of three lots, the higher bioburden average lot is used. Consequently, if one of our three lots had a significant VD_{max} that could drive the sub-dose of one lot up greater than the others, it would be used.

In our case, all three averages were similar. None of the VD_{max} were greater than a log higher than the average. Since micro-organisms are inactivated at a logarithmic rate, this could have been significant but wasn't.

Subsequent lots that were tested with a sub-dose had average bioburden populations 1/4 to 1/3 to 1/10 'significantly' lower than the overall average bioburdens that we used to reset/establish our sub-dose.

Consequently, the sub-dose for the test lots was already significantly higher. As a matter of fact, the spike in the first test lot that we sub-dosed, had one spike that was slightly higher than the overall average of the first three lots. Consequently, all micro-organisms should have been inactivated, but they weren't because resistant organisms were present.

The final sub-dose lot was even lower with an average bioburden nearly 30 times lower than the bioburden average used to set the sub-dose bioburden; and we still failed with seven growths, which reflects very high resistance organisms and some dormant spores.

Inactivation of micro-organisms occurs randomly by target hits. When we apply the sub-dose to 100 units, for example, it doesn't matter mathematically if one unit has more than another; it reduces the overall population, and that is what we have to evaluate and not bias the results with the use of VD_{max} . If VD_{max} is a concern, then other means of performing the sterility test should be considered instead, and the sterility test should not be used indiscriminately for setting a sub-dose. For the purpose of establishing a higher full dose,

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which would provide a safety factor beyond that which is truly validated, that would be acceptable as long as product safety and compatibility is not adversely affected.

If we move away from the use of average bioburden per item, then we move away from the model of Method I which ISO has adopted, and away from AAMI ISO TIR 13409 [7].

The paradoxical consideration may be that as certain individual VD_{max} move away from the average bioburden, the temptation is to use them. Using VD_{max} for establishing sub-dose can have a mathematical bias, and bias the results, and subsequent outcomes.

The history of experience of Method 1 has shown that this method has been very consistent in using its average bioburden per item for establishing sub-dose setting. Only 3% of the time, has Method 1 failed, and when it has failed, generally it demonstrated a bioburden population that had resistant micro-organism like *Acinetobacter*, which require resistance characterisation by Method 2.

When resistant micro-organisms to Method 1 are discovered the cause and source of the organism should be determined then they should be eliminated, reduced or controlled. Technical staff should not try to ignore it by applying VD_{max} so that resistant microbes are not recovered. The source of *Acinetobacter species* should be found, the best method to eliminate, reduce or controlling it should be determined, and then it should be eliminated. This is a sound science and a good quality approach.

ISO 11137 [5] Method 2 runs several sub-doses and looks for fractional positives (survivors). These fractional positives are used to calculate the final dose, for assurance of sterility. Variations of Method 2 can be used to evaluate ultra-clean bioburden as well as heavier bioburden levels. This is the most precise methodology for determining the radiation dose required.

Abbreviated validation for radiation sterilisation can be done microbiologically on a single batch basis and with a bioburden upper verification VD_{max} approach; however its limitation is material compatibility, so that much more effort and time must be taken during design control of a product, material or device. However, because of its amazing penetration, and single sterilisation process parameter of radiation dose, it can easily be validated microbiologically.

EO on the other hand is much more complex than radiation for microbiological inactivation, EO residuals and pyrogen control. It requires assessment of time, EO concentration, humidity, temperature, and vacuum/pressure. EO residuals and pyrogens (due to moisture from humidification/prehumidification) need to be assessed typically, during validation.

After more than 100 years since its discovery, radiation technology is now able to sterilise so many products, polymers, materials and configurations with irradiation without adversely or deleteriously affecting their final product quality, but it has required a lot of investigation, evaluation, qualification and validation.

A few healthcare products that have been commonly sterilised with radiation are:

- Bandages
- Baby nipples
- Body bags
- Bone cement
- Catheters
- Dressings
- Drug containers
- Gowns
- Joints
- IV Bags
- Needles
- Ophthalmic solution bottles
- Orthopaedic steel pins
- Hospital Packs
- Petri Dishes
- Plastic vials, bottles, components, pipette tips
- Protheses
- Orthopaedic Devices,
- Roller bottles
- Single use disposables (examples):
Angioplasty; Bag containers; Blood sets; Catheters; Check valves; Contact lenses; Containers; Cotton balls, gauze; Diabetes insulin assisting device; Dialysers; Drapes; Entero feeding tubes; Flexible containers; Filters; Forceps; Gloves; Gowns; Haemodialysis sets; IV sets; Manifold kits; Nasal gastric tubes; Nebulisers; Needles; Oxygenators; Oxygen masks; Plastic scissors; Plastic wires; Respirators and accessories; Scapels; Specimen containers; Sputum cups; Sponges; Stents; Surgical blades; Surgical trays, kits; Some sutures; Syringes; Swabs; Tissue culture flasks; Urinary bags; Vessel Dialators and Obturators; Waste Containers
- Woven, non-woven goods

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A dual level of probability of survivors has been accepted for 10^{-3} and 10^{-6} sterility assurance level (SAL) in the USA, depending upon a product's end use, although this remains a controversial issue in other parts of the world. However, radiation has been shown to sterilise many dry drug substances without damage. Regardless of statements in the literature, materials to be irradiated should be evaluated and validated for radiation compatibility. If much literature and information exists, steps toward prospective validation can be applied.

5.4 Gamma Radiation Facility, Equipment and Product Handling

Control of sterilisation is maintained between the two areas of the building (sterile and non-sterile). By use of a conveyor it can divide the two areas, with a permanent fence and by identifying the product as processed with the use of tags.

The building should be concrete. The cell walls should be approximately 185 cm or thicker. The building and cell should be designed and constructed by professionals.

A radiation facility must have permits and licences. Typically they should have:

- Radioactive materials licence
- Facility registration

The registration allows the facility to gamma irradiate medical devices, drug components, cosmetics, packaging materials and spices.

Other controls are, for example, the Drug Master File (regulatory file describing contents, handling, and processing of a drug).

Before the facility can have its ISO Registration, the facility must be audited by appropriate licensee and registration services. It must have EN ISO 13488 [14] (Medical Device Directive) and CEN 552 [15] approval for those wanting CE Marking approval.

A facility can be designed with at least the same technology and is operated minimally under the same quality policies and procedures as other radiation facilities.

Irradiation System: The irradiation system is a multi-pass/multi-shelf product overlap design. The product is exposed to a sealed cobalt 60, not caesium, source which emits gamma rays, where an automated conveyor system is used for transporting material through the gamma cell in carriers (totes) which hold the product.

The gamma sterilisation facility consists of several major components:

- Biological shield-cell descriptions
- Safety system
- The source system(s) – two main cells and sample cell
- The conveyor system and equipment
- Computerised documentation system (not yet paperless)

The irradiation area is heavily (biologically) shielded to absorb the gamma rays. The cell walls and ceiling consist of more than 180 cm thick high density concrete. The biological shield can be designed for use up to 5.8×10^{17} Bq of cobalt 60, as compared to previous 3.7×10^{17} Bq of cobalt 60 for a facility.

The cobalt 60 is double encapsulated in stainless steel rods. These rods are delivered to the facility in lead-shielded shipping casks from approved isotope supplies listed in the radioactive material licence. Caesium 137 has been used, but because of its water solubility, under water storage has created clean up problems. All casks are Department of Transport (DOT) approved by the supplier.

Loading casks into the cell area is accomplished by lifting them off a concrete shielding plug located in the roof with an auxilliary hoist. The cask with source material is manoeuvred into the cell on a cart.

Typically a crane is lowered through the roof and lowers the cask containing the source material into the pool and then to the bottom of a deep-water storage pool located in the floor of the cell. The pool consists of a thick steel liner and a reinforced concrete shield. The cobalt 60 or other sources are then unloaded under water from the cask and loaded into the source holders known as modules.

Typically source modules are loaded into source racks. These racks are raised and lowered by cables connected to winches located on the roof of the cell. Guide wires are attached to both ends of the racks to control their positions. The vertical position of the source racks is controlled by micro-switches built into the winches. The electrical winches have been designed to permit a controlled lowering of the racks into the pool in the event of power failure or other safety mechanisms.

During the operation of the facility, the source rack has been centred vertically to the carriers.

When access to the cell is required, the sources are lowered to the bottom of the pool where they are submerged under deionised and/or filtered water. Any failure in the system, or

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violation of the safety controls will cause the racks to be automatically lowered into the fully shielded position.

An example of a pool size is 185 cm wide, 700 cm long, and 765 cm deep. It typically consists of a 0.3 cm thick stainless steel liner within a 30 cm reinforced concrete shield that is surrounded by earth. The water in the pool is deionised and filtered, to protect the integrity of the sources, by continuously circulating the water to a water treatment area adjacent to the cell.

Typically a pool is equipped with an overflow and underflow sensing electrodes to prevent flooding of the cell or loss in the water shield.

All components of the sources and water treatment systems are constructed of stainless steel or PVC to minimise corrosion. The sources, source modules, source racks, carriers, totes, cables, pool line and other attachments are constructed of stainless steel.

A maze (non-linear and tortuous) entrance to each cell has been designed with four legs incorporating three right-angle turns to attenuate and stop scattered radiation.

There are controls for safety in the operator console located in the control room. The safety system contains both visual and audible alarms to prevent unauthorised access or other breach. A system can be designed with a relay logic and is line (mains) powered with a standby battery system for two hours in case of power failure.

Prior to leaving a cell, operators are required to perform a visual and audible check of the cell to ensure that there are no personnel located in the cell. Upon completion of this search, a key switch in the maze is activated. This begins a start-up sequence.

Another walk around is required, which activates the emergency wires in the proper sequence. After the wires are activated, a warning bell alarms. The warning bell constitutes a final warning, and also a delay timer is activated. This allows an operator several seconds to exit the maze and activate a second switch. This switch activates a safety system. The key is then placed in the console in the control room where it remains during normal operations.

Some facilities have a separate cell sample cell for dose verification experimental studies. This cell runs independent of the main cell. A sample size can comprise a space of approximately 65 m² compared to a larger main cell with a size of approximately 150 m². The product is loaded manually into aluminium trays. An overhead conveyor moves the product carriers through the gamma cell and back to the transfer site. A cycle timer controls motion that stages carriers at accumulation stops in the cell for a set time. No product transfers occur within the cell but, to achieve dose uniformity, the carriers may be rotated with the cell. Carrier rotations occur several feet from the source planes. An additional carrier system, for longer items, has been created. It processes product, and then sends it over to the processed area.

5.5 Conveyor System and Equipment

A typical conveyor system can consist of four separate conveyors: an input conveyor, a cell and maze conveyor, an output conveyor and a transfer conveyor. The product can be manually loaded into stainless steel totes. An automated system is preferred.

For efficiency, it is important to use all usable space. There should be a tote weight scale, but they are not always used. The tote is filled as per the load configuration, travels down a line roller portion of the conveyor and is then loaded onto suspended carriers from an overhead power and free conveyor, which makes multiple passes through the radiation cell both vertically and horizontally to the source racks. The multiple pass process provides greater control in delivering the desired dose and dose distribution.

The cell conveyor consists of an overhead power and moving system. The overhead conveyor transports the carriers through the gamma cell.

A suspended tote, loaded on the bottom tier of a three-tier system, can traverse the cell, transfers to the middle tier and traverses the cell, and transfers to the top tier and traverses the cell.

Typically, a tote may follow alternate paths, e.g., middle tier only, top tier and bottom tier. For example, for a 25 kGy dose, or a 17 kGy dose, the product may go through all three tiers.

A tote in closest proximity to the source (40 cm or less) can generate less heat, (e.g., from ambient to up to 12 °C) depending upon the source capacity. One facility for example could generate heat up to 43 °C, from a caesium pencilled source. Lastly, the tote is typically transferred off the carrier onto an output conveyor. This output conveyor takes only processed product.

An operator interface console typically is located in the control room that starts and stops the conveyors and source operation, including the system status indicators, and provides the controls for operation of the irradiation system.

5.6 Considerations of a Dosimetry System

The primary dosimeter system used in a contract facility may be, for example, the Far West Technology (FWT) 60 radiochromic dye thin dosimeter or red Perspex dyed chip. A secondary dosimeter system or secondary system is the polymethylmethacrylate (PMMA) dosimeter system. The primary and/or secondary dosimetry system depends upon which system is used.

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Dosimeters are calibrated at least annually or upon the use of a new lot. Dosimeter calibrations are performed with traceability to the National Institute of Standards and Technology (NIST) and in accordance with ISO ASTM standard 51275 [16] and ASTM E876-82 [17].

Calibrations require five or more dose points, selected for exposure of calibration dose, where at least four dose points have been chosen per decade of the dose range. Five or more dosimeters are submitted for each dose point selected. By determining the absorbency of the dosimeters irradiated by the calibrated source, a curve fit of parameters can be determined, through the regression analysis to be used in subsequent calculations of absorbed dose during routine production. Precision and overall uncertainty can be determined.

Dosimeters must be held and analysed under environmentally controlled conditions. During processing, dosimeters are placed in foiled pouches from the vendor to prevent exposure to UV light and then sealed in polyethylene pouches to prevent exposure to humidity. The areas in which the dosimeters are analysed are temperature and humidity (30-75%) controlled, and the lights are covered with UV protection. Dosimeters are analysed on a spectrophotometer that is calibrated every six months with tractability to the standards (e.g., NIST).

5.7 Dose Mapping and Product Qualification

Upon initial qualification of a product, a loading pattern is documented and a dose distribution map study is performed. The loading pattern describes the number, position, and the density of the product units within the irradiation tote. The dose mapping study involves placing dosimeters throughout the selected product load. The study is performed to identify the zones of minimum and maximum dose within the product load and to assess the reproducibility of the process.

The old or previous quantity and positioning of the dosimeters can be placed throughout a standard three-dimensional grid to maintain consistent dosimeter placement positions for each plane, and planes. Dose mapping methods are typically a spatial procedure. One spatial procedure is a diagonal cross measurement of radiation dose over a wide space. Any change in density requires another dose mapping study.

For example, low densities can range between 0.02 to 14 g/cm³. Medium densities range between 0.15 g/cm³ to 0.25 g/cm³, and high densities are considered greater than 0.25 g/cm³. It is preferable to buffer to 0.25 g/cm³ or greater density. This is because dose mapping will be required because the higher the density, the more radiation flow will occur.

If the source geometry, source pass conveyor or irradiation container, changes dose mapping will be required. Any removal or addition of isotope, requires further evaluation. If a significant change results, then a dose mapping study of all products may be required.

5.8 Routine Standard Dosimetry

Upon completion of the dose mapping study, the minimum and maximum dose locations are selected for routine processing. Dosimeters are placed at both the minimum and maximum dose locations in the first, middle and last irradiation container. Many providers do not always want to guarantee a less than 1 kGy for verification dose irradiation. Radiation range, for low dose, can be done in a separate cell at times. This typically provides a narrower dose range than guaranteed by a larger facility.

5.9 Processing Controls

Upon receipt of product from a production run, it is verified for count, condition and lot number. After these parameters are verified, the first and last pallet or product is assigned and tagged with an exclusive run number, tagged non-processed or equivalent, staged on the non-processed side of the warehouse, and the work order is generated and verified for accuracy. Tags could have Rad dots (labels that change with exposure to radiation). These activities are then documented on the incoming material control report.

After the work order is verified, the product is assigned a cycle time (shuffle dwell) and scheduled for processing. The product is then moved to the irradiator loading area where the identification tags are transferred to the irradiation first and last container and the product is loaded onto the irradiation system. The product is loaded by certified (trained) material handlers who again count the product, examine the condition of the product, verify the lot numbers and place the routine dosimeters. These loading activities are documented on, for example, the material handling control report.

As the product leaves the irradiation system, the conveyor carries the product to the unload station. The product is unloaded by certified material handlers who count the product, examine its condition, remove the dosimeters, tag the product 'gamma processed' or equivalent, and re-affix the run number tag. The unloading activities are then documented on the material handling control report. The product is staged on the processed side of the warehouse after completion.

After the product processing has been completed, the dosimeters are analysed by trained personnel, the absorbed dosages determined and a Certificate of Irradiation is generated.

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The Certificate of Irradiation includes the range of dose received, the product model number, lot number, run number, date processed and other information unique to the customer. All records with regard to the production run are reviewed and the Certificate of Irradiation approved by the quality assurance personnel or designee.

Upon approval of the Certificate of Irradiation, the product may be shipped per the customer's instructions. Prior to shipment the production run is again verified for count, condition and lot numbers. Shipping activities are documented and retained on a packing slip (bill of lading) or other outgoing material or product control report.

Improvements in radiation sterilisation can be achieved by means of additives and modifications in polymer chains [2, 3].

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 cobalt 60 with energy levels of 1.33 MeV and 1.17 MeV, molecular bonds are broken. The polymer can 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 that polymers with a benzene ring are generally very stable. Oxidation, caused by the presence of oxygen in the gamma-radiation process, can 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 can 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.

Electron beam and gamma radiation in sterilisation produces ionisation and excitation in polymer molecules. UV light does this to a lesser extent because of lack of penetration. Energy-rich created species can then undergo dissociation, abstraction and addition reactions in a sequence of reactions leading, ultimately, to chemical stability. This stabilisation process, which occurs during, immediately after, or even days or weeks after irradiation, often results in physical and chemical changes in the polymers as a result of the stabilising recombinations, crosslinking or chain scission that occurs. The resultant physical changes may include crosslinking, deterioration, discoloration, embrittlement, extractables, odour generation, stiffening, softening, chemical resistance, melt temperature, toxicological response and even destruction, e.g., Teflon polymer to powder.

Density, molecular weight, chain length, entanglement, polydispersity, branching, pendant functionality and chain termination all contribute to the polymer's structure/property relationship, and each of these characteristics may be modified with radiation. Knowledge and understanding about the direction and magnitude of the changes brought about to each of these characteristics, as a function of the level of radiation exposure (dose), is crucial to prediction of performance and use of irradiated plastics.

The radiation effect on the properties and performance may also differ with location within the part. As a polymer is irradiated, radicals are formed in the polymer proportional to the local dose. However, the associated stabilising chemical reactions that follow are proportional not only to the localised ionisation concentration but to the local concentration of reactants. Since the concentration of reactants differs by location (i.e., oxygen higher near exterior surfaces), the resultant radiochemical stabilising reactions are heterogeneous. In addition, orientation of molecular chains established during processing can have a profound effect on subsequent radiation damage. Molecular structures that fail during irradiation are always those molecules under the greatest combined stress from environment, load, solvent and residual moulding stress, i.e., normally at the surface.

Stabilising reactions can be grouped into four classes:

- Recombination – no change of properties
- Crosslinking – increase in strength and decrease in elongation
- Chain scission – loss of strength and elongation
- Combinations of all of the above

All of these reactions take place, to different degrees, in all irradiated polymers. The difference depends on the chemical composition and morphology of the polymer and its surrounding environment. Some of the most significant types of radiation-induced degradation are the embrittling chain scission reactions that result from interaction with oxygen or other oxidants, and also crosslinking which can be very beneficial. Free radicals tend to oxidise easily, especially when oxygen is readily available such as at the surface of the polymer. In some cases inert gas, i.e., nitrogen, argon, or a vacuum can be used to inhibit oxidation; antioxidants such as hindered amines are also useful in limiting oxidation. Fast dose rates from electron beam irradiation systems can minimise or limit oxidative degradation of polymers by limiting the time of radical exposure to oxygen. Reducing oxidative degradation through one or more of the above mechanisms is important to keep in mind for oxidative sensitive materials containing thin profiles, such as films, coatings and fibres.

Chain scissoring, as random rupturing of bonds which reduce the molecular weight, i.e., strength, of the polymer and crosslinking which results in the formation of large three-

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dimensional networks occur simultaneously, with one mechanism usually dominating, as a polymeric material is subjected to ionising radiation. If chain scission dominates, then very low molecular weight fragments, gas evolution and unsaturated bonds may appear. Conversely, if crosslinking dominates, the result is an initial increase in tensile strength and toughness, with a resultant decrease in elongation with increased dose.

The ratio of the resultant recombination, crosslinking, and chain scission is critical and will vary from polymer to polymer and to some degree from part to part based on the chemical composition, e.g., aliphatic, aromatic, morphology of the polymer (% crystallinity, molecular weight, density), the design of the part (thick *versus* thin sections), the total radiation dose absorbed, the rate at which the dose was deposited, and to some degree the post-irradiation storage environment (temperature/oxygen). The ratio can also be significantly affected by residual stress processed into the part and the environment during irradiation (especially the presence or absence of oxygen).

Another potentially negative result from the irradiation of polymers is discoloration (usually yellowing) from the development of specific chromophores during the stabilising radiochemistry reactions. Colour development, which occurs at widely differing doses in various polymers, may diminish or increase with storage time after irradiation. Often discoloration appears prior to any measurable loss in physical properties. For example, radiation will induce yellowing conjugated double bonds in PVC at a dose much lower than is necessary to cause any reduction in its physical properties.

Another undesirable effect in some polymers is odour production as a result of specific radio-stabilising chemistries. The most common polymers with post-irradiation odour are polyethylene, PVC (rancid oil odour from oxidised soybean and linseed oils in the plasticiser) and PU. If the reaction chemistries of the odours are understood, they can often be negated through the use of antioxidants, changing processing temperatures, using a polymer grade with a higher molecular weight. Odour reduction can also be improved through the use of gas permeable packaging (i.e., Tyvek, paper, filters), and post-irradiation, elevated temperature vacuum conditioning, or aeration.

A few plastics that may be adversely affected by sterilisation radiation doses, e.g., 25 kGy, can be sterilised at lower doses, e.g., 11 kGy to 24 kGy.

Some plastics sensitive to radiation are: unstabilised PP, acetals, Teflon, polyglycolic acid sutures, polymethylpentene and polyvinylidene fluoride.

Lesser effects are observed in PVC (e.g., discoloration and potential leachables), ABS, PE, polyamides and acrylics. Silicones can be crosslinked.

All plastics are affected by radiation. Some effects are favourable or negligible, while others are not.

This requires evaluation and qualification before accepting a material or polymer:

- Polyethylene is predominantly crosslinked, but acceptable to radiation. Slight odours may result, but can be reduced through modification of the formulation.
- Silicones can crosslink, but modifications can be made to minimise its effects.
- PP is both crosslinked and scissored. Embrittlement, breakage, and discoloration can occur. After about 24 months unstabilised polypropylene syringes can begin to shatter. However, radiation stable PP materials are available.
- Polymethylpentene has similar properties to PP.
- Polystyrene is very stable to radiation because of its benzene ring.
- ABS is much less resistant to radiation than polystyrene, but it can be suitable for a single dose of irradiation. It can have some discoloration (slight brownish hue).
- PVC can be made compatible to radiation, by squelching (neutralising) hydrochloric acid. However, prevention of discoloration and plasticiser leaching must be considered.
- Acetal or polyformaldehyde copolymers are sensitive to radiation and their chains are easily scissored, and the material often changed from solid to dust. Formaldehyde may be released.
- Polyamides are sensitive to crosslinking from radiation, but are suitable for a single dose of radiation.

Some general considerations to be made in selecting polymeric plastics:

- Aromatic polymers, e.g., benzene rings, are more stable than aliphatic chains.
- Look at the ratio of scissoring to 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 are unstable with irradiation.
- PVC, polymethyl pentene (PMP) and PP should be specially stabilised to improve radiation compatibility.
- High levels of antioxidants help radiation stability. In general, the antioxidant level may need to be increased if the product is to be radiation sterilised. Heat stabilisers tend to improve radiation resistance.

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- Within a given polymer class, the lower the density the greater the radiation stability.
- The elastic modulus is not greatly affected with one dose of sterilising 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 in much the same way to irradiation as the materials from which they are derived.
- Addition of colour tints can minimise the discoloration effects of irradiation.
- Modification of the polymer formula can reduce odours.
- Extra care must be taken with nucleated polymers – nucleation increases embrittlement.
- If co-polymerisation with ethylene is possible – try it.

Some examples of radiation-stabilised plastics are:

- Elastomers: silicones (peroxides and platinum cured), TPE (SEBS, TPO), natural rubber (Isoprene), EPDM, urethane, nitrile, butyl, styrene-butadiene.
- Fluoroplastics [other than PTFE and fluorinated ethylene propylene (FEP)] – polyvinylidene fluoride (PVDF), polychlorotrifluoroethylene (PCTFE), polyethylene tetrafluoroethylene (PETFE).
- ‘High-end’ engineering resins, polyetherketones (PEK), polyetherether ketones (PEEK), polyetherimide, poly phenyl sulfone,
- Polyamides, especially aromatics, 12,11, 6/12 and 6/10. Polyamide 6 (Nylon) may be the least radiation resistant.
- Polyethylene, LDPE < LLDPE, HDPE, UHMWPE.
- Polyesters (PES) and PETG.
- Polycarbonate (PC) and alloys that will change colour, unless colour stabilised.
- Polysulfone (PSF) may discolour from a yellow to a brownish hue.
- PVC flexible and semi-rigid, colour, plasticiser, and HCl acid corrected.

- Polyurethanes (PU) – eight chemical varieties – some initial discoloration.
- PP and PP copolymers (PPCO), radiation stabilised.
- Polymethylpentene (PMP), radiation stabilised.
- Polystyrene and copolymers, ABS, polystyrene (PS), styrene acrylonitrile (SAN) (ABS can slightly discolour).
- Polyacrylics [polyamide (PA), polymethacrylate (PMA), polyacrylonitrile (PAN)].
- Thermosets – epoxies, phenolics, polyimides, PU, PES.

Electronic circuit boards typically, are not always compatible, but increasing numbers are becoming compatible with irradiation with a single low dose. Premature ageing of plastics may occur due to the oxidative effects of irradiation; consequently it is always prudent to evaluate accelerated ageing of plastics to ensure that this is not a problem under real life conditions (see AAMI TIR15) [18].

Teflon despite its high heat resistance is degraded by radiation, and in general is not acceptable, although some thin films/coating and certain types of Teflons have been demonstrated to be radiation compatible to low doses.

PC is generally considered to accept one dose of radiation.

Acrylic polymers are sensitive to radiation. The effect of scissoring of the ester chain is the main effect of radiation. Polymethylmethacrylate (PMMA) has been used for dosimeters, which means radiation of this material for contact lenses is convenient. However, radiation compatible acrylics are available.

5.10 Plastic Design Considerations During Validation of Polymerised Materials for Irradiation

Validation implies that limits are established on process parameters such that predictable success will be obtained if the process remains within allowable bounds and predictable failure will occur if the process exceeds those established bounds – all of this is predicted by looking at the worst case combined effects of all environmental influences (e.g., temperature, ageing) on the component or device.

In engineering and scientific terms, the safety factor is an overall way of allowing for process variables. If all variables could be eliminated, a safety factor of one would be feasible. Most products or devices would not survive without allowing a safety factor.

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Typical safety factors are 1.5 to 3.0 depending on how well defined the engineering evaluation is made.

Typical medical device components needing a safety factor that anticipates loss of physical properties after radiation sterilisation are for example:

- Sonic welds
- IV needles may break off at the tip or thrust wing
- Interference fitted sterility protector splits in creep failure and falls off
- Luer fails in hoop stress
- Slide clamp fails in creep after 48 - 72 hours hanging
- Insert moulded interference stress cracks part

The safety factor requires re-calculation after radiation sterilisation where the polymer may have lost up to 25% elongation as determined by empirical testing on actual parts.

Expedient functional testing on the moulding floor to allow for just-in-time (JIT) delivery concept:

- Crush test related to interference stretching in luers and cylindrical parts.
- Bend-fold, twist, stretch, flex all with hand tools to relate to allowable elongation or customer misuse.
- Test both in flow axis and cross flow axis using hand tools to relate to allowable elongation or customer misuse.
- Test both flow axis and cross flow axis.
- Allow for later molecular densification.
- Allow for later radiation effects.
- Allow for later ageing and creep effects.
- Some develop a correlation factor to calibrate plier crush equivalent to '001' stretch in a luer, which becomes a new standard test.
- Crush test for satisfactory drying of materials.
- Detect early warning of vent plugging, improper drying, or moulding parameter drift out of limits

One full round of cavities is tested and then goes into a polythene bag placed on top of production box to assure the JIT receiver that once on each shift all cavities are producing good parts.

5.11 Processing Considerations for Medical Plastics to be Sterilised by Ionising Radiation

All plastics are affected in some degree by ionising radiation. Some crosslink to become stronger, some undergo scission to become weaker with reduced molecular weight. Most polymers do a combination of both these processes, creating a unique response for each family of polymers.

Most polymers are very tolerant of the mild dosages used to sterilise (200-400 Gy). This dose is nothing compared to what plastic insulation is exposed to on a nuclear reactor, for example, and exposure of polymers to high mail irradiation, which requires post irradiation aeration to eliminate or minimise numerous transient off gassing monomers, radicals, small molecules, and so on.

Either machine-made electron beams or gamma radiation from cobalt isotopes may be used. The beams provide a rapid sterilisation cycle in seconds whereas the cobalt 60 provides a slower dose over a period of hours, penetrating deeply into the load, allowing for more oxidation to occur.

Colour change is common in PVC, PC, PS, ABS and acrylics. The colour change occurs first at lower doses before any physical change can be detected.

The first and most significant change in mechanical properties is the loss of elongation to break. This is due to molecular shortening by scission and is one reason to start with high molecular weight materials.

A polymer under stress is attacked more by radiation than unstressed material is. Scissioning and oxidation effects are concentrated in the stressed zones. Therefore, test articles must have reproducible moulded-in stress and mechanically applied stress if they are to be used successfully for validation.

Polymers with great orientation in the flow axis will be strong in the flow axis, and weak in the cross-axis. Cross-axis molecular ties will be attacked by radiation more than the molecules in the flow axis. Therefore, one must test moulded parts in the cross-axis so that customer failure reports can be duplicated. This also implies that non-stressed compression moulded tensile bars or plaques will not be satisfactorily representative of actual stresses in moulded parts. To reproduce customer complaints, all validation work

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must be confirmed by testing actual parts in their weakest axis exactly as the customer complaint file indicates.

Polymers respond to all their combined environmental exposure:

- Shrinkage stress
- Moulded in stress
- Applied stress

5.12 Test Parts Used for Validation Solvent/Chemical Attack Must be Typical in all Respects of Radiation on these Environmental Exposures

- Temperature
- Regrind

Radiation does not produce toxic results. However, scissoring does invite greater extractables, pH shift, and increased conductivity due to free ions.

5.13 Control of Polymer Processing for Irradiation

When irradiating polymers, these process parameters need to be controlled:

- Mould temperature
- Melt temperature
- Cycle time
- Residence time at heat
- Regrind
- Drying
- Hydraulic pressure (injection)
- Cycle timing – automatic preferred over manual
- Vent closing
- Process monitoring devices – cavity pressure
- Water bath set-up for extrusion
- Temperature of melt and bath
- Air gap

- Haul off speed –chipping – bath residence time
- Vibration
- Eccentric cooling
- Eccentric tubing
- Grooved (leaking) tubing

All components should be made in a continuous uninterrupted run under identical conditions within established validation limits from a single lot of material with the machine running fully automatic.

Engineering design establishes component requirements of physical strength, elongation, colour, solvent bonding, sterilisation methods, cleaning, leak and pressure testing interference fits, fatigue, creep, vibration, sonic weld, radiofrequency (RF) weld, regrind use, polymer selection, molecular weight choice, alternate materials, make-or-buy component decision, use of filler and blowing agents, ageing effect.

Make prototypes and test functionality: Age device both in real time and in accelerated time and then test again.

Do a customer field test: look for failures in the field that were not seen in prototype qualification. In most cases the customer complaint file must be treated as a design requirement if you want to succeed.

Some customer complaint examples:

1. Bent thrust wings on IV spikes – 10 degree bend, 225 N break force
2. Excessive electrostatic particulates
3. Slide clamps yield over 72 hours, resulting in failure to shut off
4. Leaky air vents in microbial retentive filters
5. Leaky tubing engagements
6. Discoloration of plastics
7. Odours.

Some sources of variability in evaluating physical performance of radiation sterilised medical polymers (these ought to be considered when evaluating empirical data from different sources) are:

- Material regrind— affects molecular weight, antioxidants and hydrolysis
- Test errors – not confirmed by second or third test

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- Molecular weight – molecular weight distribution typically by gel permeation chromatography (GPC)
- Heat history
- Antioxidant change or depletion
- Pigment and pigment carrier resin
- Processing aids and lubricants
- Stabilisers for UV and radiation
- Crosslink/scission ratio
- Residual stress
- Molecular orientation – strong axis, weak cross-axis
- Moisture content
- When moulded – drying
- When irradiated –residual water and hydrophilic polymers may be hydrolysed.
- Time to sterilise
- Oxygen diffusion, dose rate, surface-core effects
- Immediate thermal conditioning, post irradiation
- Quenching, annealing, leads to geometric reduction of peroxy radicals with time
- Annealing
- Mould temperature*
- Mould filling rate*
- Pressure
- Venting
- Gating
- Runner system
- Compounding variables (PVC especially)
- Blending intensity
- Distribution of trace ingredients
- Thickness of the parts, tubing or film as it may affect surface-to-core effects and clearance time for free radicals, internal gases, and moisture vapour

- Stiffness of the polymer as it may affect recombination of free radicals due to rigidity, crystallinity or cage effect

*These two parameters, mould temperature and mould-filling rate, can affect polymer physical properties (elongation, impact, and tensile strength) more than irradiation will affect physical properties. It is therefore important to monitor the control samples carefully, even noting cavity number as that often affects performance. Warm moulds and easy filling rates produce ductile parts. Brittle parts are produced in cold moulds with tortuous filling and venting paths.

5.14 Improvements in Radiation Sterilisation Can be Achieved by Minimising Radiation Dose and Parameters to Materials, Packaging and/or Product

This step establishes the minimum permissible dose necessary to provide the required assurance of material compatibility to radiation.

This requirement is dependent upon the minimum radiation dose to inactivate bioburden and ensure that a SAL of 10^{-6} is achieved, and that the maximum dose that corresponds to the minimum dose in a carrier or treated product.

Product, packaging and/or materials should be irradiated to at least the highest radiation dose to be delivered routinely and tested to the highest useful life of the product.

With a delivery of a maximum dose of for example 40 kGy, the product and packaging will potentially be irradiated at a high dose routine dose specification of 27 kGy. The highest dose to be considered to test product, package, and/or material is 30 kGy.

Another parameter to be considered is a zero time testing. The product is subjected to 30 kGy and heat ageing at 60 °C for 12 hours to simulate worse case truck/transportation testing and real time testing for the life use of the product. This time is established on useful life of product and label claims that may be imposed, e.g., expiry dating. For example, expiry dating for Italy and France is assumed to be for five years. For field trials, expiration 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.

The effects of material, component, packaging and/or product failure rates is dependent upon temperature and stresses which are often assumed to follow the Arrhenius Law, although not always.

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In establishing accelerated ageing conditions, apply a worse case Q_{10} of 1.8 and a room temperature of 25 °C to the Arrhenius Law.

Common temperatures to be used to evaluate effects from temperature and determination of acceleration ageing are 50 °C and 60 °C, but others can be considered or used if applicable. It is important to keep in mind that a 60 °C or higher temperature may have other effects on the product other than merely radiation. Therefore a lower temperature such as 50 °C should also be used.

A stability schedule should be established to periodically test the product between zero time testing and the established three-five year life period

For example an accelerated ageing at 60 °C at 6.6 weeks is estimated to be equivalent to one year in real time. For 50 °C, at 12 weeks, a real room temperature goes up to five years. Knowing the accelerated aging periods, a schedule of real times selected for evaluations may be constructed.

5.15 Healthcare Product Biocompatibility and Sterilisation

5.15.1 A Medical Device Must Be Adequately Designed to be Safe for Its Intended End Use, After Sterilisation

Many polymers used in medical devices show varying degrees of degradation and change with radiation that may create toxic by-products or leachables. Technological advances have done much to improve a material's resistance to radiation with the use of stabilisers, additives, antioxidants, etc. These stabilisers, additives or antioxidants to improve a material's radiation compatibility may in turn be toxic. The range of biological and physico-chemical hazards is wide (e.g., silicone, latex), and medical procedures and drugs are becoming increasingly sophisticated. The evaluation and subsequent comparison of biocompatibility of materials require test methods that are meaningful and appropriate.

The evaluation of biological responses (biocompatibility) of materials is subject to limitations, inconsistencies and opportunities for misapplication and/or misinterpretation. The challenge of biocompatibility and material safety is to create and use knowledge to reduce the amount of unknowns and to help make the best possible decisions and predictions. This is a simplified approach on how to address and assess the materials and tests for device(s), for their biological and physico-chemical responses as part of the overall evaluation and development of irradiated materials and devices. Like other normative work on this subject, this guideline does not fully address the determination of the effects of devices and materials on tissues and end use but advances what approaches to take, when to test, and how to start evaluating the biocompatibility and material safety of materials of medical devices used and irradiated by device manufacturers.

5.15.2 Biocompatibility and Material Standards

Biocompatibility standards and issues are still evolving. There are the traditional ASTM, USP ISO and AAMI biocompatibility and material standards that can be applied to drug containers for biological and sterilisation safety and to defence contracts as well as numerous international governments.

Since 1987, the US Food and Drug Administration, United Kingdom and Canada have applied the Tripartite Biocompatibility Guidance for Medical Devices to set device safety standards. This has had an impact on regulatory submissions (e.g., 510 Ks). Tripartite in essence supersedes the USP in the broader view and in support of product registrations. And in 1995 the FDA adopted ISO 10993-1 [19], with some modified considerations, under its new blue memorandum #G95-1 [20] which is an international biocompatible standard providing principles governing the biological evaluation of medical devices (not cosmetics) and materials, definition of categories and greater selection of tests.

ISO guidance on standard requirements is similar to those of the FDA but includes more defined parts. The ISO standard will have an impact not only on international submissions, but also on domestic submissions, with the FDA adoption of it. The FDA recognises most of the ISO 10993 series for harmonisation, but not all. The Agency suggests additional testing for some healthcare products.

5.15.3 Definitions

Medical device: Any instrument, apparatus, appliance, material or other article, including software, whether used alone or in combination, intended by the manufacturer to be used for human beings solely or principally for the purpose of:

- diagnosis, prevention, monitoring, treatment or alleviation of disease, injury or handicap;
- investigation, replacement or modification of the anatomy or of a physiological process;
- control of conception;

and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means.

NOTES

1. Devices are different from drugs, and their biological evaluation requires a different approach. So a combination of device and drug will require added attention.

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2. Use of the term ‘medical device’ includes dental devices.

Material: Any synthetic or natural polymer, metal, alloy, ceramic or other nonviable substance, including tissue rendered nonviable, used as a device or any part thereof.

Final product: Medical device in its ‘as used’ state.

5.15.4 Categorisation of Medical Devices

The testing of any device that does not fall into one of the following categories should follow the general principles contained in this part of ISO 10993 [20]. Certain devices may fall into more than one category, in which case testing appropriate to each category should be considered (see **Table 1 Initial Evaluation Tests for Considerations** and **Table 2 Supplementary Evaluation Tests for Considerations** from FDA general program Memorandum #G95-1).

5.15.5 Categorisation by Nature of Contact

Non-contact devices: These are devices that do not contact the patient’s body directly or indirectly and are not included in ISO 10993.

Surface-contacting devices: These include devices in contact with the following:

- a) Skin: devices that contact intact skin surfaces only; examples include electrodes, external prostheses, fixation tapes, compression bandages and monitors of various types.
- b) Mucosal membranes: devices communicating with intact mucosal membranes; examples include contact lenses, urinary catheters, intravaginal and intrainstestinal devices (stomach tubes, sigmoidoscopes, colonoscopes, gastroscopes), endotracheal tubes, bronchoscopes, dental prostheses, orthodontic devices and IUD.
- c) Breached or compromised surfaces: devices that contact breached or otherwise compromised body surfaces; examples include ulcer, burn, and granulation tissue dressings or healing devices and occlusive patches.

External communicating devices: These include devices communicating with the following:

- a) Blood path, indirect: devices that contact the blood path at one point and serve as a conduit for entry into the vascular system. Examples include solution administration sets, extension sets, transfer sets and blood administration sets, catheters, and so forth.

- b) Tissue/bone/dentin communicating: devices and materials communicating with tissue, bone and pulp/dentin system. Examples include laparoscopes, arthroscopes, draining systems, dental cements, dental filling materials and skin staples.
- c) Circulating blood: devices that contact circulating blood. Examples include intravascular catheters, temporary pacemaker electrodes, oxygenators, extracorporeal oxygenator tubing and accessories, dialysers, dialysis tubing and accessories, haemoadsorbents and immunoadsorbents.

Implant devices: These include devices in contact with the following:

- a) Tissue/bone: devices principally contacting bone. Examples include orthopaedic pins, plates, replacement joints, bone prostheses, cements and intraosseous devices. Devices principally contacting tissue and tissue fluid; examples include pacemakers, drug supply devices, neuromuscular sensors and simulators, replacement tendons, breast implants, artificial larynxes, subperiosteal implants and ligation clips.
- b) Blood: devices principally contacting blood. Examples include pacemaker electrodes; artificial arteriovenous fistulae, heart valves, vascular grafts, internal drug delivery catheters and ventricular assist devices.

5.15.6 Categorisation by Duration of Contact

Contact duration may be categorised as follows:

- a) Limited exposure (A): devices whose single or multiple use or contact is likely to be up to 24 hours.
- b) Prolonged exposure (B): devices whose single, multiple or long-term use or contact is likely to exceed 24 hours but not 30 days.
- c) Permanent contact (C): devices whose single, multiple or long-term use or contact exceeds 30 days.

If a material or device can be placed in more than one duration category, the most rigorous testing requirements should apply. With multiple exposures, the decision into which category a device is placed should take into account the potential cumulative effect, bearing in mind the period of time over which these exposures occur.

5.15.7 Biological Tests - Category Descriptions

Sensitisation Assay: Determines the potential for sensitisation of a test material and/or the extracts of a material using an animal and/or human. Sensitisation is an allergic or hypersensitive response produced by repeated exposure to a material, usually dermal exposure. For products that will contact only unbroken skin, the Buehler Patch Test is usually recommended. For most other devices, the Magnusson-Kligman Maximisation Test is preferred. Japanese Sensitisation is a modification of this latter test. The Japanese require a preliminary screening extraction evaluation of a polymeric test material using four solvents (acetone, methanol, hexane, and cyclohexane:2 propanol). The optimal extract system will be used for subsequent testing.

Irritation Tests: Evaluates the irritation and sensitisation potential of test materials and their extracts, using appropriate site or implant tissue such as skin and mucous membrane in an animal model and/or human. The intracutaneous test evaluates the local dermal irritant effects of leachables extracted from the test article.

Cytotoxicity: With the use of cell culture techniques, this test determines the lysis of cells (cell death), the inhibition of cell growth, and other toxic effects on cells caused by test materials and/or extracts from the materials. The Japanese have a modification of the ISO Cytotoxicity Standard, '*Japanese Guidelines for Basic Biological Tests for Medical Devices and Materials: Part I Cytotoxicity Test (Colony Assay)*'.

This assay evaluates the cytotoxicity of leaching substances from a device by measuring the effect on colony formation in the Chinese Hamster Lung Cell (V79) culture model. Cytotoxicity is expressed as the percentage extract concentration that inhibits colony formation to 50% of the control values.

Acute Systemic Toxicity: Determines the harmful effects of either single or multiple exposures to test materials and/or extracts, in an animal model, during a period of less than 24 hours.

However, the Japanese acute systemic toxicity testing may require up to 72 hours of observation and subsequent general necropsy of the test animal.

Haemocompatibility: Evaluates any effects of blood-contacting materials on haemolysis, thrombosis, plasma proteins, enzymes, and the elements formed using an animal model (*in vitro* models are available for some procedures).

Pyrogenicity - Material Mediated: Evaluates the material mediated pyrogenicity of test materials and/or extracts. Pyrogenicity is the ability of a material to cause a fever when introduced into the blood. Pyrogenic tests are done in rabbits or *in vitro* using the LAL test. The LAL procedure must be validated for each device or material. However, no *in vitro*

alternative test (e.g., LAL) exists for detecting material mediated pyrogens. Consequently rabbits must be used for the material mediated testing.

Haemolysis: Determines the degree of red blood cell lysis and the separation of haemoglobin caused by test materials and/or extracts from the materials *in vitro*.

5.15.8 Implantation Tests

Used to evaluate the local toxic effects on living tissue, at both the gross level and the microscopic level, of a sample of material that is surgically implanted into an appropriate animal implant site or tissue, e.g., muscle, bone, for 7-90 days.

Mutagenicity (Genotoxicity): The application of mammalian or non-mammalian cell culture techniques for the determination of gene mutations, changes in chromosome structure and number, and other DNA or gene toxicities caused by test materials and/or extracts from materials. Most materials that are mutagenic are also carcinogens.

Sub-Chronic Toxicity: The determination of harmful effects from multiple exposures to test materials and/or extracts during a period of one day to less than 10% of the total life of the test animal, e.g., up to 90 days in rats.

Chronic Toxicity: The determination of harmful effects from multiple exposures to test materials and/or extracts during a period of 10% to the total life of the test animal, e.g., over 90 days in rats.

Carcinogenesis Bioassay: The determination of the tumorigenic potential of test materials and/or extracts from either a single or multiple exposures, over a period of the total life, e.g., two years for rats, 18 months for mice or seven years for dogs.

Pharmacokinetics: Determination of the metabolic processes of absorption, distribution, biotransformation, and elimination of toxic leachables and degradation products or test materials and/or extracts.

Reproductive and Developmental Toxicity: The evaluation of the potential effects of test materials and/or extracts on fertility, reproductive function, and prenatal and early postnatal development.

USP Biological Tests for Classification of Plastics – Tests involve systemic injection, intracutaneous toxicity and intramuscular implant as described earlier. Tests required for Classes I - VI are given in **Table 5.4**.

Table 5.4 Classification of Plastics								
Class						Extract	Animal	Dose
I	II	III	IV	V	VI			
X	X	X	X	X	X	SC	Mouse	50 ml/kg-IV
X	X	X	X	X	X	SC	Rabbit	0.2 ml/Rabbit IC
	X	X	X	X	X	A:S (1:20)	Mouse	50 ml/kg-IV
	X	X	X	X	X	SC	Rabbit	0.2 ml/Rabbit-IC
		X		X	X	PEG-400	Mouse	10 Gg/kg-IP
				X	X	PEG-400	Rabbit	0.2 ml/Rabbit-IC
		X	X	X	X	CSO	Mouse	50 ml/kg-IP
			X	X	X	CSO	Rabbit	0.2 ml/Rabbit-IC
			X		X	Impl.	Rabbit	4 Strips/Rabbit
SC = Sodium Chloride CSO = Cottonseed Oil A:S = Alcohol: Sodium Chloride PEG = Polyethylene Glycol 400						Rabbit: USP Intracutaneous Test Rabbit: USP Muscle Implantation Test Mouse: USP Systemic Injection Test		

5.16 Purpose and Meaning of Biocompatibility Testing of Medical Devices and Materials

A medical device must be adequately designed to be safe for its intended end use and intended host (patient). Simply put, biocompatibility is the ability of a device to get along with the host (patient). The device should not inflict harm upon its host and that host will not inflict harm upon the function of the device. The device should not release any harmful substances to the patient that could lead to adverse effects.

The range of biological hazards is wide. The tissue interaction of material cannot be considered in isolation from the overall device design. The best material for tissue interaction may result in a less functional device, tissue interaction being only one characteristic of a material.

When designing a biocompatibility test outline, an aware and knowledgeable professional will often have to consult other applicable and available publications, information and sources (standards and International Pharmacopoeia, Medline, Toxline, MSDS, etc). Where the material is intended to interact with tissue in order for the device to perform its function, evaluation of combined reactions takes on dimensions not generally addressed in standards and guidelines to date.

The medical device professional needs to ensure that the device or material will not inflict harm upon its host and that the host will not inflict harm upon the device (which might then in turn cause the device to negatively affect the host).

An assessment of biocompatibility draws on tools used in the field of toxicology, the study of poisons, and in material characterisation.

5.17 Additional Material Biocompatibility Considerations

There are many degrees of biocompatibility responses. Many toxic substances taken in small doses may actually be stimulatory, but at higher doses they become toxic. Consequently, materials may have a dose-response relationship; however, many classical dose-response toxicological tests were developed for a pure chemical agent or drug, and may not be fully applicable to biocompatibility testing of devices. Biological responses of medical devices are generally more complex, because the materials are often not pure and not reflective of a dose-response relationship of a drug, for example.

Biocompatibility has been described as a chemical interaction between the material and the tissue, and the biological response to medical devices is an unusual test subject in toxicity testing.

Often a biomaterial is a complex entity (formulation), and the material's toxicity is mediated by both physical and chemical properties. Toxicity sometimes comes from leachable components, and the chemical composition of a material is often not fully known. Toxicological information on the material and its chemical composition is seldom available, and the possible interactions among the components in any given biological test system are seldom known.

Biocompatibility cannot generally be defined by a single test. It is highly unlikely that a single parameter will be able to account for the total biocompatibility. Therefore it is frequently necessary to test multiple biocompatibility parameters.

It is important to design a test with enough samples to draw legitimate conclusions. For example, the ISO biocompatibility requires three samples, not just one.

Suitable positive and negative controls should be used to produce a standard response index for repeated tests. ISO doesn't require controls for the intracutaneous irritation test, but the variability of the test requires that they be applied.

The use of an exaggerated challenge, such as using higher dose ranges and longer contact duration or multiple insults that are many factors more severe than the actual use condition, is important in differentiating what might be subtle or acausal responses in less rigorous tests.

Diversity of the materials used, types of medical devices, intended use, body contact, exposure and potential harm present an enormous challenge to develop well-defined biocompatibility and material safety testing programs.

Experience gained in one application area is not necessarily transferable to another application. For example, two materials having the same chemical composition but different physical characteristics may not induce the same biological responses. Also, toxicity may come from leachable components of the material due to differences in formulation and manufacturing procedures.

The same applies to different biocompatibility tests or sometimes slightly different responses.

Selection and application of the biological tests to be used is important.

5.18 An Abbreviated Discussion of Material Biocompatibility Tests

A matrix of the newly FDA adopted ISO 10993-1 [19] Biocompatibility Tests for Consideration with some additions is shown in Tables 1 and 2 by its Centre for Devices and Radiological Health (CDRH) (Blue Memorandum ~G95-1) (See Section- 2.4.5.2 *Matrix*).

Tests can be classified as screening, advanced, final, or to other terms. The matrix consists of different device categories and contact durations. These are sub-divided into body contact that includes the site of contact between the device and the body, the contact duration and the associated tests to be considered or supplemented. ISO defines in detail the type of tests to be considered and their criteria.

The guideline does not state which tests must be performed for compliance; however, specified tests cannot be regarded as irrelevant. But the tests listed will be the FDA's default requirement. Therefore, the relevance for all tests must be considered, and a rationale or justification for not performing it must be developed.

If published biocompatibility testing for a material on a device exists, the original biocompatibility test may be precluded. Minimal testing may be applied to verify that the existing biocompatibility testing is valid. For example, change in manufacturing: sterilisation may be evaluated with minimum testing to show that the change in biocompatibility doesn't rationally exist for the material already tested.

A typical example of a screen test is a tissue culture test. Tissue culture (cytotoxicity) is a method for toxic screening. It is very different from animal testing. It is a model, and it is more sensitive than an animal test because it is isolated to just a few specific cells. It does not include the aspects of healing or long-term effects that animal testing can monitor. Cytotoxicity often responds to chemical insult(s).

An even more sensitive toxicity test than cytotoxicity is Microtox, which uses bioluminescent bacteria to measure toxicity built up in an aqueous environment, but there are some known toxins it cannot detect. Another test frequently referred to, as a screen test is the *in vitro* haemolysis test. The haemolysis test can be seen as a type of haemocompatibility test, as a means of detecting haemoglobin from broken red blood cells.

Advanced tests are often *in vivo* tests (inside animals) or more complex *in vitro* (outside animal) tests. The acute systemic toxicity test is an example of animal test.

This animal test considers the toxicity of extractables from materials on the systems of animals. Testing of systemic toxicity in animals most commonly affects the central nervous system, but can also relate to the cardiovascular and other systems or the entire body. Macroscopic findings may include loss of weight and even death. Other common animal tests are intracutaneous reactivity, irritation and implant. Together, the systemic toxicity, intracutaneous reactivity and implant have been used in the past as USP Plastic Bio tests, Classes I-VI - Ref. USP/NF Section (88): *Biological Reactivity Tests, In Vivo*.

In vitro mutagenicity testing can generally be used as a precursor to carcinogenicity. Mutagenicity affects the germ cells, but can be screened in short tests by bacteria (AMES test). Teratogenicity is characterised by deformities in offspring. This test involves multiple generations of animals.

Biological reactions that are adverse for a material in one application may not be adverse for the use of the material in a different application. Biological testing relies mostly upon animal models and some *in vitro* testing, e.g., cytotoxicity. Material cannot, therefore, be conclusively shown in all cases to have the same tissue reactions to humans, if for example only cytotoxicity testing were applied. In addition differences between humans suggest that some patients may have adverse reactions even to well established materials and some animal species are consequently used that are more sensitive than others to certain substances, so as to cover human response diversity. For example, the guinea pig is more sensitive to allergens, and is therefore an animal of choice for sensitisation tests. Age can also influence toxicity. Young animals poorly metabolise some toxicants, resulting in marked susceptibility. Potential effects of a toxicant may not manifest themselves in one sex but rather in the other. Consequently, it is important to consider the test model being used.

5.19 Assessing Material Risks by Other Means

The hazard presented by a substance with inherent toxic potential can only be manifested when fully comprehended. Therefore, risk that is actual or potential harm is a function of toxic hazard and exposure. The safety of any leachables contained in the device or on the surface can be evaluated by determining the total amount of potential harmful substance,

estimating the amount reaching the patient's tissues, assessing the risk of exposure, and performing the risk *versus* benefit analysis. When the potential harm from the use of biomaterial is identified from the biocompatibility tests, this potential may be compared against the availability of an alternate material and/or test(s) for assessment of its safety and effectiveness.

5.20 Some Introductory/Design Considerations

Various materials may be evaluated for biocompatibility in medical devices but plastic polymers are only highlighted in the Tripartite and ISO Guidelines, ISO 10993-1.

Other material type considerations are:

- Rubber/elastomers
- Ceramics
- Carbons
- Metals and alloys
- Textiles
- Wood
- Composites
- Solvents
- Cleaners, Wipes
- Lubricants, Slipping Agents
- Dyes/inks/colorants

5.20.1 When to Consider Testing

A variety of considerations must be made to determine when to test:

- New material, component, product
- Change of a significant vendor
- Product development – material selection for new product
- Final product check prior to commercialisation
- Replace material in commercial product

- An intended change of the end use of material, component or device
- Change in manufacturing process(es), i.e., sterilisation
- Change in manufacturing site
- Change in mould or mould process, or site
- Change in resin supplier
- Change in physical configuration
- Surveillance
- Non-conforming condition or situation
- Qualification lot release testing
- Scheduled monitoring of biological safety
- Expiration/stability testing
- Two or more materials juxtaposed to another material may effect the properties of the others
- Vendor combines two or more materials and sells them as a unit
- Some materials may be unstable alone
- A material by itself does not fairly represent the final device
- Contaminants are being introduced during the manufacturing process
- It is impractical to test material alone
- Mixture of more than one chemical entity, e.g., formulation or a coloured plastic
- Composite/laminate

5.20.2 When Not To Perform Full Biocompatibility Testing

Considerations for not performing full biocompatibility testing are:

- Material is outside a category or classification of intended use, e.g., surface device (non contact), non contact to fluid path device
- When there is sufficient documentation that the material(s) are identical to one/or several predicate (proven) device(s)
- When the material(s) processing used are identical or worse case to one or several predicate device(s)

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- The risk of the material(s)' relationship to the device is low
- The material/device is well recognised with a long history of use
- The manufacturing doesn't introduce any toxic contamination to be considered in the cognizant professional's eyes
- That there is information in file, with statements of the material's identity identical to the predicate device
- That there is information in file, with statements of the material's processing techniques identical to the predicate device

5.20.3 Biocompatible Consideration and Other Points

- Toxicological character of material(s)
- Final product, manufacture, contaminants, residues, leachables, degradation products/ by products
- Human exposure to the device and nature, degree, frequency, duration of exposure to the body
- All potential biological hazards
- Follow good laboratory procedures (GLP), documentation review test, i.e., testing variables; species differences
- Changes to materials, chemistry, formulation, processing packaging, configuration, sterilisation, storage, intended use, or new adverse reactions
- Accuracy and predictive values of the test
- Controls and reference materials
- Proper interpretation

5.20.3.1 Why Consider All Available Material Information and Data

What is the business objective for the material/device?

- To be first in marketplace?
- To improve on a competitor's product?
- To change and/or improve one's own product?

- To introduce a ‘me-too’ product
- To introduce a less expensive product?
- To expand or focus the product line?
- What the directions for use are?
- What the indications/contraindications are?
- What the labels are?
- What the target market is?
- What will be claimed, what are the featured functions, benefits, and outcomes?
- What the major competitive products or procedures are?

5.20.3.2 Some Mechanisms of Material Safety Adulteration or Errors

- Residual monomers
- Residual solvents/cleaners
- By products from irradiation
- Sterilisation residuals
- Mould release agents
- Coatings
- Inadvertent contaminants (e.g., pesticides, pyrogens)
- Bacterial endotoxins, material mediated pyrogens
- Particulates
- Degradation by-products
- Leachables-formulation additives,
- Residuals
- Physical and configuration characteristics
- Emissions
- Microbes

5.20.4 Processing Factors To Be Considered Which May Affect Materials

A variety of factors must be carefully considered that may effect material quality and compatibility, a few examples are as follows:

- Additives
- Sterilisation method or process parameter changes
- Reprocessing or re-sterilisation
- Extrusion
- Cleaning
- Coating, dipping
- Adding regrind
- Curing, annealing
- Machining, polishing
- Corona treating
- Grinding, regrind
- Passivation, surface treating
- Casting
- Investing
- Moulding
- Surface finishing
- Release agents
- Time
- Atmosphere, environment, temperature
- Pressing
- Solvent bonding

5.20.5 Approaches and Strategies to Address Material Testing

Various approaches to addressing testing exist. A few examples are:

- Gather information on the material and the device
consider all related materials used in manufacturing, processing
- Perform literature search
early step in material investigation

goal to save time, money, resources, and animals

prepare a 'white paper' a written report of data

- Use vendor data
 - description of test article
 - name of test and test method(s)
 - name of laboratory
 - test results
 - test report
 - test interpretations
 - material safety data sheet (MSDS)
- Use historical data
 - build library of data for each material
 - arrange data so that it can be searched
 - computerise data for easy access
 - use same material in many devices
 - reuse biological safety data when possible
- Conduct new tests
 - least costly method(s)
 - most common method(s)
 - bulk of safety data

5.20.6 Some Considerations and Consequences of Testing a Whole Device or Assembly

There are consequences for testing a whole device or assembly. Some advantages and disadvantages are as follows:

Advantages:

- The test article more closely resembles the finished product
- Fewer tests are needed
- Interactions of different materials are stronger than properties of a single material

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Disadvantages:

- Toxic effects of some materials may be masked or diluted by other materials
- Positive (toxic) results cannot be related to a particular material but multiple materials

5.9.6.1 Advantage of Material Based Testing

- Makes conceptual sense
- Lends itself to phased or clearance testing
- Data can be applied to multiple devices
- Can be computerised
- Can search materials by biological properties
- Can search for materials by approved uses
- Can search for devices by material content
- Can search for assemblies by material content
- Database available to material professionals
- Can generate safety reports by material
- Can generate safety reports by device
- Can correlate test results to clinical results
- Can correlate test results to complaint history

5.20.7 Condition(s) of Material/Component, Assembly or Device for Testing and Preparation

The condition(s) of the material/ component, assembly or entire device and preparation can influence the test, timing and results.

- Determine if only material/components are to be used or if it is the assembly or full device
- Material/component, assembly or device is clean, yet reflective and representative of manufacturing environment:

Consider mocking up, representing or simulating the manufacturing process

Add regrind or other additives, if applicable

Simulate cleaning, polishing or other processes, if applicable

Label, package, and store samples as applicable

- Material/component, assembly or device has been double sterilised
- Material/component, assembly or device is stable and inert (sterilant residuals eluted)
- Material/component, assembly or device is purchased ‘as is’ from a vendor
- Material/component, assembly or device functions as a unit, and can be replaced more or less independently of other materials in device
- Independent material/component, assembly doesn’t influence biocompatibility in device
- Material component, assembly or device can be measured in surface area, and/or weight (if acceptable). Ideally, measurement of surface area and testing by surface area is preferred; however, the additional calculation of weight of the surface area measured sample provides optional information for alternative dose response analysis.

Note: weight is used only after surface area of a sample cannot be calculated or standard requires weight. Classically dose response is a good toxicological means to characterise a pure chemical substance.

- Consider extractable chemicals of concern (e.g., potential leachable, or class of chemicals that could create biological hazard such as carcinogen). Note: if a leachable substance is contained, and does not migrate to the surface at time of test, the results will appear to be satisfactory for a short time, but over a longer period of time and if the material is biodegradable after a long period of time, migration may occur. Device(s) intended for longer duration should be considered for testing at longer period of times.

5.20.8 Consider appropriate testing requirements and extractions

Considering what appropriate testing requirements and extractions is of prime importance for patient biocompatibility and revealing toxicity or unusual qualities that are not anticipated.

- Consider what makes up a test article:
Material/component, assembly or device
Generally contract laboratory includes positive/negative controls
Sponsor may include a clinically accepted device as a control

Table 5.5 Some Typical Plastic Extractions, Ratios and Tests		
Plastic extractions	Ratio	Test
60 cm ² /extract	(>0.5 mm thick)-3 cm ² /cm ³	Cytotoxicity
120 cm ² /extract	(<0.5 mm thick)-6 cm ² /cm ³	Intracutaneous
4 g per extract	0.2 g/cm ³	Mutagenicity
		Systematic injection
	10 ⁻¹ x 1 x 10 mm strips/2 cm ³	Muscle implant
	12-16 - 1 mm diameter x 10 mm	
	180 cm ² /extract (>0.5 mm thick)	Sensitisation
	360 cm ² /extract (<0.5 mm thick)	
	12 g per extract	180 cm ² or 6 g

- How many samples are needed. Some recommendations are shown in Table 5.5.

For updates or changes in extractions see the latest Pharmacopeia, ISO 10993 Standards or specific National Regulatory criteria

Note: weight is used only if the surface area of a sample cannot be calculated or if the standard requires weight. Classically dose response is a good toxicological means to characterise a pure chemical substance.

In use and potential solubility of chemicals or material extracts of concern. Examples are:

- Saline (0.9% sodium chloride)
- Saline/alcohol (1 in 20 ml saline)
- Polyethylene glycol PEG 400
- Cottonseed oil/vegetable oil
- Dimethylsulfoxide (DMSO)
- Minimum Essential Media (5% serum)
- Culture media (10% or less serum)
- Blood
- Simulated drug or drug product vehicle
- Water (distilled)

- Alcohol (1 in 10 ml water)
- Maximise extraction conditions. Some examples are:

In vivo Conditions or simulations or most severe conditions

Seven days (plastic implant)

120 hours at room temperature

96 hours at 37 °C

72 hours at 50 °C

24 hours at 70 °C

Typical *in vitro* temperature conditions

24, 48, 72 hours at 37 °C

One hour at 37 °C (direct blood contact=)

Physico-chemical

One hour at 121 °C (plastics)

Two hours at 121 (elastomers)

30 minutes boiling (Japanese)

Other Conditions as Appropriate (e.g., Good Laboratory Practices (GLP))

Ideally contract laboratory should perform GLP

Tests at least annually(where applicable)

Other Rule of Thumb For Temperature Extraction:

Use maximum temperature that will not degrade material

Extract at 121 °C for materials that are steam autoclaved

Extract at temperature below melting/softening point

5.20.9 Biocompatibility and Material Safety Screening Tests

The Technical Professional will issue requirements, as applicable. Some examples are:

- Physical/Chemical - form, fit, function and formulation of the product

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- Category – type of product, process and how it interacts with the patient
- Screening tests – preliminary tests to confirm likelihood of passing more difficult and sensitive tests
- Both physicochemical and biocompatibility – a combination of different type tests that when taken together favourably indicate a likelihood of an acceptable outcome
- Alternative tests are physico-chemical tests (for information only)

Other physical/chemical characterisation(s) to be provided or considered are:

- List all related materials to be used with component, assembly, or device
- Determine molecular weight (MW), in order to assess any change in MW after irradiation
- Analysis of monomers and oligomers of polymers, particularly after irradiation
- Consider characteristics of fillers, polymer additives – these ingredients may cause deterioration of materials but may also have toxicity
- Consider the extent of crosslinking and/or scissoring due to radiation. The response to radiation can improve or destroy a plastic material's properties for use
- Consider any mechanical/chemical properties that influence testing
- Consider extractable chemicals of concern (potential leachable in significant excess of diminutive quantities) or a class or group of chemicals, chemical entities that could create environmental issue or human response (e.g., off gassing of mail)
- Biological hazard. *Note:* if a leachable substance is contained, and does not migrate to the surface at time of test, the results will appear to be satisfactory for the short time, but over a longer period of time and if the material is biodegradable after a long period time, migration may occur. Device(s) intended for longer duration should be considered for testing at longer period of times.

5.20.10 A Technical Review Can Be Made After the Screening Test

Material physico-chemical is equivalent to that of currently qualified product and will be released pending completion of routine requirements.

Include rationale or justification for not performing other tests to be considered.

Equivalence to a currently qualified material/device cannot be verified without additional testing. Proceed by testing product as described under surveillance or advanced/confirmatory tests.

Surveillance (some examples)

- Simulated tests.
- Modified physio-chemical tests

5.20.11 Advanced or Confirmatory Tests

Professional will determine relevant testing (e.g., adopted from ISO 10993-1, other standards, knowledge of material and biological hazard, information gained during literature review, vendor data, screening, historical review, etc.). Some examples for external supplemental tests are: Particulates, Contaminants, Customised, Chronic testing, Carcinogenicity Testing, Reproductive Testing and Biodegradation.

5.20.12 Review Data

Data that has been generated needs to be assessed. The following needs to be considered:

- Designated person(s) who will document and compile data and report
- Professional who will verify adequate documentation of data and compilation, and issue review
- Professional who will review, analyse and interpret data

Some other considerations are:

- Were sample preparation(s) and test method(s) constant and adequately documented?
- Have adopted ISO and/or other standards and test requirement been met for type of tissue contact and for duration of exposure? Have all required screening and alternative testing been carried out and passed? Have all final tests conducted and passed?
- Have some tests not been done or failed?
- Is the material considered biocompatible, based on historical use or standards (material precedent, preapproved or grandfathered)?

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- Material biocompatible based on comparably used materials (some tests failed, but still may be available for particular job)

Is the material considered biocompatible based on labelling or restrictions of use, e.g., warnings, contraindications:

- type of sterilisation or processing method that is acceptable or not
- material not considered biocompatible
- is test profile adequate given the labelling
- was unnecessary testing performed
- does material device demonstrate safety, effectiveness and efficacy.
- does repeat, larger sampling, or multiple batch testing need to be performed?

5.20.13 Some Considerations for Accepting Higher Levels of Toxicity May Exist

Some examples are:

- Does the product benefit outweigh product risk?
- Is the company willing to accept costs of litigation, publicity, loss of sales?
- Is the product less toxic than its major competitors?
- Has material been used for similar intended use without history of clinical complaints or adverse reactions?
- Could labelling and/or directions for use be adequate given potential for use, (e.g., if safety isn't designed in, can it be labelled in?

5.20.14 Test interpretation may include some customised response(s)

Respond to what the business objectives for the material/device are:

- Be original or first in marketplace
- Improve on competitor's product
- Improve or change on own product
- Introduce a me-too product
- Introduce a less expensive product

- Expand or focus the product line
- What the applicable directions for use are
- What the applicable indications are
- What the target market is
- What is to be claimed
- What the featured functions, benefits, outcomes are
- What the major competitive products or procedures are

5.20.15 Documentation

Some documentation considerations relating to biocompatibility are:

- a) Issue report memo, test report, protocol with clearance from the Professional.
- b) Memo to File (Regulatory Submission)
 - Investigational device exemption (IDE)
 - 510 K
 - Pre-market approval (PMA)
 - Foreign Submissions or Safety/Effectiveness Support
 - New Drug Application (NDA)
 - Clinical Trials
- c) Directions for Use (DFU)
 - Intended and indicated uses
 - Quantity of dose, if applicable
 - Frequency of administration
 - Time of administration
 - Route of administration
 - Preparation for use
- d) Package Inserts and Labelling
 - DFU
 - Intended and indications for use

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Product description

Hazards

Warnings

Contraindications

Adverse effects

Precautions

Cautions

Reprocessing, reuse

e) Advertising

Claims of non irritation

Claims of hypoallergenicity

Claims of desirable biological responses (e.g., non pyrogenic)

Claims of sterility, cleanliness

f) Litigation or letter/documents to prevent litigation

Biocompatibility or material compatibility will soon be a subset of a larger healthcare compatibility where not only will the product and materials have to be compatible to the sterilization method and patient, but also compatible to drugs or disease mitigating ingredients added to materials and product, as is being observed with combinational products (e.g. devices and drugs). Consequently those who have compatibility experience in both drugs and devices will become desirable. This scope will not be included herein, but as a near future topic.

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14. EN ISO 13488, *Quality Systems - Medical Devices - Particular Requirements for the Application of EN ISO 9002*, 2001.
15. CEN 552, *Sterilization of Medical Devices - Validation and Routine Control of Sterilization by Irradiation*, 1994.
16. ISO ASTM 51275, *Practice for Use of a Radiochromic Film Dosimetry System*, 2002.
17. ASTM E876-89 (1994)e1, *Standard Practice for Use of Statistics in the Evaluation of Spectrometric Data*, 1994.

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18. AAMI TIR15, *Ethylene Oxide Sterilisation Equipment, Process Considerations and Pertinent Calculations*, 1988.
19. ISO 10993-1, *Biological Evaluation of Medical Devices*, 1997.
20. *Use of International Standard ISO 10993 'Biological Evaluation of Medical Devices Part 1: Evaluation and Testing*, Blue Memorandum G#95-1, FDA, Rockville, MD, USA, 1995.

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EO is useful for sterilising heat and radiation sensitive materials, polymers and plastics [1, 2, 3]. It is still one of the predominant methods of sterilisation used in the healthcare industry and second to steam sterilisation in hospitals, and equivalent to radiation in the medical device industry. EO sterilisation acquired this position with the advent and popularity of using heat sensitive polymeric materials for medical devices and special surgical instruments and supplies such as sutures.

Originally EO was used for the decontamination of spaces, but it has become the predominant means of sterilising heat sensitive polymer materials in healthcare products.

Some effects of EO sterilisation on materials in healthcare products are:

- Compatible with most polymeric materials
- Some materials are sensitive to the humidity of the EO process. For example, certain hydrophilic coatings
- Some materials can be more compatible with other methods under certain moulding, configuration, and load conditions
- Certain extremely temperature sensitive materials are not compatible at its higher temperatures (e.g., > 60 °C)
- Residues may be toxic - often requiring long degassing times
- Penetration is limited through thick gas diffusion barriers, and some mated surfaces.

EO sterilisation is a gaseous method. It is an ideal gaseous sterilant because of its characteristically high diffusivity and permeability. It is a ring ether compound, with relatively no charge. One of its disadvantages is toxic residuals, (e.g., ethylene oxide, ethylene chlorohydrin, and the less toxic ethylene glycol). Other significant characteristics of this chemical are its low volatility (10.8 °C), its ring structure, its moderate chemical reactivity that is enhanced by relative humidity, and its significant compatibility with most plastic materials.

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It typically sterilises through alkylation of side chains of enzymes, DNA/RNA, (e.g., OH, COOH, SH, and NH). Alkylating chemicals, (e.g., EO, methyl bromide, glutaraldehyde, or formaldehyde) have been referred to as radiomimetic poisons because their biological effects closely resemble that of ionising radiations.

Its disadvantages are its high toxicity, flammability, explosivity, carcinogenicity, reproductive toxicity, and high cost of handling and equipment. These disadvantages have been principally overcome with tightened equipment control, non-flammable gas mixtures, environmental control, detoxifying scrubbers, facility designs, worker training, and administrative controls. The benefits of EO as a sterilant continue to outweigh its inherent risks. For example, neither radiation nor steam sterilisation can sterilise electronics, or biomaterials without damage or destruction. Radiation can't sterilise acetals or Teflons. EO can sterilise drug eluting stents that radiation and steam would damage.

To obtain successful sterilisation with EO requires an understanding of its process parameters and the interrelationships between them and the products [2].

6.1 Cycle Phase Parameters of Ethylene Oxide Sterilisation

EO sterilisation consists of several cycle phase parameters:

- Air and gas barrier removal or reduction.
- Humidification with relative humidity with a dwell period.
- Injection of EO and sometimes other gases, (e.g., CO₂, nitrogen, HCFC).
- EO concentration, temperature control or other criteria during the exposure phase.
- Evacuation and air washes to remove EO gas.

Preconditioning and post cycle aeration are used to aid the sterilisation process. Preconditioning facilitates the eventual humidity conditioning of significantly dry product loads, (e.g., winter months and desert environments), and dehydrated bacterial spores, and post cycle aeration facilitates the removal of toxic residuals from materials treated with EO.

Each of these cycle phase parameters need to be considered:

- Air and gas barrier removal are often the initial and post evacuation steps of the cycle(s). The deeper the vacuum the better the opportunity of moisture or humidity diffusion taking place. Deep vacuums also remove sufficient air so that subsequent EO gas injection will not pass through significant flammable limits or explosive conditions. There are times however, when deep vacuums are not advisable such as

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when vacuum pumps are not sufficient to draw deep vacuums, and the stress load produced in attempting to pull deep vacuums would be detrimental to the pumps, and the products and packages being sterilised could explode. This tends to happen when EO gas mixtures are used that are non flammable and non explosive, and pulsed steam is admitted under vacuum so that air is removed, diluted or displaced by the action of steam injection and removal.

- Prehumidification is possibly one of the most significant phases of the process, because humidity appears to be synergistic for EO inactivation of spores, and particularly desiccated or extremely dry spores. In the latter case the humidity may help to facilitate penetration of EO through the cortex for the bacterial spores. Humidity may help to activate the germination of the very dormant dry spores. Also, humidity is likely to enhance the alkylation of the DNA/RNA amine groups by EO.
- Without adequate humidification, sterilisation with EO may not be achieved.

Inadequate humidification during the process has been the major contributory cause of most microbiological failures of standard EO processes.

Prehumidification is performed before injection of EO gas because the moisture molecules diffuse slower than the EO molecules. Moisture molecules are polar and have a higher boiling point than EO which is a non polar, rapidly volatile ether chemical, with a low boiling temperature of only 10.8 °C. Prehumidification can be performed by injecting steam until a certain pressure is reached or a humidity level is achieved. Typically after injection, a dwell period is made to allow for the diffusion of the moisture, however, pulsing or match flow of the steam and vacuum removal can speed this process up. Prehumidification is also used to heat the load up prior to injecting EO gas.

Dynamic steam pulsing is one of the best ways to heat the load up, rather than by static humidity dwell that can take a long time to cause a temperature change.

EO sterilisation is one of the gentlest methods for sterilising complex instruments and delicate materials. Because of this, it is frequently used in hospital sterilisation and custom pack sterilisation.

Some cautions:

- Porous or plastic materials require aeration for at least 24 hours before contacting skin or tissues (see operator's manual). Metal items can be used immediately.
- Low cost equipment provides 12 hour cycles at room temperature above 20 °C but meets Occupational Safety and Health Administration (OSHA) safety standards and is

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effective for processing dental instruments. Large chamber sizes hold many instruments or packs and reduce cycle numbers, but are more costly. Manufacturers should be consulted to obtain detailed information and ventilation requirements.

- Expensive equipment provides shorter cycles of three hours at 50-71 °C.
- EO is not presently validated for hand piece sterilisation, but shows promise.
- Oil can defeat sterilisation, so hand pieces should be cleaned but not oiled before EO sterilisation.
- Room temperature sterilisers should remain above 20 °C throughout their operation.
- Gas cannot penetrate closed glass containers at any temperature, or polyamide bags at room temperature.
- Use only types of packaging specified by the manufacturer (see operator's manual).
- Instruments must not be wet, but should be freshly cleaned and damp before processing (again, consult manufacturer or operator's manual).
- Store BI spores for testing EO as directed by the manufacturer.

6.2 Ethylene Oxide Processing Cycles

Some types of EO processing cycles are:

- 100% EO cycles with/without nitrogen are useful for industrial products, commodities where cost is of concern, but which require intrinsically explosion safe equipment and instrumentation.
- Standard EO/HCFC cycles provide for a safe gas mixture and are useful in equipment and facilities that are of non-explosive construction.
- Balance pressure cycles or air displacement cycles are useful for products that would be damaged due to vacuums or changes in pressure.
- EO/CO₂ (high pressure) cycles are useful as a non-ozone depleting gas, but require a high-pressure process (> 0.2 MPa).

A number of other EO processing methods may involve humidification, preconditioning, or aeration. The selected process method varies with the end product/packaging type

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configuration, characteristics. Ernst and Doyle prepared an excellent review of chemical and physical factors of EO sterilisation [2]. One of the limiting factors for EO is its limited ability to diffuse into the innermost areas of a few products or spaces that require, sterilisation within a reasonable time frame. The Russians used an EO/methyl bromide gas mixture that helps penetration through PE films.

Methyl bromide has been used as a sterilant mixture with EO as a potentiator for Russian spacecraft sterilisation. Methyl bromide is probably 10% as effective as EO, however in combination with EO, the mixture works faster than either simply added together [4]. This is probably because the methyl bromide facilitates penetration/diffusion of EO across non-polar films.

Compared to EO alone or chlorine dioxide, the mixture is a better penetrant, and diffusive, under effective use conditions. The mix is less dangerous, less flammable and has less explosive potential unlike EO alone or chlorine dioxide.

Methyl bromide is an ozone depletory gas. Because it will be banned in 2005, a replacement gas like methyl iodide, without ozone depletion is likely to prove to be beneficial in the near future.

EO is used less in hospitals and other public facilities in California, where it is deemed to be a carcinogen, a reproductive toxicant, and limited through environmental and other regulations. However the need for EO has not diminished in the healthcare industry where a myriad of different materials, in custom packs, medical devices are needed to be urgently sterilised. Healthcare products that have been commonly sterilised with EO include: artificial kidney machines, bronchoscopes, catheters, cauterisers, custom packs, cystoscopes, dialysers, disposable dialysers, endoscopes, oesophagus scope, heart lung machines, heart pacemakers, infant incubators, IV sets, laserscopes, nebulisers, otoscopes, petri dishes, rubber gloves, rubber drain and feed sets, rubber sheeting, rubber tubing, scalpel blades, sigmoidoscopes, speculae, suction pumps, sutures, test tubes, thorascopes, angiogram trays, aortogram trays, lumbar puncture trays, paracentesis trays, thoracentesis trays, tracheotomy trays, sputum trays, stopcocks, syringes, urethoscopes, urinary bags, vials, drug eluting stents, prefilled syringes, angioplasty devices and defibrillators.

Ethylene oxide will sterilise most plastic materials including: acetals (Delrins), elastomers – silicones (peroxides and platinum cured), thermoplastic elastomers (styrene ethylene butylene styrene block copolymer, thermoplastic elastomer - olefin), natural rubber (Isoprene), ethylene propylene diene monomer rubber, urethane, nitrile, butyl, styrene-butadiene, Teflons (polytetrafluoroethylene and fluorinated ethylene propylene), polyvinylidene fluoride, polymonochlorotrifluoroethylene, PETFE, 'high-end' engineering

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resins (polyetherketone, polyetheretherketone, polyetherimide), polyamides (Nylons – both aliphatics and aromatics, 12,11, 6/12 and 6/10), polyethylene, low density polyethylene, linear low density polyethylene, high density polyethylene, ultrahigh molecular weight polyethylene, polyesters (PES) and glycol modified polyethylene terephthalate, polycarbonate and alloys, polyglycollic acid, polysulfone, polyvinyl chloride (PVC; flexible and semi-rigid), polyurethane (eight chemical varieties), polypropylene and its copolymers, polystyrene (PS) and its copolymers, acrylonitrile-butadiene styrene terpolymer, styrene acrylonitrile, polyacrylics (PA), polymethacrylate (PMA), polyacrylonitrile), thermosets - epoxies, phenolics, polyimides, polyurethanes, and polyesters. However, some EO gas mixtures with freon may craze some plastics, (e.g., polycarbonate, acrylics), and polystyrene petri dishes have been known to stick together due to high relative humidity in the process. Polyacrylics can distort if the process temperature exceeds 62-66 °C.

EO effectiveness and efficacy is limited because of diffusion barriers, process time, toxic residuals. Parametric release is difficult to achieve uniformly with this method, but it is possible.

Because EO is deemed to be a potential human carcinogen and reproductive toxicant as well as an irritant and sensitiser, its use is limited. On line scrubber, gas emissions, worker exposure, and end users are significant considerations in the use of EO sterilisation.

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 biological indicator testing reduce the use of this process on a practical basis.

6.3 Industrial Qualification of Ethylene Oxide Sterilisation

Validation of EO frequently follows an overkill methodology [5]. An example of validating and revalidating EO is described in Section 6.3.1.

6.3.1 Validation (Example): Ethylene Oxide Sterilisation Validation Protocol for Healthcare Medical Care Product Devices at Contractor(s)

6.3.1.1 Purpose

The purpose of this procedure is to validate the full EO sterilisation for healthcare product devices in EO sterilisation chamber.

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This validation protocol is designed to:

- Demonstrate the effectiveness of the process for lethality and to produce a safe and acceptable product for its intended use.
- Demonstrate the relative resistivity of devices to the EO process and the suitability of an external BI for routine sterilisation.
- Demonstrate the appropriateness of the overkill method.
- Demonstrate the reproducibility of the process.

6.3.1.2 Scope

Sterilisation validation will typically employ the overkill approach of method C of ISO 11135 [6] and EN 550 [7].

- The validation will include: a fractional sub-process, and three half and full cycle runs, for an overkill process.
- This validation applies to a specified chamber, and process cycle parameter specification.
- This typically applies to all devices EO sterilised for industrial healthcare.
- This will complete the validation status of the product, and validate the new process.

6.3.1.3 Reference Documents

Contract (Ref: Agreement for EO Gas Cycle Sterilisation Services), if applicable.
Biological challenge of ethylene oxide sterilisation cycles from AAMI 11138 [8].
Validation of bioburden procedure with % recovery from AAMI ISO 11737-1 [9].
Product sterility and bacteriostasis and fungistasis from AAMI ISO 11737-2 [10].

As applicable, biosafety procedures, e.g., AAMI ISO 10993-1 [11]: cytotoxicity, acute systemic, irritation, sensitisation, pyrogen, haemolysis, chronic toxicity, carcinogenicity, genotoxicity, etc.

EN 550 Sterilisation of Medical Devices and Validation:

- AAMI ISO 11737-1:1995, *Sterilisation of Medical Devices – Microbiological Methods – Part 1: Estimation of Population of Micro-organisms on Products*.
- AAMI ISO 11737-2:1998, *Sterilisation of Medical Devices – Microbiological Methods – Part 2: Tests of Sterility Performed in the Validation of a Sterilisation Process*.

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- AAMI/TIR 14:2004, *Contract Sterilisation for Ethylene Oxide*.
- Sterilisation Process Cycle Parameter Specification.
- ANSI AAMI ISO-11135:1994, *Medical Devices-Validation and routine control of ethylene oxide sterilisation*, [6].
- AAMI TIR 15-1997, *Ethylene Oxide Sterilisation Equipment, Process Considerations, and Pertinent Calculations*.
- AAMI TIR 16:2000, *Process Development and Performance Qualification for ETO sterilisation-Microbiological Aspects*.
- ANSI AAMI ISO-10993-7:1995, *Biological Evaluation of Medical Administration – Part 7: Ethylene Oxide Sterilisation Residuals*, [12].
- AAMI TIR 19, *Guidance for ANSI/AAMI/ISO 10993-7:1995, Biological Evaluation of Medical Devices Part 7: Ethylene Oxide Sterilisation Residuals (1st Edition and Amendment)*, [13].
- AAMI TIR 20:2001, *Parametric Release for Ethylene Oxide Sterilisation*.
- ANSI AAMI ISO-11138-1, *Sterilisation healthcare products - Biological Indicators – Part 1: General*, [8].
- AAMI TIR 28: 2001, *Product Adoption and Process Equivalency for Ethylene Oxide Sterilisation*.
- CFR Federal Register, *Proposed FDA levels for EO Residuals*, June 23, 1978.
- Product sterilisation and release.
- Sterilisation load configuration pallet patterns or equivalent pallet configuration and equivalent density.
- *United States Pharmacopoeia* [14] (revision as applicable), or other compendium, (e.g., *Martindale* [15]).
- ANSI AAMI ST 29 – 1988, *Residual Ethylene Oxide in Medical Devices*, as appropriate or needed, [16].
- Sterilisation validation document (SVD).
- Relative resistivity, comparison of new to validated master product.
- Product control procedure, sterile and nonsterile product.

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- Handling and control of product sterilisation samples.
- Procedure for transfer of unsterile product/components.
- Guideline for isolate characterisation/identification of unknown micro-organisms.
- Bacteriostasis and fungistasis test of product/medium.
- Validation of bacterial endotoxin test - general guidelines.
- Bacterial endotoxin test for medical devices.

6.3.1.4 Technical Review and Responsibilities

This review includes a comparison of product design, materials, palletising, and packaging to existing products (or family representatives). Consideration is given to the hardest to sterilise models, e.g., process monitoring device(s), and unit/model.

The technical review finds the proposed product, materials, and packaging configurations equivalent to existing product, then they can be assigned to an existing cycle for validation purposes: one short cycle, three half and three full validation cycle runs are required on a selected representative product(s) from the product family type.

If during the technical review potential non-equivalency is revealed, then further development work is required.

A. Development of the technical rationale for relative resistivity and other testing.

- Selected models of devices, (i.e., representative models of each family), will be BI seeded in their most difficult to sterilise areas such as in bag, in sealed sites, mated surfaces, in hollow area(s) that may be remote to gas diffusion and with spore strips. Only a single BI will be placed in each individual device.
- Liquid inoculation of fluid path only testing, is not considered at this stage and may not be performed, except for a contingency situation.
- When the bioburden is low, a comparison of relative resistivity that includes both store strips and liquid inoculation can create inconsistent resistance results.
- If the bioburden is high, liquid inoculation may be performed. Then, the liquid inoculum will comprise a minute spore level for the worse case location that simulates the bioburden level. The difference between 10^6 inocula and minute inocula level will be distributed over the remainder of the device.

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- Dual fluid paths are only performed to evaluate worse case bioburden levels in remote, difficult to sterilise areas, and for distributing liquid inoculate on the product not empirically but with some rationalisation.
- If the results demonstrate greater resistance than expected, then sub-process D-value analysis using the Stumbo Equation and Halverson-Ziegler Equation, will be performed for the development of a new cycle. Three half and three full cycle validation runs will be run minimally in a longer (or stronger) cycle.
- If the results indicate less resistance than the most severe case to sterilise the model, then the normal three half and three full cycle validation runs are required for the most severe case device.
- For non routine or field trial product release during and under the umbrella of validation, the Manger of Technical Services or his equivalent can issue instructions for lot by lot individual sterilisation product release, on the basis of adequate safety and effectiveness and sterility assurance demonstrated, (e.g., 10^{-6}), and product safety, (e.g., functionality and EO residuals).

B. Sterilisation validation will use the overkill approach of method C of ISO 11135 [6] and EN 550 [7].

A process challenge device (PCD) will be adopted into a validated cycle. Adoption is based on:

- Bioburden results and product sterility.
- The relative resistivity of the sterilisation BI of PCD in comparison with routine products.
- Consideration of any remote model product is applicable because of its larger surface and porosity (which has not been previously shown for relative resistivity).
- Other standard product devices are similar except for various considerations.
- The product will be palletised according to a selected pattern for the established method. The pallet must be filled to simulate the most dense load and pallet configuration.

The following testing is required to establish the validation of process and chamber or sterilisation of a healthcare product:

- Presterilisation bioburden analysis on the first (three*) production lots and minimally on the most severe and representative product. The % recovery will be determined. This assures an estimate of total bioburden. [* Note: lots of past bioburden data may be applied, if applicable.]

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- The singular sub-process includes relative and comparative resistivity among product types, and potential recovery of BI. From at least the worse case model and minimally a single pallet with BI.
- Three half-cycle validation (as applicable, after a technical review).
- Three full cycle validation.
- Thermocouple temperature measurement and distribution (minimum of three per pallet).
- A singular RH sensor measurement and distribution (minimum of one per pallet) will be performed on initial load and other RH measurements and distribution will be performed subsequently.
- Pyrogenicity, as applicable.
- Functional (to include double sterilisation).
- Cytotoxicity (optional, as applicable).
- ISO mouse safety (optional, as applicable).
- Haemolysis test (ASTM test) (optional, as applicable).
- EO residuals after the full cycle. The maximum EO residual level (domestic) is 25 ppm and 2 mg EO residual level (ISO), for certain applications (e.g., blood), but other levels and situations exist.
- An annual audit by the contractor or manufacturer, which includes a review of their vessel:
 - Commissioning (installation qualification),
 - Physical qualification, and possible process development.

C. Technical Review and Design Considerations

The technical review for qualification(s) or rational for performing validation(s) should be made and documented as per a set procedure and/or with an SVD. The procedure and/or SVD will specify the requirements for qualification, as appropriate for the purpose or objective described, and as approved by the validation microbiologist or other designee and the technical services manager or other designee.

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- Product may be released prior to completion of validation if adequate test data (e.g., if this protocol or previous protocols have half and full cycle data to demonstrate 10^{-6} probability of survivor, and safety risk, (e.g., EO residuals) is acceptable as per the protocol. Independent product release can be approved by the Technical Services Manager or other appropriate designee.
- Responsibility for commissioning, calibration and the maintenance of equipment, for the operation of sterilisation, is that of the contractor, or manufacturer. The contractor or manufacture shall assign qualified personnel to carry these steps out.

6.1.3.5 Test Samples

Describe product types and number of units and cases as applicable.

Describe BI types and number of units and cases as applicable.

6.1.3.6 Equipment, Process, Materials, and Products

Describe contractor or manufacturer, steriliser chamber and process parameters and any modified cycle parameters if applicable:

- Contractor or manufacturer, RH sensors - calibrated to standards, (e.g., NIST).
- Contractor or manufacturer, thermocouples, calibrated to NIST.
- BI, 10^6 *Bacillus subtilis* or *atrophaeus* (*var niger*), strips, with certificate. Meets ISO 11138-1 [17] and/or USP or other applicable compendium or standards.
- Contractor or manufacturer preconditioning, (e.g., 8-20 hours) for subprocess, (e.g., shorter or longer hours for a full cycle typically between 42-52 °C, 50-80% RH), or other specified conditions).
- Contractor aeration-preconditioning: abbreviated time for subprocess, (e.g., longer hours) for standard process, (e.g., 40-54.4 °C) or as specified by other conditions.
- List of designated devices and details.
- The PCD consists of BI spore strips or liquid spores inoculated into difficult to sterilise products or item(s) with a known population of micro-organisms, namely spores that can be used subsequently in routine testing of the sterilisation cycle.

6.1.3.7 Procedure

6.1.3.7.1 Pre Sterilisation Bioburden

Bioburden: Product Devices. Test by fluid path or total immersion depending upon the label claim and end use of the device. For example, if the device is an IV administration set, then fluid path may be recommended. If the device is for long-term implantation, then immersion and wash is involved. Test devices for bioburden and spores (include heat shock unless already heat shocked and individual samples). Other types of micro-organisms to be considered are fungi and anaerobes.

Select representative model(s) from representative products. Test 10-40 units, for example: 10 for total aerobes, 10 for spores, 10 for fungi, 10 for anaerobes, or fractionals of 10 minimum units for each type of micro-organism. The 10 spore samples can be from 10 samples that have 10 *B. atrophaeus (subtilis)* spores (BI) that have been sealed in a polar or non polar film representative of the packaging, as necessary, placed in the fluid path or exit or enclosure of the product, so that the spores can't escape, but water vapour can cross the barrier to them at the most severe, distant location. Incubate the bioburden for 2-4 days at 30 to 35 °C followed by 3-5 days at 20-25 °C (for fungi). Report the total number of micro-organisms per lot for fluid path and/or total content, as well as the number of aerobes, anaerobes, fungi, organisms and spores. The report should characterise any predominant or unusual micro-organism(s). The test laboratory or other lab will calculate and report the total recoverable bioburden and spores to the nearest cfu. Per test laboratory bioburden should include consideration of % recovery on calculation of the final bioburden count.

Note: Additional testing may be performed in the future if total count bioburden are extremely high, in order to determine what levels of BI liquid inoculum would be required in inaccessible areas, and what remaining levels will be reasonably distributed on the rest of the device.

If BI spores are evaluated, verify that resulting spores are at a higher level than the bioburden, and use recovered numbers to assess subsequent exposure times.

6.1.3.7.2 Relative Resistivity Study and Sub Fractional Cycle

- The product devices and any other family models will be BI seeded in the most difficult to sterilise areas, (e.g., in tubing, below product, in bag, in hollow area, mated surfaces, within biodegradable implantable material, at any pressure fitment area(s). Only a single BI will be placed in individual devices.

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- Product will be packaged and palletised according to documentation.
- The product will be EO sterilised for a fractional cycle for 30 minutes, or less if determined from spore bioburden levels. All testing in chamber or equivalent vessel and sterilisation process, (e.g., 100% in order to make consistent and reliable comparisons). The process and time conditions will be according to cycle parameters, or as per a modified EO gas cycle, with the exception of gas dwell at 30 minutes or at a maximum of 45 minutes.
- BI (spore strips) are seeded in the most difficult to sterilise areas in a tight fitment, remote as diffusion areas or barriers such as in tube with non breathing ends, where applicable, for routine placement. A BI may be placed adjacent to the device, for information purposes to see if an alternative position of BI is needed during heating and conditioning during manufacturing.
- BI: Ten BI of each model are typically placed in eight corners of a pallet and two in the centre of at least one pallet. All locations will be labelled. Note: There will be representative product devices and PCD at each pallet location.
- BI may be refrigerated after sterilisation. They are tested by a test laboratory procedure. BI will typically be put on test within four hours of the end of the sterilisation process. They are incubated at 30-35 °C, for 7 days and longer for up to 14-21 days for information, e.g., slow growers.
- Optional. Three thermocouples per pallet or a total of greater than 10 thermocouples for the entire load are required. Two thermocouples per pallet may meet ISO 11135 [6] criteria. Not more than two thermocouples should be allowed to fail. Include thermocouples in preconditioning, sterilisation, and ambient aeration sections.
- Optional. Relative Humidity: %RH distribution will be performed during preconditioning. RH during conditioning in the steriliser is not required, when already previously performed.
- Product sterility by fluid path or total immersion, in accordance to the product claim and use or based on technical considerations. Review previous testing for history.
- Bacteriostasis/fungistasis: Methodology by test laboratory test that includes micro-organisms such as *Bacillus subtilis*, *Clostridium sporogenes*, *Candida albicans* or other organisms that reflect the sterility test methodology applied.

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- If the results demonstrate greater resistance than selected validated hardest to sterilise models, then a sub-process analysis must be performed from the sub cycle for calculation of a new exposure time for a new cycle:
 - Three half cycle validation runs
 - One full cycle validation run

If the results indicate less resistance than the most severe case to sterilise model then, only a half and one full cycle validation run is required.

6.1.3.7.3 Half Cycle Sterilisation Parameters

Cycle: half the exposure time of typical process parameters, unless they are wide, (e.g., greater than 10 °C, 15% RH, and 7500 Pa. If the parameters are wider, then apply minimum parameters for a most severe condition or possibility, and maximum parameters if there is potential product vulnerability.

- Typically use the most severe case BI locations per pallet, (e.g., eight corners and two in the centre).
- Thermocouples and RH measurement typically placed per sub process requirements.
- Cycle parameters - at a minimum half of exposure. Evaluate minimum preconditioning time and maximum time between preconditioning and steriliser.
- Apply abbreviated hours minimum degas aeration time.
- Typically one lot from only one run will be run as product sterility and bacteriostasis/fungistasis (B/F), unless performed at sub process fractional cycle and/or on the basis of previous product sterility tests and B/F performed and reviewed.
- Ship BI and sterility samples in cooler (blue ice) or refrigerated to lab, within the shortest period possible.

6.1.3.7.4 Full Cycle Performance Sterilisation Run - Typical Criteria

- Three nominal cycle parameters per process parameter, including nominal preconditioning time and maximum period between preconditioning and steriliser.
- Double the number of routine BI per load.

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- Thermocouples and RH measurement per fractional/sub process requirements.
- Pyrogen test, ten samples of worse case, which may include all components or components that haven't been previously evaluated. Test for enhancement and inhibition, to validate new materials for the pyrogen test.
- Review previous data as well.
- Safety as needed in lieu of old USP Safety, e.g., perform acute systemic injection test, fluid path surface extraction for 50 °C for 72 hours, or simulate worse case use condition. Number of samples: one of each model or models that haven't previously been evaluated. Review previous data as well.
- Cytotoxicity as needed (Test laboratory test: ISO Minimum Essential Medium (MEM) Elution with MEM extraction for 48 hours and 37 °C. Tissue: L-929 Mouse fibroblasts. Observation: 48 hours. Number of samples: one of each model that hasn't been previously been evaluated, but test in three flasks. Review previous data.
- Ethylene oxide residuals: (test methodology per AAMI Guidelines, consider ISO 10993 Part 7 [12]) includes EO, ethylene chlorohydrin (ETCH) and ethylene glycol (ETG). Healthcare test or test laboratory specified procedures. Review previous data and determine if ETG is required again, and which models need to be evaluated.
- Six samples per extraction day of each significant model type, e.g., representative product, worse case model, representative predominant product.
- Take samples from the cold location of the load, each day. Refrigerate samples during shipping to contract laboratory.
- Test at selected post sterilisation aeration times required to reach 25, 250 ppm (domestic), and 20 mg, 2 mg, or 0.2 mg dose (International) of EO residuals as required. Residual levels for ETCH and ETG should be considered where applicable.

To verify that there is no possibility of sensitisation demonstrate EO residual per device to be less than 250 ppm.

- Perform additional testing if time to reach 250 ppm or 20 mg for EO and other limits, if not demonstrated initially; testing may be stopped if at two or more consecutive days indicate meeting limit.
- Haemolysis test, as needed (test laboratory test). One sample medical device, e.g., product and/or representative product.

6.1.3.8 Acceptance Criteria

6.1.3.8.1 Bioburden

- 100 cfu/device or less, normative, without any further characterisation.
- If greater than 100 cfu, perform gram stain or spore test to evaluate for possible spores.
- Less than 100 cfu gram positive rods (sporeformers), and/or spores.
- If spores are greater than 100 cfu, then further investigation is needed.

Value Run and Relative Resistivity Study

- Cycle: Meets process parameters and 35-45 minute exposure time or less if pre-determined.
- BI: Demonstrate no survival and/or partial survivals. If thermocouples: Not more than two thermocouples are allowed to fail. Include preconditioning, sterilisation, and aeration, if applied: <18 °F or 10 °C, if applied).
- Relative humidity: ±15% (as applied).
- Product sterility: Sterile, no growth, or allowed repeat test in accordance with the USP.
- Bacteriostasis/fungistasis, non bacteriostasis and non fungistasis for *Bacillus subtilis*, *Clostridium sporogenes*, *Candida albicans* or other appropriate organisms.

6.1.3.8.2 Half Cycle

- BI: No growth
- Relative humidity: ±15%, if applied
- Thermocouples: ±5 °C, if applied.
- Minimum degas time between runs: 24 hours
- Meet parameters of EO gas cycle, and exposure half the normative or full cycle

6.1.3.8.3 Full Cycle Sterilisation Run Performance Criteria

- Full exposure time and meet cycle parameters.

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- Steriliser chamber: validate steriliser number.
- BI: No growth.
- Relative humidity: $\pm 15\%$.
- Thermocouples: $\pm 5^\circ\text{C}$.
- Non pyrogen and non inhibitory (to validate LAL for routine use, at least three inhibitory test lots need to be performed).
- Safety: no failures, no significant clinical responses nor mortalities.
- Cytotoxicity: non toxic (no significant observation of lysis, creneation (cellular shrinking), cell damage or swelling).
- Haemolysis: non haemolytic (no significant lysis [e.g., $<5\%$ haemolysis]).

6.1.3.8.4 Ten EO residual samples: 250 ppm (domestic), or 20 mg dose (International), or other limits of EO, ETCH, ETG as specified per AAMI/ISO 11939-7 and TIR 19.

- Must pass finished product test requirements.
- Comments: Product may be released prior to completion of validation if adequate test data, e.g., if this protocol or previous protocols have half and full cycle data to demonstrate 10^{-6} probability of survivors, and safety risk is acceptable as per protocol. Note: independent product release can be approved by Technical Service Manager, prior to completing this.
- Documentation: to include all signed off document(s) in support of above requirements: with review and approval.
- All information necessary for the completion of protocol must be compiled and found acceptable. Process will be considered validated as part of the device master record for the models included and qualified with completion of the full protocol.

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The next five pages give examples of typical Sterilisation Validation Documents (SVD) - for providing additional details and specificity not provided in procedure.

STERILISATION VALIDATION DOCUMENT (SVD)		
Steriliser Cycle(s) (ID) and Year:		Date:
Sterilisation Validation Document (SVD) References:		
Title: e.g., example Validation of Steriliser #1		
Objective/Purpose: (e.g., To perform a performance qualification on a specific device with a minimum and three performance normative runs)		
Product(s):		Fill Volume(s/Size):
Viscosity/Density:		Formulation:
Load Configuration:		Cycle (ID):
New Drug Application (NDA):		Audit:
510K:		Other:
PMA:		
Cycle Criteria:		
New Product:	Modified Product:	Packaging Change:
New Cycle:	Retest/Requalification:	New Steriliser:
Modified Vessel:		Controller Change:
New Manufacturing Change:		Modified Cycle Process:
New Requirement(s):		Criteria:
Studies:		References:
Installation(Commissioning):		
Empty Vessel/Operational:		
Evaluation of Closures:		

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Biovalidation Studies:	
Container (Cold) Location:	
Load (Cold) Location(s):	
Sub(Dose)/Minimum (BI)Cycles:	
Maximum (Stability) Cycles:	
Normal(Heat) Distribution:	
Normal(Heat) Penetration:	
Requalification/Audit:	
Other(s):	
Equivalence to current qualified Process, Product, Package or Other:	
Equivalence to current product, process, package cannot be verified without additional testing:	
Special Criteria:	
Validation Approach:	Bioburden:
Bioburden Resistance:	BI Type, D/z-value:
Relative Resistance:	
Half/Sub/Verification Runs:	
Adoption:	In Vessel D _v :
Alternative Process(es)/Approach(es):	
Other:	
Validation Specialist Date Technical Services Manager:	
Date:	

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STERILISATION VALIDATION DOCUMENT	
Steriliser Cycle (ID) and Year:	
Type of Runs, # Runs, Cycle, Numbers, Locations and Position of Temperature Distribution Probes:	
Type of Runs, # Runs, Cycle, Numbers, Locations and Positions of Temperature Penetration Probes:	
Type of Runs, # Runs, Cycle, Numbers, Locations, Positions of Biological Indicators:	
Special Post Sterilisation Requirements:	
Studies: Types, Cycles, Numbers, Locations, and Positions of Other Measurements:	
Technical Review/Rational(s):	
Validation Specialist Date:	
Technical Service Manager Date:	

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STERILISATION VALIDATION DOCUMENT		
Steriliser Cycle (ID) and Year:		
Results (information attached):		
Studies:(as applicable):	Status (as applicable):	
Installation Qualification:		
Empty Vessel/Operational:		
Evaluation of Closures:		
Bio Validation:		
Container (Cold) Location:		
Load (Cold) Location(s):		
Minimum Cycle Limits:		
Maximum Cycle Limits:		
Normal (Heat) Distribution:		
Normal (Heat) Penetration:		
Requalification:		
Other(s):		
Other(s):		
Environment:	Bioburden:	Bioburden (thermal) Resistance:
D-value (bio/BI):		Relative Resistance:
Half Cycle/Sub-Process Verification:		Adoption Consideration(s):
Full Cycle/Heat Penetration/Distribution/Dose Mapping:		
Other(s):		
Deviations:		
Anomalies:		
Discrepancies:		
Non Conformance:		
Technical Review/Rational or Comments (attach)		

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Documents (as applicable): ☐ Statement of Final Product Specifications (as applicable) ☐ Statement of Equipment ☐ Calibration of Biological Indicator ☐ Physical Evaluation of Container and Closure Systems (as applicable) ☐ Microbiological Evaluation of Closure Systems (as applicable) ☐ Product Temperature Mapping (as applicable) ☐ Minimum Cycle Limits (Heat Penetration)(as applicable) ☐ Maximum Cycle Limits (Heat Penetration)(as applicable) ☐ Closure Sterilisation (as applicable) ☐ Bio Information ☐ Qualification Specification Sheet ☐ Bioburden ☐ Thermal Relative Resistance ☐ Product Sterility ☐ Bio Laboratory Report(s) ☐ Qualification Specification Sheet ☐ Run Summary for Cycle ☐ Copy of PLC Record ☐ Copy of Steriliser Circular Chart ☐ Copy of Data Logger ☐ Data Trace Records or Summaries of Records ☐ Steriliser ☐ Vessel Cart Loading (BRP) ☐ Raw Data (Data Tracer Disks; set points, Calibration Data,) ☐ Qualifications Probe/Bio Reports-Heat Penetration/Distribution Tables ☐ Fo Calculations-Summary of Cycle Runs ☐ Validation Study-Bio Validation Study ☐ Sterilisation Process Qualifications Certification ☐ Executive Summary Report ☐ Process specification ☐ Other(s)
Each of the applicable study aspect(s) shall be summarised by the study workers and reviewed by Validation Specialist and Plant Validation Committee. Upon completion of work, a written addendum will be formalised. The addendum shall be reviewed and accepted by the Validation Committee. All summaries, raw data, and test notes shall be included as part of the final validation record.
Validation Specialist: Date:
Technical Service Manager: Date:

6.3.2 An Example of a Revalidation Test Protocol

6.3.2.1 Purpose

This protocol is for the annual validation of sterile disposable products and production. Ethylene oxide (EO) sterilisation cycle based on EN 550 [7], AAMI/ISO 11135 [6] and AAMI ST 29 [16] guidelines.

6.3.2.2 Scope

Revalidate chambers for sterile disposable products using an EO cycle compliant to EN 550 [7] and AAMI/ISO 11135 [6].

6.3.2.3 Reference Documents (examples)

- Procedure, Manufacture of Products for Evaluation
- Procedure, General Sterilisation
- Work Instruction, Sterilisation
- Procedure, Validated Sterilisation Suppliers and Cycles
- Work Instruction, Sterilisation Validation
- Work Instruction, Environmental Bioburden Study and Monitoring
- Work Instruction, Particulate Testing
- Title 21 CFR, Part 58: *Good Laboratory Practices*
- Title 21 CFR, Part 820: *Good Manufacturing Practices*
- AAMI/ISO 11135, *Medical Devices - Validation and Routine Control of EO Sterilisation*
- AAMI ST 29, *Recommended Practices for Determining Residual EO in Medical Devices, as needed or appropriate*
- AAMI/ISO 10993-7:1995 *Biological Evaluation of Medical Devices – Part 7: Ethylene Oxide Sterilisation Residuals* [12]
- AAMI/TIR No.19 *Guidance for ANSI/AAMI/ISO 10993-7:1995* [13]

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- BS EN 550 *Sterilisation of Medical Devices – Validation and Routine Control of EO Sterilisation* [7]
- BS EN 556-1 *Sterilisation of Medical Devices – Requirements for Medical Devices to be Labelled ‘Sterile’* [17]
- AAMI/ISO 11737-1:1995 *Sterilisation of Medical Devices – Microbiological Methods- Part 1: Estimation of Population of Micro-organisms on Products* [9]
- AAMI/ISO 11737-2:1998 *Sterilisation of Medical Devices – Microbiological Methods – Part 2: Tests of Sterility Performed in the Validation of a Sterilisation Process* [10]
- AAMI/TIR No. 14:1997 *Contract Sterilisation for Ethylene Oxide* [19]
- ISO 11138-2:1994 *Sterilisation of Healthcare Products – Biological Indicators – Part 2: Biological Indicators for Ethylene Oxide Sterilisation* [20]
- USP 24:1999 *Sterility, LAL, Bacteriostasis/Fungistasis* [14]
- AAMI/TIR 15:1997 *Ethylene Oxide Sterilisation Equipment Process Considerations and Pertinent Calculations* [21]
- AAMI TIR 16:1998 *Process development and performance qualification for ethylene oxide sterilisation - Microbiological aspects.*
- AAMI/TIR 20:2001 *Parametric Release for Ethylene Oxide Sterilisation*

6.3.2.4 Contents for an Appendix

- Appendix A: Specify half-cycle EO process specification for EO sterilisation. To reflect a developed process.
- Appendix B: Specify full-cycle EO process specification for routine production to reflect a developed and documented process to be revalidated.
- Appendix C: Fractional-cycle EO process specification for routine production. To reflect a developed process.
- Appendix D: Process challenge device – BI test strip inserted into a product package. Note: The product may be replaced with one BI.
- Appendix E: Sterilisation chamber configuration for validation runs: product, BI, temperature and humidity sensor placement.
- Appendix F: Type of packaging, (e.g., flexible packaging).
- Appendix G: Type of packaging, (e.g., tray packaging).

6.3.2.5 Reference Laboratory Test and Contract Documents

- Bioburden recovery
- Bioburden test
- Sterility test, USP Procedure [14]
- Bacteriostasis/fungistasis USP
- AAMI sterility tests
- Ethylene oxide, ethylene chlorhydrin, ethylene glycol by gas chromatography
- Bacterial endotoxins test USP [14]
- Equipment equivalency procedure
- Process cycle specification (full cycle)
- Process cycle specification (half cycle)
- Process cycle specification (fractional cycle)

6.3.2.6 Sponsor

Manufacturers.

6.3.2.7 Sponsor Study Coordinator

Quality engineer, manufacturing engineer or other designee.

6.3.2.8 Sterilising Facility

Contract or manufacturing facility.

6.3.2.9 Testing Facility

In-house or contract laboratory.

6.3.2.10 Background

- Products were previously validated by a contractor specified in the chamber reference protocol. Sterilisation processes in the selected chamber were also previously validated under sterilisation protocol numbers for specified products.

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- The contract EO site, is to be audited for compliance to GMP, ISO certification and acceptability as a contract EO sterilisation site.
- The contract laboratory is to be audited for compliance to GLP and ISO certification. The procedures are reviewed and acceptability as a contract testing facility is determined. Other laboratories used are also audited.
- The process cycle is validated to ISO 11135 [6] and AAMI guidelines. The EN 550 standard [7] requires removal of biological indicators and sterility test samples from the load prior to aeration. EN 550 also requires refrigeration of these samples if not at the laboratory and in-test within four hours. Manufacturers must repeat all of the revised cycles and change the sample removal/handling to comply with the requirements of EN 550.
- The process cycle is validated to EN 550, AAMI/ISO 11135 and AAMI ST 29 guidelines (as applicable) [16].
- Early cycles should have been validated to EN 550, AAMI/ISO 11135 and AAMI ST 29 guidelines. Reference previous sterilisation validation.

6.3.2.11 Objectives

- This validation study will be performed in accordance with EN 550 and AAMI/ISO 11135.
- The configuration of the sterilisation load is five pallets of a simulated product load. Products from the defined families (See Section 6.3.2.13) will be seeded into the half cycle load to validate a minimum biological effectiveness of 10^{-6} Sterility Assurance Level (SAL) as per BS EN 556-1 [17].

6.3.2.12 Proposed Study Dates

- Estimated Starting Date
- Estimated Completion Date
- Estimated Final Report

6.3.2.13 Product Families

- Quality engineering, the disposable - production team and the study coordinator must evaluate the characteristics of the sterile disposable products for their resistance to EO sterilisation. Device construction, product complexity, and packaging are a part of this evaluation.
- Product list - as applicable.
- The product used for sterility testing will be chosen to be representative of the design geometry of the device and the handling steps in manufacturing that might affect product bioburden. Packaging for this family is either support cards or sleeves, pouches and display boxes. There are no significant differences in the ability of EO to penetrate the packages. Samples of this product will be included with each fractional-cycle validation load for sterility testing.
- Process challenge device. The product packages will be used to hold the BI carrier and inoculated device (See Appendix as applicable).
- In certain cases liquid inoculation will be used to place difficult to place BI.
- Each package will be clearly marked 'Biological Indicator'. The packages will be numbered consecutively. Two BI will be submitted as control samples. There should be three sets (groups) of BI: one for the fractional-cycle, some for the half-cycles, and some for the full (routine) cycle, as appropriate.

6.3.2.14 Protocol Design

- This study will follow the 'over-kill' method recommendations from EN 550 [7] and ISO 11135 [6]. One half-cycle will be tested with no survivors for each chamber. It will be processed with maximum volume. A test cycle will run in the chamber. One fractional-cycle will be processed, if necessary, from which survivors can be recovered. For this revalidation of sterilisation, the fractional-cycle test is required to demonstrate BI recovery. Sterilisation cycle parameters are specified in Appendices, as applicable.
- Rationale for Exclusion of Fractional-Cycle (when deemed reasonable): The fractional-cycle is used primarily to demonstrate BI recovery, but secondarily to show relative resistance between challenge devices and natural occurring flora in the product. Unless the manufacturing process/product or some other process has significant changes, the studies that have been performed to date should be sufficient

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to show the resistances. Additionally, product sterility will be performed in the fractional study to show that the resistance of the natural flora is less than or equal to the process challenge device (since all product flora should be dead, in comparison to the PCD).

- Each cycle test will contain a full load of pallets of product, using the product-loading configuration planned for routine production. This represents the maximum intended chamber load and the products most difficult to sterilise. Biological and sterility test samples will be placed at representative product locations based on typical process temperature variations in a load. Product density for the validation will be with either a normal production load or a simulated product load that is representative of the actual routinely-processed loads. A mix of normal and simulated products may be used.
- A minimum of ten bioburden samples from at least one lot of each family type product will be submitted for bioburden testing prior to sterilisation in accordance with EN 550 [7] and ISO 11135 [6]. These products have been selected as the most representative product due to the diversity and number of manufacturing operations for the assembly of the product. The purpose of this testing is to demonstrate that the natural flora associated with each composite test sample does not exceed the resistance limitations represented by BI that have been inoculated with approximately 1.0×10^6 spores of *Bacillus subtilis* (*var. niger* or *globigii*).
- For the fractional and half-cycle pallets, two Datatrace temperature and one relative humidity sensors per pallet minimum, BI, and product test samples (fractional) will be placed in representative sites throughout the load (See Appendix) to challenge the validation sterilisation cycle parameters. Product temperature sensors are required for temperature monitoring of pre-conditioning fractional and half-cycle. Subsequent sterility testing of the biological indicators must demonstrate a six logarithm reduction of spore population.

The fractional cycle will not provide a six log reduction, unless manipulated statistically or it fails.

NOTE: One or two positive strips could occur, and demonstration of 6 logs still occurs, depending upon the initial spore population.

- For the fractional-cycle run, 20 product samples (minimum) for sterility testing, and 38 packaged BI will be placed as shown in Appendix E. Two non-sterilised spore strips will be submitted as a viability control. Bacteriostatic/fungistatic testing will be done after incubation of the sterility test samples is complete.

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- An EO five-point residual decay curve will be determined. Samples for this test are to come from routine production loads. There will typically be three locations – for variation in absorption and desorption - and these can be sampled at day 0, 1, 3, 5 and 7. Samples should be taken, for two full-cycles following each half cycle with three products from each of selected locations on each sample day.
- Product/package functionality will be determined as per the packaging integrity study.
- Pyrogen testing will be determined as per the validated method.
- Biological indicators. The BI employed in this study will be spore strips of *Bacillus subtilis* (*var. niger* or *globigii*) with a population of approximately 1.0×10^6 spores. The lot number and expiration date will be recorded at the time of use.
- Stretch wrap, plastic strapping, and plastic netting will be left on for all validation runs. This will be standard practice for normal production runs once the sterilisation process has been validated.
- Product sterility and BI testing will be done as per Section 6.3 on samples from the fractional cycle and BI testing for the half-cycles. Bacteriostatic and fungistatic testing will be done for each family per Section 6.4 and BI viability testing as per Section 6.2. Incubation time is to be a minimum of seven days for all testing unless otherwise specified.

6.3.2.15 Special Experiments

Special experiments may be required when new products or packaging are planned. These tests will be conducted at a research laboratory site. To validate the EO penetration to the material used for the new product or packaging, product samples and *Bacillus atrophaeus* (*(subtilis (var. niger or globigii))*) BI will be placed in three half-cycle validation runs. After the samples are processed, these samples and BI will be removed and sent for sterility, bioburden (presterilisation), EO residuals, MEM elution, pyrogen, and BI population verification testing. If all tests pass, the new product will be biologically validated for routine EO sterilisation.

NOTE: BI population verification samples need to be unprocessed control BI.

6.3.2.16 Test Sample Handling

Each fractional (1) and half-cycle (2) requires packaged BI, plus two control samples. Also, fractional cycles require product samples from each of the product families. The

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EO residual panel requires product samples from product families, which will be run on a full cycle. Additional BI will be needed for subsequent full-cycles.

NOTE: These samples will be produced under representative manufacturing conditions.

The manufacturer will send the BI, product sterility test samples, and product bacteriostatic and fungistatic test samples for the fractional-cycle validation run and others as applicable. The product (when needed) and BI samples are to be visibly marked for sample placement by the steriliser personnel in each of the validation runs. Samples will be removed by the steriliser personnel. Manufacturer's markings will clearly indicate sample type, run number and numerical indications.

After the validation run, the representative of the steriliser manufacturer will remove the product samples and the packages that contain the BI and send them to the laboratory as soon as possible. Sample refrigeration at 2-8 °C is required until testing begins unless testing begins within four hours. The following information should accompany the samples:

- Manufacturer's sterilisation validation cycle type,
- Steriliser's sterilisation run number and date,
- Sample quantity and sample type, and
- Test sample submission form, as appropriate.

Any production product included in the half-cycle(s) that is to be released as 'STERILE' will be re-sterilised with a regular production cycle with 10 biological indicators as is standard practice.

6.3.2.17 Re-Validation Criteria

Cycle Challenges: To re-qualify an EO sterilisation chamber for use on the manufacturer's sterile disposable devices, the following are required:

- The cycle parameters used in the two half-cycle qualification runs are as described in an Appendix . The run is for biological validation using 'most severe case' expected process parameters. The fractional exposure time for cycles where BI survivors are expected, can be tentatively D_v minutes times (Log of BI population - 2 logs), but this is dependent upon certified BI laboratory survival resistance and contract or manufacturer cycle parameters and profile (see Appendix).

The total bioburden count data will indicate that the product challenge does not exceed the 10^2 or other contamination level that is below resistance of the biological indicators used and thus an overkill cycle will provide an adequate safety factor for product sterilisation.

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Any process deviations from specified parameters will be documented by the steriliser manufacturer's representative to the quality representative.

- The product samples, the BI, and the product temperature and humidity sensors are to be distributed in the designated locations in the chamber (Appendix).
- The shipper/sterilisation box configuration will be as given in an Appendix.
 - The cycle printout shall indicate that all cycle parameters were met.
 - Chamber temperature variation should not exceed $\pm 10^{\circ}\text{C}$ from the nominal cycle temperature during the cycle.
 - Chamber temperature variation should not exceed $\pm 5^{\circ}\text{C}$ from the nominal cycle temperature during the gas exposure part of the cycle.
 - Temperature and relative humidity distribution at the end of preconditioning and conditioning shall not exceed $\pm 5^{\circ}\text{C}$ and $\pm 15\%$ RH, respectively. During gas exposure, the temperature distribution shall not exceed $\pm 5^{\circ}\text{C}$, unless there is an accepted deviation beyond the limits.
 - The product sterility test should show that the product is sterile.
 - All exposed BI samples must be negative, except for the fractional cycle. The fractional cycle shall demonstrate that BI are recoverable.

6.3.2.18 Documentation

The following documentation of adherence to the cycle parameters will be supplied by the representative steriliser manufacturer in order to provide a consistent, traceable means of documenting the sterilisation data of the validated cycle:

- Preconditioning temperature and relative humidity charts for the room.
- Preconditioning fractional and half-cycle product humidity and temperature studies for the biological validation run.
- Certificate of analysis of EO concentration from their supplier and gas concentration used.
- Chart for each cycle displaying pressure, temperature and time.

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- Temperature and humidity profiling requirements:

Printout of temperature and relative humidity readings at all positions showing the maximum and minimum readings for each time period. Actual cycle parameters must be within the specified parameters of the cycle.

Diagram showing location of temperature and relative humidity sensors in pallet relative to chamber load configuration.

Diagram for the pallet showing sample and spore strip placement defining front, centre, and rear.

- Certification of calibration of temperature and relative humidity measuring equipment.
- Certification of calibration of cycle parameters chart recorder.
- Documents identified as required in B3.5 of EN 550 [7] and AAMI/ISO 11135 [6].

6.3.2.19 Summary

- The bioburden information for the samples will serve to demonstrate that the bioburden on the sample does not exceed 100 cfu or other levels that are below or equal to the resistance limitations characterised by biological indicators that have been inoculated with 1.0×10^6 spores of *Bacillus atrophaeus* ((*subtilis* (*var. niger* or *globigii*)).
- Successful completion of the fractional-cycle run will require that some BI positives occur from any of the test sites sampled. If there are one or two positives product, an investigation should be performed to ascertain if there was a laboratory test error and if statistically its relative resistance is below that of the BI challenge. Additional qualification runs may be required after appropriate modifications in the cycles are made if no BI or sterility positives occur.
- Successful completion of the half-cycle run will require that no BI positives occur from any of the test sites sampled. Additional qualification runs may be required after appropriate modifications are made to the cycles if BI sterility positives occur.
- The validation experiment dwell time will be designed to provide a minimum SAL for products of 10^{-6} .
- Acceptable bacteriostatic/fungistatic test results should be documented.

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- The completion of the validation will allow a manufacturer to release products and/or lots by doing sterility testing on ten *Bacillus atrophaeus* ((*subtilis* (*var. niger* or *globigii*)) biological indicators. All BI must be negative in routine production.

6.3.2.20 Contents of the Final Report

- Summary report and interpretation by study coordinator, signed by the VP of Quality Assurance & Regulatory Compliance or other designee.
- The validation protocol
- Results of bioburden testing
- Certification of EO used in sterilisation
- Sterilisation records
- Biological test reports (Product and BI)
- Additional tests: packaging, EO residual, and pyrogen
- Data required by B5.6 of BS EN 550 [7] and AAMI ISO 11135 [6]
- Letters to record, if applicable
- Sample building documentation (manufacturing evaluations).

6.3.2.21 Archives

The final results of the sterilisation validation should be retained in the archives.

6.3.2.22 Routine Product Release

Once the process is confirmed to be validated for these products, the acceptance and release criteria for routine production lots will be as follows:

- Routine cycle charts and load documents must be received by the Quality Control department prior to product release from quarantine.
- Steriliser certification that sterilisation process parameters were met.

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- Satisfactory BI sterility testing [i.e., typically minimum 10 BI per load (plus two positive controls)] will be used to corroborate sterility. The laboratory will send the results for testing to the quality assurance department or other designee prior to product release.
- Process cycle specification for routine production (See an Appendix).

6.3.2.23 Revalidation: (Periodic re-evaluation of Validated Processes)

AAMI/ISO/EN guidelines recommend that sterility re-qualification be done on an annual basis. Unless production/process changes dictate otherwise, a half-cycle with associated product and spore strip sterility tests will be conducted annually to re-validate the sterilisation process.

Process Challenge Device (PCD) - Biological Indicator Test Strip inserted into a Package, or in a remote location or difficult location of a device.

NOTE: For the process challenge, the device shall be replaced with one BI, whenever feasible.

6.4 Guidance on EO Sterilisation Process and Statistics

6.4.1 Relative Humidity and Its Role in Sterilisation Processes

Relative humidity 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 to existing in-house dynamic environmental conditioning (DEC) sterilisation processes:

1. Basically RH is necessary for effective EO sterilisation, no matter what the cycle. Scientifically, Kaye and Phillips [22] demonstrated that the microcidal action of EO was 10 times faster at 28% than 97% RH, and four times faster at 28% than at 65% RH, during gas exposure. Currently %RH is indirectly measured (steam pressure) before exposure.

Other investigators, Ernst and Shull [23] presented data to show that sterilising efficiency increased with increased relative humidities.

2. Gilbert and co-workers [24] and others demonstrated that microbiocidal activity was minimal when spores were extremely dry or desiccated and that high RH were

required to reduce the resistance of desiccated or very dry spores. This desiccated state could not be readily overcome by high relative humidity level unless 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. Phillips [25] 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 has consequently led to the use of high humidities. The humidity necessary to kill the *Bacillus subtilis* spores and preparation by Statens Serum Institut of Copenhagen, which is recognised officially by the Nordic Pharmacopoeia is at least 76% RH at 20 °C, the constant humidity condition to dissolve sodium chloride in sweat or at 60 °C, would be minimally 75% RH. Several years ago, Sweden asked that we inactivate their spores and preparation. Only a dynamic 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. They accepted our cycles without full inactivation of their BI, on the basis that we had such extremely low bioburden in the fluid path of our product.

3. Most sterilisation processes inject steam and measure relative humidity during preconditioning (before and outside the vessel), and during prehumidification or during dynamic conditioning, before admission of EO. This is deemed to be 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:
 - a. the number of water molecules even in a highly humidified environment is overwhelmed by the greater number of EO molecules
 - b. the diffusivity of EO surpasses that of water vapour.
 - c. water readily reacts with EO and carbon dioxide through hydrogen bonding which creates aggregates that impede diffusivity of water vapour.
 - d. molecular interference such as air pockets and expanded heat sealed plastic bags prevent effective permeation of water vapour.

Example of %RH determination and development:

%RH = inches/mm Hg of saturated steam (x 100)

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Saturated steam pressure at the specified temperature is obtained from a steam table.

Modifications of this formula, using temperature data, can be developed into formulae that match actual RH readings.

%RH distribution should not exceed 30% RH during most of preconditioning at the end of conditioning. Ideally, the %RH should be $\pm 15\%$ of a reference point.

6.4.2 Product Temperature

Product temperature does not have the influence on sterilisation time, that it does with heat:

- Sterilisation, particularly steam sterilisation: typically a rise in 10 °C in product/process temperature will halve the sterilisation time. The temperature required typically for EO to reduce time by 10% is about 50 °C.
- Product temperature penetration distribution should not exceed 10 °C during most of preconditioning, at the end of conditioning, during gas exposure, and aeration. Ideally, the temperature should be ± 10 °C of a reference point.
- Readings can be made at the beginning, middle, end of each phase condition (preconditioning, end of conditioning, gas exposure, aeration).
- For an empty vessel run, temperature distribution should be ± 3 °C throughout the vessel.

6.4.3 Ethylene Oxide Concentration

Ethylene oxide concentration determines the base line exposure time. The higher the concentration the faster the sterilisation process; however, higher concentrations will eventually lead to higher EO residuals. Typically if EO concentration is doubled, the time to sterilise is reduced in half, but barriers will definitely influence the concentration of EO needed to reach a remote bacteria site.

Determination of EO gas concentration (two methods):

- Ideal gas law - subtract pressure from residual air, humidity, and nitrogen
- Gas weight/volume; note: ounces of gas/cubic feet = mg/l $\times 1000$

6.4.4 Inactivation Factor and SAL: A Microbiological Statistical Expression of Sterilisation Effectiveness

Calculation of spore log reduction (SLR) is made by the method of Stumbo, Murphy and Cochran [26].

$$SLR = \log_{10} N_o - \log_{10} N_u$$

Where: N_o = initial population per cycle = population/strip $\times$ # strips

N_u = population per container after heat treatment

$N_u = \ln r/q (\times r)$ (Halovrsen Ziegler most probable number formula)

r = total replicate samples

q = number of negative replicate or sterile units

In the event that no survivors occur, for calculation of worse case, one survivor will be assumed to have occurred.

$$\text{Inactivation Factor} = SAL (\text{half cycle}) \times 2$$

$$SAL = \log^{-1} (2 \times \log_{10} N_o - \log_{10} N_u)$$

Where: N_o = initial population per spore strip

N_u = population per cycle

$N_u = \ln r/q$ (Halovrsen Ziegler most probable number Formula)

r = total replicate samples

q = number of negative replicate or sterile units

Bio TCDT_{bio}: a microbiological statistical expression of sterilisation lethality

Calculation of TCDT_{bio} (for determining the similarity of biological and physical measurements of lethality) is made according to:

$$\text{Thermal Chemical Death Time (TCDT}_{\text{bio}}) = D_{\text{subprocess}} (\log_{10} A - \log_{10} B)$$

SAL: a microbiological expression of probability of denoting a non sterile unit

The intent of SAL is to show that the cycle will process solutions with a probability of a nonsterile unit of 10^{-6} ('overkill' if bioburden is assumed to have a resistance of $D = < BI$ D-value and a 12 log BI inactivation or greater and bioburden population is assumed to be 100 cfu per unit)

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$$N_u = \log^{-1} ((\log N_o) - \text{Exposure Time}/D_{\text{subprocess}})$$

Biovalidation verifies that physical measurements of lethality are in reasonable agreement with lethality measured biologically.

6.5 Guidance on Designing and Developing Sterilisation Parameters

Sterilisation validation can be performed as: overkill, bioburden or a combinational approach.

Overkill approach: BI with limited bioburden with minimal analysis. A sterilisation design based upon low uncertainty of risk of failure:

$$\text{TCDT} = D_v \text{ BI } (\log 6 + 6 + *) \text{ or } 12 \text{ logs or } >12 D_v$$

(with worse case D-value from sub process run)

(*When testing or confirming with samples, always include the log number of the samples.)

Bioburden probability approach: bioburden and limited or no BI, only probability determined, but heavy reliance on bioburden levels and resistance.

A sterilisation design based upon maximum product microbial bioburden and resistance, load size and simple 10^{-6} .

$$\text{TCDT} = >D_v \text{ isolate (max log bioburden + log } 10^{-6} + R$$

Where: R is the number of units per year.

Where optimisation of bioburden recovery and bioburden EO resistance is required, combination of overkill BI and bioburden probability approach - considers relative resistivity between the two.

Sterilisation design is based upon low uncertainty and maximum product microbial bioburden and resistance, 10^{-6} , including number of products.

$$\text{TCDT} = D_v \text{ BI } (\log N_o + 1 + R) = \text{or } >D_v \text{ isolate (max Log bioburden + R + log } 10^{-6}$$

Where: $D_v \text{ BI} > \text{maximum product isolate.}$

R is the maximum number of units per batch

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The combinatorial approach is sometimes the best because it matches up BI with bioburden resistance. BI is a useful tool to confirm physical product thermal history and results.

Develop sterilisation cycles on the following basis:

Heat up and preconditioning time - generally do not contribute much to total microbial inactivation, but it is the key towards obtaining faster heat up and understanding thermal, %RH uniformity.

Gassing time and time at exposure contributes to bringing product to final equilibrium temperature and %RH. Generally the last half of exposure period has significant influence on comparing inactivation at lower or higher temperatures.

Aeration time is very influential on the contribution of total inactivation. Often more than half of BI inactivation is achieved during the last half of exposure and cool down.

Minimise the time between unloading steriliser and loading aerator (e.g., < 1 hour).

Note: however, wide temperature distribution in aeration leads to cold spots for EO gas desorption (high EO residuals). In validation, measure load temperature, determine air and pressure changes as applicable.

For occupational safety purposes, prior to eight hours in aeration, (e.g., 4 hours), remove samples from the load.

Minimum cycles should consist of lowest process time, but consider temperature, EO concentration, %RH as well, however, times between preconditioning and steriliser and between steriliser and heated aeration are needed to control process.

6.6 Ethylene Oxide Sterilisation Can be Improved by Increasing Sterilising Temperatures and Using Heated Aeration as Part of the Overall Process

An increase in sterilising temperatures to 70-80 °C for EO that is used with steam formaldehyde, could reduce EO concentration, process times, and reduce residuals. According to the recent ISO 10993-7 [12], ethylene glycol is not deemed a significant residual, as EO and ethylene chlorohydrin are. Since the higher temperature and moisture at 70-80 °C would create more ethylene glycol, it should not be a significant problem as previously considered.

Improvements of plastics with heat stabilisers and co-polymerisation as are already used for radiation would enhance the number of plastics that could be sterilised at these increased temperatures.

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Sterilisation or further deduction in treated microbes occurs with elevated temperatures when EO gas is removed with heat and aeration. Consequently minimisation of total process time can be achieved, simultaneously.

Radiation can sterilise many materials but it is limited by a few materials and products such as acetals, Teflon, glass, electronics, that may appear in some medical or health care products. Also, radiation cannot be used for repeated sterilisation of items as required in hospitals, because of material degradation.

However, radiation is a method that can nearly always be relied upon to sterilise even the most difficult to penetrate, configured and tough to sterilise areas of some products, components such as stopcocks, powdered gloves, sealed lumens, impermeable crystals, etc.

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7 Dry Heat Sterilisation/Depyrogenation for Extremely Heat Tolerant and Non Liquid Materials

Dry heat sterilisation is one of the oldest sterilisation methods, but it is infrequently applied in the medical device industry, except in the pharmaceutical area where it is used as part of aseptic processing. It is used in sterilising dental instruments to minimise the corrosion of sharp items. It is commonly used in laboratories for depyrogenation of glassware to be used in pyrogen testing. 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. The Russians used an ethylene oxide (EO)/methyl bromide gas mixture [1, 2]. The US discovered that dry heat and ionising radiation were synergistic. Dry heat sterilisation has been generally reserved for materials and products that cannot withstand steam or for reason of depyrogenation.

7.1 Typical Products, Polymers, and Materials that are Dry Heat Sterilised

Healthcare products, polymers, materials that have been sterilised with dry heat include:

- Acetals
- Ceramics
- Cutting edge instruments
- Electronics
- Metal instruments and trays
- Metal needles
- Petroleum
- Polymers – acetal, polymethylpentene, polypropylene, some Nylons and Teflon
- Powders
- Glass syringes
- Glass suction containers
- Glassware – test tubes, flasks, vial oils – glycerine
- Earlier spacecraft circuit boards, component, metals, polymers, and materials*
- Silicone- prosthesis, implants

*Today however, NASA must use methods other than dry heat for sterilisation of today's electronics and other materials. These products and materials make the spacecraft lighter and smaller. This allows for the craft to be launched on smaller, cheaper launch vehicles.

Table 7.1 Some dry heat parameters	
Temperature, °C	Time, minutes
190	6-12 (with rapid recirculation)
180	30-60
170	60
160	120
105-135	Overnight - 24 hours
<i>Time will change with load size and items being sterilised. Typically time starts when items have reached sterilisation temperature.</i>	

Dry heat sterilisation typically requires high temperatures/time (see Table 7.1). The Russians used an EO/methyl bromide mix that did not use high temperatures, and could be used for more heat sensitive materials and electronics.

At extremely high temperatures there can be deleterious affects on many products, polymers or materials, however one can be assured of the destruction of pyrogenic substances/materials. Some of the disadvantages of dry heat sterilisation are:

- Heating is slow
- Longer sterilising times compared to steam
- Can be used for a very limited number of materials
- Limited packaging to allow for heat transfer

The transfer of heat by steam (sterilisation) at 121 °C is 12 times more effective than with hot air.

Dry heat sterilisation is generally carried out by hot air oven or infrared tunnel [3], but heated autoclaves without steam in the chambers have been used.

For packaging, a Nylon type sterilisation pouch is available for dry heat sterilisers. Also, there is a high temperature Nylon tubing in a variety of widths that is heat-sealed. For packaged devices, or products, the cycle time is longer to allow the heat to penetrate the packaging material. That is why the steriliser must have a 'packaged' cycle, not just a single time and temperature. Also, the packaged instruments must be 'loosely' loaded into the steriliser so that the heat can penetrate the packages. If packed too tightly the packages in the centre may not be sterile.

Dry heat has been suggested as the contributing cause of sterilisation by some atmospheric plasma process conditions because of the extreme temperatures achieved. The mechanism of inactivation of micro-organisms by dry heat is considered to be primarily an oxidative process, however lower temperatures with drier dehydrating conditions can accelerate inactivation by dry heat.

Because of its extremely high temperature requirements, e.g., 160-180 °C, dry heat has not been the preferred method of choice, except for special end product uses and needs.

In hospitals or central supply units many of the items that could be dry heat sterilised, come in sterile disposable form, however, dry heat is used most often today in dental offices for instruments, and in laboratories for oily, petroleum and powder materials.

7.2 Potential Inactivation Mechanisms of Dry Heat Sterilisation

Classically, inactivation of microbes by dry heat has been described as oxidation, however during spacecraft sterilisation research, the level of moisture in the bacterial cell profoundly affects the rate of inactivation. It was found that under extremely dry conditions, of less than 0.1 A_w, spores could be inactivated more rapidly [4].

It has also been found that DNA can be denatured and mutated, which can be another mechanism of dry heat inactivation. A review of apoptosis may shed further light on dry heat inactivation. Apoptosis can be defined as ‘gene-directed cellular self-destruction’ or programmed cell death. There are many ways of detecting apoptosis by flow cytometry and more common ones in use in this laboratory. Further details and practical issues such as staining protocols may be found in any standard text.

Apoptotic cells can be recognised by a characteristic pattern of morphological, biochemical and molecular changes, which may be broadly and chronologically defined as morphological changes. Morphological changes include:

- Cell shrinkage
- Cell shape change
- Condensation of cytoplasm
- Nuclear envelope changes
- Nuclear fragmentation
- Loss of cell surface structures
- Apoptotic bodies
- Cell/spore detachment

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Functional/biochemical changes include:

- Free calcium ion rise
- Bcl-2/Bax protein interaction
- Cell dehydration
- Loss of mitochondrial membrane potential
- Proteolysis
- Phosphatidylserine externalisation
- Lamin B proteolysis
- DNA denaturation
- 50-300 kb cleavage
- Intranucleosomal cleavage
- Protein crosslinking

It is recognised that calcium increases heat resistance, so that calcium loss should reduce its resistance to heat. Furthermore, spore dehydration also appears to reduce spore resistance to dry heat, as well as DNA denaturation. During sporulation, protein crosslinking, proteolysis, loss of mitochondrial membrane potential occurs, this could result in programmed dormant spore formation. These changes and others may contribute to dry heat inactivation through apoptosis. It may be that sporulation is a similitude of apoptosis that has developed a means for overcoming the process (death) by surviving, resulting in spore activation and germination. Knowing the mechanism of spore inactivation by dry heat may help to enhance the process.

7.3 Dry Heat Sterilisation

The dynamics of dry heat sterilisation can be improved by using convection ovens with significant circulation [5]. Basic dry heat ovens are merely heated baking chambers that allow air to circulate by gravity flow (gravity convection). Only good quality ovens made for professional use should be used. Forced draft (mechanical convection) ovens suitable for clinical use should be selected from well-calibrated equipment with FDA premarket approval or less expensive, high quality, equipment rated for industrial use. Heat must range above 160 °C. Individual dental instruments must actually reach above 160 °C for 30 minutes to achieve sterilisation. However, much additional time is needed to heat the chamber and instruments to that temperature, depending on the wattage of the unit. An oven thermometer measures only oven temperature, not instrument temperature. A thermocouple wire and pyrometer are needed to monitor instrument temperature.

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Mechanical convection (fan-operated forced draft ovens) may require an additional 0.25-0.5 hours to heat instruments (total time = 45 to 75 minutes), more or less depending on the wattage and the load size at a range of 170-175 °C. Standardisation is carried out with a pyrometer and verification with BI spore tests placed inside the bags.

Gravity convection ovens (have no fan or blower) may require 0.5 to 1.5 hours (1-2 hours total time) to heat a lightly wrapped, properly spaced load of instrument packs to sterilisation temperature. Time required in use will also depend upon the efficiency of the oven for its size, the size of the load, and how the load is packaged. A time of 60 to 90 minutes may be required to sterilise a medium load of lightly wrapped instruments in an oven set at a range of 165-175 °C. Paper, foil, or high-temperature Nylon wrap or bags should be used for dry heat sterilisation. Prolonged higher temperatures may melt the solder that holds instrument tips in place.

Dry heating temperatures fluctuate 5-10 °C above and below the setting during a cycle, so a 'range' rather than a specific temperature must be set. Without careful calibration, more sterilisation failures are obtained with ordinary gravity, convection dry heat ovens and with home-type mechanical convection ovens than any other type of steriliser. The only accurate way to calibrate a sterilisation cycle in most of the relatively inexpensive professional medical or professional industrial dry heat ovens is by using an external thermocouple wire attached to a temperature gauge (pyrometer). The sensing end of the wire is extended inside the oven and tied to an instrument in a centrally located pack to measure its exact temperature. Pyrometers are available from scientific supply companies. For continued use, the end of the probe wire is tied to an instrument left in a package in the steriliser as a control.

Caution: instruments cannot be added during a sterilisation cycle without restarting the timing. Special Nylon bags, foil or paper wrapped packs, or metal trays should be used for instruments. Packs/trays should be placed at least a centimetre apart to allow heated air to circulate.

Rapid dry heat sterilisation processes do exist that use forced draft or mechanical convection. The equipment must have FDA premarket approval. Heat must reach instruments long enough to heat surfaces to oxidise spores. Forced draft ovens that circulate air with a fan operate with rapid changes, using 6 minutes for unwrapped and 12 minutes for wrapped instruments, (e.g., Cox Dry Heat Ovens).

Hot bead sterilisers are not suitable for sterilisation of devices for re-use between patients; they are limited to use for re-disinfecting items during an endodontic treatment.

Vacuum ovens and lower temperatures can improve dry heat but not without development, evaluation, qualification, and validation. It is recognised that dry heat inactivation can

be enhanced by reducing moisture within the cell and the cell environment. Vacuums can help to evaporate moisture and help to drive the removal of moisture from cells and the immediate environment. It has been shown that dry heat is effective at temperatures as low as 66-88 °C through dry heat under vacuum, although the time needed for inactivation is excessive, (e.g., days). If there was a means to remove or extract bound water in bacterial spores, the time to inactivation could potentially be shortened. Some dry sterilisation requirements for validation and control can be found in the USP [8], PDA [9] and AAMI ST 63 [10].

7.4 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 dose to deliver to the package a 10^{-6} Sterility Assurance Level (SAL) but the package has to maintain this sterility level (10^{-6}) as well a minimum of 10^{-3} sterility assurance level for topical application use [6].

This can be determined through microbial challenge to pharmaceutical products that lie within moisture proof barrier containers like 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 that with most medical devices in breathable packages would result in failure, except for packaging, like the Nylon type sterilisation pouch available for dry heat sterilisation. For radiation most packages must be breathable and porous to allow for degassing of odours. For EO, chlorine dioxide, ozone, and hydroperoxide, porous packages are needed for permeation of the sterilants.

For evaluating the stability of packaging, maximum sterilisation must be delivered. If the product package can be resterilised, then it must be evaluated. The other parameters to be considered are zero time testing and heat ageing at 60-65 °C for 12 hours 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.

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The time of useful life of product should be determined and label claims may be imposed, e.g., expiry dating - internationally. Expiry dating is 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.

Common temperatures used to evaluate effects from temperature and determination 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 real room temperature goes up to five years, then a schedule may be tentatively constructed as follows:

- Zero time: includes 60 °C for 12 hours.
- 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 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.
- Two years at room temperature.
- Three years at room temperature.
- Four years at room temperature.

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Other more precise and applicable approaches toward looking at packaging stability may be obtained from AAMI TIR 15, *Material Qualification* [7].

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 when used will not compromise the safety of the patients using the product.

Sterile packaging can be cost efficient, effective, efficacious and safe provided that:

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 are considered.
3. The safety and assessment risk needs of the patient are met over an acceptable time period (e.g., expiration date).

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8

Alternative Methods of Sterilisation of Healthcare Products, Polymers and Materials

Over the years, newer methods of sterilisation have emerged, but none of them have had the staying power or uses of the classical or traditional sterilisation methods such as steam, dry heat, irradiation, low steam - formaldehyde or EO [1, 2].

All of these techniques have been around in one form or another for more than 50 years. The sterilising efficacy of steam, dry heat, steam - formaldehyde, irradiation, glutaraldehyde, and ozone have been known for more than 100 years. Advances, technological improvements and keeping up with the state-of-the-art is what has continued to make these classical methods viable and predominant. Less compatible and flexible agents such as dry heat, ozone, beta propriolactone, formaldehyde, propylene oxide, phenol, methyl bromide, chlorine and chlorine derivatives, glutaraldehyde, sulfur dioxide, ultraviolet light, iodine, and others have been used but were not flexible or compatible enough to become major players in the healthcare sterilisation field, however, sterilisation processes that are capable of inactivating all micro-organisms are great but not without limitations [3]. Only a few processes are capable of sterilising medical devices and healthcare products to a low risk of contamination of 10^{-3} to 10^{-6} .

New sterilisation processes such as hydrogen peroxide, plasma, chlorine dioxide, peracetic acid, ozone, microwave, and bactericide, pulsed light (e.g., PureBright) and plasma and sporicide can be good but are not without complications [4]. Not every hospital has sterilisers to use these processes for reprocessing, i.e., repeat sterilising. And some devices and packages cannot be easily penetrated. More current methods like electron beam and radiation have great penetration capabilities, but are not adaptable for typical hospital applications because they will eventually affect materials adversely after repeated reprocessing. Radiation frequently damages electronic components. Conventional methods like EO, glutaraldehyde and steam formaldehyde may be useful for sterilising electronic devices, but are extremely toxic, hazardous to handle, and leave toxic residuals. Use of traditional methods like gravity and flash steam sterilisation is limited because of excessive heat and material damage and deformation and moisture interference to certain materials and electronics. So what is left, are lesser methods of sterilisation in healthcare products [3, 5, 6].

8.1 Healthcare Products

Components that have been traditionally sterilised with dry heat are oils-glycerine, cutting edge instruments, metal instruments and trays, metal needles, powders, glass syringes, glass suction containers, glass, metal containers, and parts for use in subsequent aseptic filling techniques. But other heat stable polymers at validated lower temperatures could also be sterilised.

8.2 Gaseous Ozone

Most recently gaseous ozone sterilisation has been introduced but caution must be considered, because of its properties.

Ozone:

- Is an oxidising and bleaching agent.
- Has adverse chemical effects.
- Causes adverse changes in steel, brass, latex, and other polymers.
- Is not recommended for plastic devices.
- Has to be generated on-site.
- As an oxidising agent, does not have a penetration capability like EO - it is a surface sterilant.
- Has Occupational Safety and Health Administration (OSHA) exposure levels of 0.1 ppm 8-hour time weighted average (TWA).
- Has Immediately Dangerous Levels to Health (IDLH) of 5 ppm.
- Has a reportable quantity (RQ) in case of a release of 1 pound Superfund Amendments and Reauthorisation Act (SARA) Section 302 Environmental Health Services (EHS).
- Has limited penetrating capabilities in the presence of organic matter.

8.3 Gaseous Formaldehyde

Gaseous formaldehyde has been around for many years but is used less than it was previously because of toxicity, odour and carcinogenicity. Gaseous formaldehyde:

- Is still used in Asia, Japan, India, etc.
- Is used in room fumigation and in vapour cabinets.

- In the form of dry formaldehyde gas has been shown to be penetratable [7].
- Is considered to be a potential carcinogen – it has an IDLH of 20 ppm.
- Many Asian countries do not have standards or worker exposure. There may be no RQ standards in Asian countries.

8.4 Low Temperature Steam Formaldehyde

Low temperature steam formaldehyde has many characteristics of steam sterilisation but at lower compatible temperatures. Low steam formaldehyde:

- Is used in European countries, UK, Sweden, Holland, Germany instead of EO.
- Requires higher temperatures: 65-85 °C (150-185 °F) and higher relative humidity.
- Is not used in the United States *per se* (except for some very remote applications).
- Has OSHA worker exposure levels of 0.75 ppm 8-hour TWA and 2 ppm 15-minute short-term exposure limit (STEL).
- Is considered to be a potential carcinogen – it has an IDLH of 20 ppm.
- Has an RQ in case of a spill of 100 pounds under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA).
- Requires dynamic vacuum pulsing of steam-formaldehyde to improve penetration.

8.5 Formaldehyde/Solvent/Alcohol

Formaldehyde/solvent/alcohol sterilant has been used in place of steam sterilisation. Formaldehyde/solvent/alcohol is:

- Used in small dental table-top sterilisers, in the US, and primarily in the West to minimise corrosion and dulling of sharp instruments.
- A vapour at elevated temperature (132 °C), and minimal pressure of 0.14 MPa.
- Is mainly used for unwrapped dental instruments.
- Is not appropriate for heat and/or moisture-sensitive medical devices.
- Has OSHA worker exposure levels of 0.75 ppm 8-hour TWA and 2 ppm 15-minute STEL.

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- Is considered to be a potential carcinogen – it has an IDLH of 20 ppm.
- Has an RQ in case of a release of 100 pounds under CERCLA.
- Alcohol also has OSHA exposure levels.

8.6 Glutaraldehyde

Glutaraldehyde is commonly used in place of formaldehyde and frequently is a liquid sterilant. Glutaraldehyde is:

- Used in aqueous solutions.
- A dialdehyde with less toxicity than formaldehyde.
- A high level disinfectant/sterilant (if left in solution for within the specified time).
- Used for unwrapped items only.
- Used for sterilising animal tissues and enzymes.
- A cause of hazardous residuals.
- Is a mucus membrane irritant – it causes contact allergies.
- Can take between 7 and 12 hours to sterilise.

A new American Conference of Governmental Industrial Hygienists (ACGIH) set a threshold limit value ceiling limit (TLV-C) of 0.05 ppm as of May 1997.

Only ozone and formaldehyde have re-emerged as possible revised sterilants. Iodine has continued in use, but mainly as an antiseptic and for emergency sterilising of water [8]. But certain *Pseudomonas* species have been shown to actually survive in iodophors (iodine releasing solution) that are otherwise capable of killing spores.

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9

More Recent Alternative Methods of Sterilisation of Polymer Products

In recent years, oxidising agents and processes have been improved for applications in the healthcare industry. These agents include hydrogen peroxide, peracetic acid, ozone, performic acid, sodium hypochlorite, and chlorine dioxide. A comparison of some of these oxidising alternative sterilisation technologies are given in the following sections.

9.1 Peracetic Acid

Peracetic acid started life as a liquid area decontaminant, and has been refined for use in product sterilisation. Peracetic acid breaks down into acetic acid, water and oxygen all of which have a low toxicity. It is a popular alternative to glutaraldehyde.

Peracetic acid:

- Is a wet process.
- Is a strong oxidising agent produced from acetic acid and hydrogen peroxide.
- Is extremely reactive and consequently a very hazardous chemical.
- Is predominantly used with endoscopes but increasingly applied to other items, e.g., reverse osmosis membranes.
- Requires sterilisation equipment to be designed as a closed system.
- Requires rinsing with a neutralising agent.
- Has been approved by the Food and Drug Administration (FDA) and the Environmental Protection Agency (EPA) for processing endoscopic equipment as a liquid peracetic acid solution of 0.2%.
- In a sterilisation system, a 0.2% buffered solution at 50-55 °C, the peracetic acid is circulated through and around the devices for about 12 minutes. This is followed by an automated rinse to remove the sterilant from the products.

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- Requires specifically designed trays or containers which are used for positioning the instrumentation.
- As with any liquid process, monitoring is a problem. It requires use of *Bacillus stearothermophilus* biological indicators.
- Has a by-product, acetic acid, which has an Occupational Safety & Health Administration (OSHA) and NIOSH exposure limit of 10 ppm 8-hour TWA.
- Immediately Dangerous to Life or Health (IDLH) level for acetic acid is 50 ppm.
- Has an RQ of a release of 1 pound under SARA Section 302 Extremely Hazardous Substances (EHS).

9.2 Vapour Phase Hydrogen Peroxide

Low temperature vapour phase hydrogen peroxide is quite efficacious, however it could leave peroxide residuals and have limited penetration compared to EO.

- The system is currently being sold in Europe; a FDA regulatory submission 501k was withdrawn in the US.
- Is designed to sterilise unwrapped metal instruments only; it is basically used for unwrapped dental instruments.
- Has an OSHA and NIOSH exposure standard of 1 ppm and is listed in the OSHA *Standard for Air Contaminants* and requires monitoring.
- The RQ is 1 pound in concentration > 52% under SARA Section 302 EHS.

9.3 Chlorine Dioxide

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

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

- Is unstable and classified as a hazardous substance.
- It has an OSHA extreme limit of 0.1 ppm for an 8-hour TWA and a NIOSH limit of 0.1 ppm 8-hour TWA plus a 0.3 ppm STEL.
- It has an IDLH level of 5 ppm.
- Investigations are ongoing for industrial application but not for healthcare facilities.

Peracetic acid and hydrogen peroxide have been combined with plasma(s) for synergistic responses and reduction of concentration of the individual agents [1, 2].

9.4 Peracetic Acid/Hydrogen Peroxide Plasma

Peracetic acid plasma is similar to hydrogen peroxide plasma but has acetic acid, oxygen, and water as by-products.

- The system has been on the market and is sold as an alternative to ethylene oxide (EO).
- Chemically, peracetic acid is the equilibrium mixture of hydrogen peroxide 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 peracetic acid is introduced into the sterilisation chamber under a deep vacuum. In phase two, a nonflammable mixture of hydrogen, oxygen and a carrier gas is subjected to microwave electromagnetic energy to create the plasma.
- Peracetic acid vapour breaks down to hydrogen peroxide 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, peracetic acid/hydrogen peroxide 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 hydrogen peroxide is 1 ppm for an 8-hour TWA.

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- OSHA exposure standard for acetic acid is 10 ppm for an 8-hour TWA.
- The IDLH is 75 ppm.
- The RQ on hydrogen peroxide is 1 pound and peracetic acid 1 pound under SARA Section 302 EHS.
- The steriliser would be classified as a Class II medical device, requiring performance standards. At this point, no performance standards are established.
- Hydrogen peroxide at 35% or more is a toxic substance.

According to the Agency for Toxic Substances and Disease Registry (ATSDR) and Sax's Dangerous Properties of Industrial Materials [3], there are adverse health effects with both acute and chronic exposures.

9.5 Hydrogen Peroxide Gas Plasma

Hydrogen peroxide vapour by itself has been shown to be sporicidal, but in combination with plasma its activity is enhanced and the plasma eventually facilitates the breakdown of hydrogen peroxide residuals into water and oxygen.

- The system is available commercially and sold as a replacement for EO.
- The system uses a cartridge, called a pillow, with ten (10) unit dose cells that contain a 58% solution of hydrogen peroxide.
- Hydrogen peroxide gas plasma is generated in the unit under a deep vacuum with electrical power from radiofrequency energy to convert vapour to plasma.
- Unlike EO, which is an alkalising agent and penetrates through packaging and most devices, hydrogen peroxide gas plasma is an oxidising agent and has only surface contact capability.
- A variety of devices can be subjected to the process with material evaluation and qualification.
- Certain packaging material is provided by the manufacturer. No textiles or cellulose material common to a healthcare facility central service department can be used with the system.
- Items longer than 12 inches with a lumen are not recommended for the process without the use of an adapter, currently unavailable in the US.

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- Sterilisers would be classified as Class II medical devices, requiring performance standards. Unlike EO gas sterilisers, which have performance standards developed through ANSI/AAAMI, the hydrogen peroxide system does not currently have a specific performance standard.
- Hydrogen peroxide has an OSHA and NIOSH exposure standard of 1 ppm for an 8-hour TWA, is listed in the OSHA Standard on Air Contaminants, and requires monitoring.
- The manufacturer does not recommend environmental monitoring.
- Reports from the field indicate there have been health problems associated with the system.
- Hydrogen peroxide at 35% or more is a toxic substance - this system uses a 58% solution.
- According to the ATSDR and *Sax's Dangerous Properties of Industrial Materials* [3], there are adverse health effects with both acute and chronic exposures.
- The IDLH is 75 ppm.
- The RQ is 1 pound in concentration > 52% under SARA Section 302 EHS.
- Enclosed system generates hydrogen peroxide gas plasma (from 58% hydrogen peroxide).
- Effective for sterilisation.
- Sterrad can sterilise during a 45 minute cycle.

Of all the sterilants described, it is interesting to note: that ozone, hydrogen peroxide and peracetic acid all have the capability of producing breakdown products that are not toxic. For example:

Ozone breaks down to oxygen,
Hydrogen peroxide breaks down into water and oxygen,
Peracetic acid changes to acetic acid, water and oxygen,
Plasma actually improves their breakdown, and
Chlorine dioxide does not breakdown into non-toxic residuals.

Later on I will discuss methyl iodide, a new pesticide agent, that breaks down into methane and iodine which are of low toxicity. Iodine is an antiseptic, compatible with skin tissue [4].

9.6 Low Temperature Hydrogen Peroxide Gas Plasma

Plasma is considered to be a fourth state of matter, and different from chemicals in solids, liquids, and gases. A plasma usually consists of a reactive cloud of ions, electrons, free radicals, and other neutral species. Plasmas can be produced at very high temperatures, or from low temperatures in strong electromagnetic fields (the so-called 'glow-discharge' plasmas). The Northern lights are a natural example of plasmas.

The process must pass the barrier of packaging materials by using a gas-diffusion phase to allow gas to penetrate to all parts of the load before the plasma is created. Hydrogen peroxide gas must diffuse to all parts of the load to be effective and the load of items must not interfere with subsequent plasma formation. Although this system is widely used in the US, there are still limitations with its use because product cleaning prior to sterilisation needs to be well controlled. Sterrad can have an adverse loss of strength effect on latex. Loss of strength of rigid 70D pellethane polyurethane can occur with both Sterrad and Plazlyte (peracetic acid and plasma). Nylon was not adversely affected by the hydrogen peroxide plasma process. The Sterrad system is marketed as a replacement for EO. This process using just hydrogen peroxide can degrade Nylon, like many other oxidising agents.

Oxidising agents and plasmas are typically not compatible with a variety of materials and products such as: cellulose products, cotton, paper towels, certain packaging materials, muslin, dressings, organic materials, water, biomaterials, electronics or electrical equipment and wadding. In the case of cellulosic materials the agent will be absorbed leading to complete sterilisation of the device.

Plasmas and hydrogen peroxide cannot sterilise as many plastics and devices, as EO and they are limited, because they have less permeability than EO. When designing materials for devices using plasmas and oxidisers, it is best to avoid decomposers - such as silver, copper, and copper alloys - and absorbers, such as polyurethane, Nylon, ethyl vinyl acetate and cellulotics.

Some of the materials that hydrogen vapour can sterilise are:

Metals: some aluminium, some chlorine and stainless steel

Non metals: ceramics, silica and glass

Some polymers and elastomers: polyethylene, polypropylene (PP), PP copolymer, polymethylpentene, polyphenylene oxide, Tefzel [ethylene tetrafluoroethylene (ETFE)], fluoropolymers [PTFE, copolymer of poly tetrafluoro ethylene and perfluoralkyl vinyl ether, fluorinated ethylene propylene (FEP)], chlorinated polyvinyl chloride, polyvinyl chloride, polystyrene, polycarbonate, polyethylene glycol terephthalate (PETG).

If low temperature hydrogen peroxide gas plasma is used for sterilising disposable products (single use), which are not reprocessable, more materials (polymers) could be acceptable.

9.7 Chlorine Dioxide

Chlorine as a vapour is useful as a hospital sterilant and as an area fumigant against anthrax. Chlorine dioxide was investigated in the mid to late 1980s and into the 1990s [1, 5, 6]. Chlorine dioxide without plasma can be relatively reactive. The process uses a cycle similar to that of EO sterilisers, with vacuum air-removal followed by a dynamic prehumidification phase to humidify the product load to an RH of about 70%. At the end of the preconditioning phase, chlorine dioxide gas is added to a concentration of 30 mg/l. Then nitrogen is added to a pressure of 80 kPa. A gas exposure time of about 60 minutes is typical. At the end of the cycle, the chlorine dioxide is removed using a four-pulse dynamic air exchange.

Chlorine dioxide can be corrosive to metals and incompatible with some plastics, i.e., polycarbonate, yet it has been used to sterilise medical devices such as contact lens and overwrapped foil suture packages.

Other medical applications are possible. One of its chief advantages is that it is not a carcinogen, and it does not react the same as the other oxidising agents. Also because chlorine dioxide does not have the chemical solubility of EO - there are no significant levels of residual sterilant within the product material, which indicates it doesn't have the penetration that EO has. Chlorine dioxide is not flammable in air at the concentrations used, as EO is.

The sporicidal effect of chlorine dioxide on standard spore strips has been demonstrated in independent studies at 100 ppm and a four hour exposure through two layers of porous materials. Chlorine dioxide gas has been successfully applied to a 410,600 m³ facility in a single treatment.

9.8 Gaseous Ozone

Gaseous ozone is typically highly reactive and has poor penetration but recent applications, for example, accelerating diffusers, allow for improved penetration and material compatibility. Gaseous ozone is a very strong oxidising and bleaching agent. It has been used for sterilising water for a long time, and for decontaminating working areas. It was used in the late 1930s and 1940s in cold storage of preserving fruit. It has been approved

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by the EPA as a sterilant for medical applications, but it is limited in product, polymer and material use, because of its reactivity [1]. It has adverse chemical effects and causes changes to steel, brass, latex, and other polymers. It is not recommended for plastic devices, however, it has been used to sterilise endoscopes. Ozone has to be generated on-site, like chlorine dioxide. As an oxidising agent, it does not have a penetration capability like EO - it is a surface sterilant. Its OSHA and NIOSH exposure level is only 0.1 ppm for an 8-hour TWA. Its IDLH is only 5 ppm. The RQ in case of a release is 1 pound SARA Section 302 EHS.

The sterilising capacity of ozone has been known for a long time. It has been frequently used in decontaminating and sanitising water, but its use as a gaseous sterilant is limited because of its instability. Recent advances have made the generation of ozone a more practicable proposition, and commercially available sterilisers have been developed by companies such as the Cyclops Company. Gaseous ozone requires humidity to be effective. Cyclops developed ozone generating technology that is small and highly efficient. Their technology allows the development of ozone sterilisers that are compact, easy to operate and cost effective in both hospital and manufacturing settings.

Ozone sterilisation is environmentally compatible. No toxic chemicals are purchased for use in the steriliser and no harmful emissions result from the process. All that is needed is oxygen, electricity and a small amount of water. An ozone generator located inside the machine converts the oxygen into ozone during sterilisation. After sterilisation is complete, the ozone is converted back into non-toxic oxygen before it is released into the environment. The major limitation of full use of ozone has been its extreme reactivity, and lack of deep penetration of soiled materials. It fails to inactivate microbes that are encrusted in or surrounded with organic matter.

An agent capable of inactivating anthrax in buildings could be gaseous ozone. Because of its high reactivity and instability, it was not used during the Anthrax attack in the Hart Building in Washington, DC. However, unlike other chemicals, ozone sterilisation may be improved under cold environments. The ozone may be less reactive, more stable, and more permeable by reducing ambient temperatures to cold environments. For years, ozone has been used in cold storage rooms for maintaining fruits preserved in a storage environment.

If the reactivity and stability of the ozone can be decreased by reducing the temperature, and other conditions, it could penetrate deeper into organic matter before reacting or destabilising into oxygen. Penetration of ozone may be controlled by vacuum in the chamber, or enhanced by adding humidity. At completion of exposure time, oxygen is allowed to flow through the chamber to purge the ozone. Cycle time may be up to 60 minutes depending on the size of the chamber or load.

9.9 Liquid Sterilants

Liquid sterilants by their nature have limitations. They typically cannot sterilise a product within a package without leaving residuals. Typically items are immersed in the sterilants and then removed, exposing them to extrinsic contamination. But sometimes they are the only method applicable and available for a specific end use, e.g., biomaterials. If handled correctly they can effectively sterilise without adventitious (accidental) contamination afterwards.

9.9.1 Glutaraldehyde within Closed Systems

Glutaraldehyde is a dialdehyde [4]. It is used in aqueous solutions, e.g., 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 on mucus membranes. The 1997 ACGIH Ceiling Limit is 0.05 ppm TLV-C. 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. However, because of lack of *in situ* packaging sterilisation, it is difficult to achieve/maintain sterility, and its activity, and thus effectiveness as a sterilant has been questioned.

In recent years, glutaraldehyde has been used to sterilise biomaterials such as porcine heart valves under sterile environmental assembly conditions, and subsequently used as a preservative to maintain sterility. In this case the glutaraldehyde can act both as a protein crosslinker, and 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.

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 hours of exposure time for it to fully sterilise materials. Its rate of sterilisation can be increased by increasing 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.

9.9.2 Peracetic Acid

Peracetic acid is a strongly oxidising solution of acetic acid and hydrogen peroxide. It is typically a wet process. It has been used as a vapour [4]. It is extremely reactive and consequently a very hazardous chemical. It has limited use basically for endoscopes and other qualified items. The equipment is a close designed system. It requires rinsing with a neutralising agent. A liquid peracetic acid solution of 0.2% has been approved by FDA and EPA for processing endoscopic equipment. The buffered solution at 50 °C to 55 °C is circulated through and around the devices for about 12 minutes. 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* biological indicator (BI) is used with peracetic acid. Acetic acid is the by-product and has an OSHA and NIOSH exposure limit of 10 ppm for an 8-hour TWA. The IDLH for acetic acid is 50 ppm. The RQ for peracetic acid in case of a release is 1 pound under SARA Section 302 as an EHS.

The use of peracetic acid/plasma process has come into question, because of its effect with copper and other substances, in ophthalmic injuries. Ophthalmic cannulas (small-lumen instruments) may have nickel- or chrome-plated brass hubs. This method uses a vapourised mixture of peracetic acid, acetic acid, and hydrogen peroxide in combination with low temperature. The vapour was removed with argon, oxygen, and hydrogen gas.

Brass can be oxidised to yield copper and zinc compounds. Preliminary results using inductively coupled plasma atomic emission spectrometer analyses performed at CDC revealed copper and zinc in the cannulas sterilised in the Abtox Plazlyte (peracetic/plasma) system. When this rinsate was infused into human and rabbit corneas, corneal decompensation occurred. The use of the Abtox system was discontinued at the St. Louis VAMC, and ophthalmic instruments were sterilised by steam autoclave.

Peracetic acid in a 0.2% solution requires a 12 minute exposure to achieve sterilisation using the SYSTEM 1 Sterile Processing System. Acecide, another peracetic acid based formulation, claims to achieve high level disinfection with a five minute exposure in a 0.3% solution.

9.10 Chemiclave

The Chemiclave is a formaldehyde/alcohol vapour chamber process used primarily in small 'table-top' dental sterilisers [7]. It is a vapour at elevated temperature (132 °C), with minimal pressure of 0.14 MPa. Mainly used for unwrapped dental instruments, the

chemiclave is appropriate for heat and/or moisture-sensitive medical devices. The OSHA worker exposure levels - for formaldehyde are 0.75 ppm for an 8-hour TWA and 2 ppm for a 15-minute 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 CERCLA. Alcohol also has OSHA exposure levels. Its advantage is that it 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 soluble lubricants. Steam sterilisation is not necessarily the optimal method that preserves handpiece function best for all dental handpiece designs.

The Chemiclave alcohol/acetone/formaldehyde vapour must penetrate thin packs and condense on dry instruments to kill spores. It requires - 131 °C and 0.14 MPa pressure, about 30 minutes 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 vapour. When possible, the steriliser must be cool before opening door to reduce fumes. Not suitable for towel packs.

9.11 Aseptic Processing

One method of sterilisation not discussed is aseptic processing, because it is not the preferred method of sterilisation. In general, it is preferred to sterilise products in their final configuration and packaging to minimise the risk of microbial contamination. Products designed for aseptic processing generally consist of components that have been previously sterilised by one of the previous terminal sterilisation methods.

Aseptic processing frequently incorporates other methods of sterilisation, such as steam, EO, dry heat, and filtration.

9.11.1 Filtration

Sterilisation by filtration refers to the removal of viable microorganisms by the use of filters [1]. Sterilisation by filtration is a practical, yet last resort method of sterilising liquids or drugs, that can't be heated or irradiated. Because it is like liquid sterilising

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methods, it borders on being a non-terminal sterilisation method. It is difficult at times to assure a SAL of even 10^{-3} probability of survivors, because of the general way it is used and applied. Sterilisation by filtration is commonly used in the pharmaceutical area for sterilisation of drugs that would be adversely affected by steam heat, but there are other healthcare products, and devices containing liquid that require this approach [1]. It is also commonly used in the sterilisation of air for clean rooms and other spaces. The method is also used in some devices as means of assuring against adventitious or accidental contamination during use. The method may be used in producing contactable rinse solutions.

The types of filtration may be further delineated by the types of filters used:

- Porous (membrane) filters
- Depth (probability) filters
- Charged or absorptive filters

Filtration can also be performed using reverse osmosis or ultrafiltration.

The type of sterilisation by filtration can also be described by the filter size, rating, or grade:

Membrane: 0.45 μm , 0.22 μm , or 0.1 μm pore size

HEPA: 99.99% efficient

The currently accepted standard for most liquid sterile filtration is the 0.22 μm filter, but the suggested filtration level of 0.1 μm is being 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.

9.11.2 Sterile Assembly

Sterile assembly is a means of putting together sterilised parts, components, product or packaging under a sterile, controlled environment. It requires personnel to wear sterile gowns (bunny suits) and gloves, to use sterile isolation hoods, laminar flow benches, sterile tunnels, etc. But, it provides a means of sterilising materials that can not be sterilised terminally in place, such as porcine heart valves, enzyme detector kits, heat labile drugs, etc.

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10 Potential Applications and Developments of Sterilisation Techniques

10.1 Chlorine Dioxide – Another Look?

After being used to sanitise the buildings of the US House of Representatives contaminated with anthrax, chlorine dioxide may be used as a sterilant. But whether it can sterilise inside a computer, or other complex pieces of equipment or products, is not fully known. It is a good surface sterilant with limited penetration as a reactive gas. Yet its parameters and sequence are very similar to low temperature ethylene oxide (EO). Chlorine dioxide has sporicidal properties and can be used at 27 °C to 35 °C, at a concentration of 10-50 mg/l, and 70-80% relative humidity [1]. Its limitation is formation of residuals; however, gaseous chlorine dioxide can't be scrubbed with sodium thiosulfite ($\text{Na}_2\text{S}_2\text{O}_3$) to remove these residuals. Residual levels of well below 1 ppm can be achieved and are usually undetectable. In the case of the decontamination of the congressional buildings, a white residue was observable. However, chlorine dioxide can also be used as a liquid sterilant, as well as a gaseous agent. This dual state may provide unique opportunities for sterilisation. Its advantage over many other sterilants is its lack of carcinogenicity and reactivity when compared to other common oxidising agents such as ozone, bleach and hydrogen peroxide, and it is likely to have greater penetration than these common oxidising agents.

Peracetic acid has been used in the past as a vapourised sterilant. Acetic acid is enhanced with hydrogen peroxide to form peracetic acid, which can break down to non-toxic acetic acid, water and oxygen. Together their individual concentrations may be decreased, resulting in lower residuals.

10.2 Heat Sterilisation – Something Old but with a Look to the Future

Heat sterilisation is an evolution of sterilisation techniques used in pharmaceutical applications, aseptic processing, spacecraft sterilisation and dental sterilisation. In many ways, it has been a victim of its own success. Traditionally, it has been characterised primarily by its extremely high temperatures to depyrogenate extremely heat-resistant pyrogens, or to sterilise extremely resistant pathogenic thermophiles which other methods like radiation are not required to fully destroy or demonstrate!

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Its simplicity and low capital cost make it a very cheap, attractive and more useful method than is commonly considered [2]. Standard, steam and dry heat sterilisation are considered separately.

Steam sterilisation is typically a surface sterilising method. Steam itself does not penetrate as dry heat does. When using steam for sterilisation, conditions are reached much faster, (e.g., 12 times), than with dry heat due to the condensation of the steam. However, the effect of steam sterilisation is measured by its ability to inactivate microbial spores upon contact with the heat of condensation and moisture, so that we do not look for inactivation of microbes or spores that do not come in contact with the moist heat of steam. For dry heat we look for inactivation of micro-organisms by their exposure to dry heat, in areas often impenetrable to steam. If we were to combine the two methods, we could sterilise surfaces where most of the microbes are likely to be encountered, more effectively with steam, and sterilise areas impenetrable to steam more effectively by use of the dry heat.

Because dry heat is often used for objects that are damaged by high levels of moist heat, e.g., electronics, other agents capable of faster heating than heating air can be used, (e.g., the Chemiclave). The only problem with the Chemiclave is that it relies on formaldehyde to do some of its killing. It does not rely solely on alcohols, ketones, or ether to heat and kill.

The use of saturated methanol may be an improvement, because it has one of the highest heats of condensation below that of steam, and it does not have any toxic residuals *per se*. It is not a sensitiser, carcinogenic or genotoxic. Many years ago during spacecraft sterilisation research, methanol was considered to be an acceptable material solvent vapour to be used with formaldehyde, because it minimised the polymerisation of formaldehyde, and it did not damage most plastics, polymers or create adverse effects on other materials as did other chemicals, and solvents such as ISOPropyl alcohol. Methanol can be purchased that is very dry; it is used to determine the presence of moisture, so it has the excellent dehydrating properties of dry heat, as well as heating properties with a heat of vapourisation just below that of steam. In other words condensating methanol could deliver heat much as condensating steam does, but actually with less sudden heat, so that it may be more compatible and kinder to heat-sensitive drugs contained in containers. Thus it may be an improvement over steam, where the steam's higher heat of condensation may result in heat degradation of some drugs and materials or distortion of some plastic polymers, while methanol may not.

The ancient and classical process of dry heat for preservation and decontamination has been around since we learned to use fire to prevent the spread of diseases many centuries ago, perhaps even two millennia.

Coincidentally you may have already used dry air heat at home when baking at 150-180 °C for two to four hours, because the effect of dry heat on micro-organisms may be

equivalent to that of baking or steam sterilisation when processing bottles of food in a steam pressure cooker.

Dry air heat sterilisation is probably the least used and least recognised method of sterilisation in the medical device industry. Dry heat has been predominantly used in spacecraft sterilisation, for pharmaceutical sterilisation of glassware containers, powders, ointments and oils; steam has been used in a very limited way in the food, parenteral and hospital areas, but also for the sterilisation of medical devices.

Dry heat sterilisation exposure times can range from as short as 6-20 minutes at 190 °C to 30 minutes at 160 °C or as long as 12-16 hours between 105-135 °C, depending upon the product and the bioburden that is being heated and sterilised.

Steam sterilisation has not been used frequently for medical devices except for reprocessing. It has been used for as little as three minutes at 134 °C, and for as long as 30-40 minutes at 115 °C. Parenteral drug products can be sterilised for as little as eight minutes at 121 °C. With extrapolation and validation, steam sterilisation may be achieved at temperatures as low as 100 °C with an EO like exposure of hours.

The dry heat sterilisation of 190 °C for six minutes is part of the NASA success story with its spacecraft sterilisation. Using the NASA report, Cox was able to design the fast effect and economic sterilisation of dental and medical instruments. However, above 191 °C some caution should be applied. The temper of some metals may change at 105-135 °C, and so 12 hours of exposure may be considered too long, but if you consider an EO process with aeration or transportation for contract radiation, it may be competitive. Flaming loops may be sterilised in seconds at 1800 °C, but this may incinerate most organics. Continuous dry radiant heat air transferred from conveyor or tunnel ovens can sterilise glass vials in seconds to minutes at very elevated temperatures as part of the aseptic processing system, but the items must not have any heat diffusion or hidden barriers.

The 121 °C for eight minutes is part of a Food and Drug Administration – Good Manufacturing Practice (FDA-GMP) sterilisation story, which allowed for use of less heat to prevent degradation of drug solutions like glucose, amino acids, etc.

The use of the classical method of steam is limited because excessive heat from condensing steam can deform and melt heat labile materials and cause wetting and wet spots (water blush), and interfere with electronics. Without the moisture from steam, the dry heat method becomes more attractive and useful for electronics, heat stabilised plastics and rubber (silicone and thermoplastic elastomers), and materials that are blushed (wetted) and damaged by high levels of moisture. There is an interest in using dry heat to sterilise dental devices, to prevent the corrosion of metals and the dulling of the cutting edges of instruments. Dry heat has proven to be successful already in the sterilisation of silicone

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artificial prostheses such as mammary glands and other implants over the past 25 years, where EO sterilisation would have left high toxic residuals. Dry heat has been successfully used in dental, laboratory, pharmaceutical and spacecraft applications. It has been used in the medical device and diagnostic industry - an idea suggested for sterilisation of silicone implants some 25 years ago.

Dry heat sterilisation has been used where damage would occur, (e.g., with oils, electronics) by moist heat or radiation, or from impermeability and non-compatibility with EO, or alternative processes. And, when one realises that excessive dry heat temperatures of 160 to 180 °C are not necessarily needed to be effective, but that temperatures between 105 to 135 °C can work, there is a better case for the use of dry heat in sterilising medical devices. Similarly, if lower temperatures of steam can be used, many more polymers could be sterilised.

In the UK, steam sterilisation at 115 °C has been used. With use of formaldehyde, steam sterilisation has been reduced to 70-80 °C.

According to Martin Favero [3], previous head of CDC activities in spacecraft sterilisation and planetary quarantine: 'Steam autoclave and dry heat sterilisers are among the most reliable and error-free methods available for use in hospitals.' According to the FDA, use of dry heat and steam methods convey inherently greater sterility, assurance, confidence and reliability than liquid chemical sterilants.

With sufficient time and loss of moisture, dry heat sterilisation can be achieved at lower temperatures than those used conventionally or traditionally.

This Author has observed inactivation of 10^6 spores at dry heat temperatures of less than 100 °C (e.g., 75 °C), in the presence of an active dehydration medium that displaces or reorientates water from the bacterial spore. Davis and co-workers found that survival of spores at 60 °C was lower under vacuum than at atmospheric pressure. At 88 °C there was no growth under vacuum after four to five days [2]. Consequently dehydration plays a greater role in dry heat sterilisation than is typically realised. The influence of moisture with heat is also often overlooked.

The presence of moisture could cause denaturation or coagulation of protein. The following basic example of egg coagulation is a easy way to understand the influence/effect of moisture on protein coagulation/denaturation with heat [4, 5]:

Albumin plus 56% water – coagulates at 56 °C

Albumin plus 25% water – coagulates at 74-80 °C

Albumin plus 18% water – coagulates at 80-90 °C

Albumin plus 6% water – coagulates at 145 °C

Albumin plus <1% water – coagulates at 160-170 °C

Consequently, theoretical inactivation of non-thermal resistant microbes may be possible at temperatures as low as 56 °C, although it is the Author's opinion that heat temperatures of 66 °C or greater may be needed to achieve inactivation of most spores, but with additional dehydrating potentiation. Note: any dry heat sterilisation less than 160 °C needs to be further developed and validated.

Combining sequencing between wet and dry heat sterilisation under certain conditions may provide additional opportunities not observed with either alone. For example, since the transfer of heat by steam sterilisation at 121 °C is 12 times greater than with hot air, a load may be initially heated by steam, followed by dry heat to minimise and prevent damage by excessive moisture of steam over time.

Also the use of microwaves to heat may be advantageous. Microwaves have been used with steam to reduce the sterilisation time. Thermal irradiation and ionising irradiation have been found to be synergistic.

In the device area, if steam sterilisation was validated in the same way as radiation, through resistance modelling, bioburden populations without thermophiles and anaerobes, and resistance, much lower levels of temperature and time could be demonstrated and verified!

Only a few plastics and materials have been recommended for traditional dry heat sterilisation: ethylene-chlorotrifluoroethylene copolymer (ECTFE), ethylene-tetrafluoroethylene (ETFE), TEFLON like fluorinated ethylene propylene (FEP), TEFLON like perfluoroalkyl (PFA), polymethylpentene (PMP), silicone, glass and metals. With lower temperatures, e.g., 105-120 °C, additional polymers could be tentatively processed and used such as: acetal, Cellophane, polycarbonate, high density polyethylene (HDPE), polypropylene, polypropylene copolymers, Mylar, some phenolic plastics, polysulfones, some specific polyesters, e.g., Tyvek, some aromatic polyurethanes (PU), silicone, and possibly some other thermoplastic elastomers and other materials could be sterilised (see **Table 10.1**).

Some materials such as ABS, acrylics, acrylonitriles, aliphatic PU, typical cellulotics, polystyrene, low density polyethylene, rigid polyvinyl chloride, glycol modified polyethylene terephthalate (PETG), styrene-acrylonitrile (SAN), etc., may be damaged by high temperature heat but, unlike ionising radiation's typical damage of acetal, unstable PP and Teflon, these would be good candidates for heat. Some PU which can be hydrolytically attacked by steam and potentially by radiation, can be treated by low temperature dry heat as there is no moisture to cause such a breakdown of aromatic PU. 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 and Tyvek. Cuprophane used in dialysers that is sensitive to moist heat and radiation may be compatible with low temperature dry heat. PVC tubing can be blushed and clouded by steam sterilisation above 75 °C, but cleared by dry heat oven at 110 °C. Material molecules are typically more stable in low temperature heat than in radiation.

Table 10.1 Potential low but heat tolerant sterilisable polymers and materials polymer or material type tentative maximum heat temperatures	
Polymer	Maximum heat temperatures
Acetal (ACL), delrin, or polyoxymethylene	up to 121 °C (dry)
Aluminum	up to 190 °C (dry)
Cellulose acetate (non load)	up to 120 °C
Cellulose acetate butyrate (non load)	up to 130 °C
Glass	>190 °C
Grease	(depends upon the type of grease) (dry)
ECTFE	up to 150 °C
ETFE	up to 150 °C
Petrolatum gauze	up to 160 °C
High density polyethylene (HDPE)	up to 120 °C
Metals (note some metal temper may occur above 160 °C)	up to 190 °C (dry)
Muslin	up to 160 °C
Nylon (polyamide heat stabilised grades)	up to 130 °C
Paper (varies depending upon paper)	up to 160 °C (dry)
Polycarbonate (PC)	up to 134 °C
Polyetherimide	up to 134 °C
Polyetherketone (PEI)	up to 250 °C
Polyethylene terephthalate copolymer (PETG)	up to 170 °C
Poly 4-methyl-pentene-1 (PMP)	up to 170 °C
Polypropylene (PP)	up to 135 °C no stacking
Polyphenylene oxides (PPO)	100-148 °C
Polypropylene copolymer (PPCO)	up to 120 °C
Polysulfone (PSF)	up to 160 °C
Polyurethane (PU-aromatic)	varies considerably depending upon grade and load
Polyvinyl chloride tubing (flexible-non load, varies)	up to 120 °C
Polyvinylidene fluoride (PVF)	up to 125 °C
Silicones	up to 200 °C
Teflons	up to 170 °C
Polytetrafluoroethylene (PTFE)	up to 170 °C
FEP	up to 170 °C
PFA	up to 170 °C
<i>Deflection/maximum temperature can vary with formulation changes.</i>	

Although considerable research has been performed on most methods of sterilisation, heat would have been neglected until it became necessary to find a way of sterilising the probes used in space exploration, and to sterilise foods. More recently, the Cox dental dry heat steriliser has incorporated heated air transfer laminar flow that forces air through a chamber at the rate of 0.91 km per minute and achieves a 12 log or 10^{12} spore inactivation in only six minutes at 190 °C [2]. A chemical vapour dry heat steriliser exists that can sterilise in 20 minutes at 132 °C. Dry heat tunnels and ovens are typically used in the pharmaceutical and biotechnology industries.

Heat sterilisation can be a candidate for a parametric release process, which is as easy to use as ionising radiation where only thermal lethality delivered is needed, instead of dose.

With parametric release, inexpensive in-house heat sterilisation could become competitive with contract radiation that requires a one- to three-day turnaround for scheduling, processing and shipping. Reduced biological indicator (BI) incubation and rapid BI may shorten the time of release.

The low heat process should be validated with exposure times that are competitive with EO sterilisation and aeration or better. Times for dry heat typically vary with the temperature that the item is exposed to and the final temperature achieved. Some examples are provided in Table 10.2.

Table 10.2 Some possible alternative time-temperature relationships for dry heat	
Temperature	Time
150 °C	150 minutes
140 °C	180 minutes
121 °C	Overnight, (e.g., 12-15 hours)
111.7 °C (105-135 °C)	30 hours
88 °C*	4-5+ days** (vacuum)
Unwrapped and predried items have been shown to be effective at six minutes and wrapped items effective at 12 minutes at 190 °C with increased airflow and velocities with forced air and recirculation.	
*Has been shown to be as effective as low as 60 °C under vacuum but three times longer than at 88 °C or approximately 12-15 days.	
**May require longer times for an SAL of 10^{-6} .	
Note: None of these conditions must be considered effective unless validated.	

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Time of processing can vary significantly depending upon the level of processing sophistication used [6, 7].

For example the use of DMSO medium could enhance dehydration, dry heat sterilisation of spores – decreasing, the time and temperature parameters. However, DMSO is a solvent and thus harmful for many polymers.

Time of processing can vary considerably with steam sterilisation as shown in Table 10.3.

Table 10.3 Some additional future time-temperature relationships for moist heat	
Temperature	Time
121 °C	8-15 minutes
118 °C	18-24 minutes
115 °C	30-40 minutes
110 °C	100-120 minutes
104 °C	6.6-9 hours
100 °C	17-25 hours
74-80 °C*	4-8 hours
66-74 °C*	3-4 days
<i>*Hypothetical sterilisation time for inactivation of non-thermal, non-anaerobe spores, as required for validation with radiation, for sterilisation for dry (non-growth supporting) medical devices and hospital products.</i>	
<i>Note: None of these conditions should be considered unless validated. Times may change depending upon the bioburden resistance, load size and the items to be sterilised and the validation approach used (e.g., bioburden versus overkill approach).</i>	

10.3 Pulsed-Light Sterilisation

This process was introduced about ten years ago and uses intense pulses of light. It is marketed as the PureBright system (PurePulse Technologies, San Diego, CA, USA [8]. The intense light includes ultraviolet light, but that alone is not responsible for the total inactivation of microbes. Its lamp encompasses broad spectrum wavelengths, from ultraviolet to infrared, with an intensity of somewhere of 20,000-90,000 times greater than sunlight. It can sterilise in seconds. PureBright is similar to the spectrum of sunlight

at sea level but with two additional differences. The first difference is that it delivers a spectrum 90,000 times more intense than sunlight at the earth's surface. The other difference is that the UV wavelengths between 200-300 nm are normally filtered out by the earth's atmosphere, but not with PureBright.

The process successfully kills spores, micro-organisms, viruses, and deactivates enzymes. Its effectiveness depends in part on the ease with which the organisms to be killed can be directly exposed. Microbes on smooth, continuous surfaces or transparent materials are easily killed compared to those that are hidden or covered.

The cost of the process is inexpensive. Parametric release may be practicable, but the number of lamps, their configuration and the flash rate depend on the particular application, and this requires investigation. The economics of the final process are as low as one cent per m² of surface sterilised. Potential applications for this process could be for aseptic fills, terminal sterilisation of drugs and devices packed in transparent containers. The technology may sterilise blood plasma derivatives and other blood components, biopharmaceuticals and vaccines. PureBright can sterilise a variety of clear liquids and transparent plastic packaging used in both the medical industry and the commercial water market. PureBright is compatible with polyamides, PP and most polyethylenes.

Any degradation photoproducts of the process would need to be searched for and evaluated. But they appear to be no less than those obtained from sunlight. Since sunlight can cause ageing of polymers, etc., and the light is 20,000-90,000 times more intense than the sunlight, this would need to be considered and investigated.

10.4 Iodine – Something Old, Something Used, Something New

Iodine has been long been known to be an antiseptic, which means it is more compatible with human tissues than most sterilants [9]. It has been recognised as an emergency sterilant. It can kill or inactivate spores. However, as a liquid it has a difficult time penetrating most items, and it can stain.

However, if it could be transported/transferred as a vapour to a bacterial site on another compound, it may be extremely flexible and advantageous. This may be done with iodomethane, which can be vapourised and breaks down in the presence of light and with time to iodine.

Recently, iodomethane has been patented by the University of California [10, 11] for replacing methyl bromide as a fumigant and a pesticide. It does not stain until the iodine is released. Iodine can be released by exposing the iodomethane to light for a certain

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time. It is possible to vapourise iodomethane and allow it to penetrate various areas, e.g., computers in areas that need to be decontaminated and inside tubing that needs to be sterilised. Having similar properties to methyl bromide, it is likely to have good to excellent penetration. It may also inactivate microbes by alkylation, as methyl bromide can. It is not listed as a carcinogen by the International Agency for Research on Cancer (IARC), but is listed as a carcinogen by the State of California but only by the route of injection. The current consensus by the EPA and other similar organisations is that methyl iodine is no carcinogen. EPA has accepted it as a pesticide with limits. However it does have mutagenic capabilities. Since it has similar properties to methyl bromide, it may not ultimately be a human carcinogen. As it is a liquid, it will be easier to handle than gases such as methyl bromide, chlorine dioxide and EO.

Given time and light, much of the iodomethane will break down into iodine which also has mild oxidising sterilising capabilities of its own. If high energy pulsed light (e.g., pure pulse), which can inactivate microbes, is used to break down iodomethane more effectively, there may even be some synergism between these two agents. The iodine created may be used as a preservative after sterilisation has been achieved, much like those used in drugs and parenterals.

If iodine is needed to be neutralised, a process similar to that used in chlorine dioxide could be applied. If the properties of iodomethane are allowed to occur, iodomethane may become a novel way to decontaminate, sterilise and preserve.

Iodomethane is not yet recognised as a sterilant by regulatory bodies, but iodine is recognised by some organisations. Iodomethane has been patented as a pesticide by the University of California, and it is being limited by the EPA as a pesticide. It is not a regulated ozone depleting chemical and it is replacing methyl bromide as a pesticide in limited application. At this point its availability and cost will improve.

If iodomethane is similar to methyl bromide, it may not be compatible with natural rubbers and other materials, but will be compatible with a host of resistant materials, polymers, biomaterials and products. To be accepted as a sterilising agent, iodomethane should be evaluated in accordance with the EPA, FDA and ISO 14937-2000 [12] and with other international regulatory bodies as applicable.

Like chlorine dioxide, iodomethane may be used as a liquid sterilant as well as a vapour. Potential applications may be similar to those of EO, chlorine dioxide or formaldehyde sterilisation. It may also have beneficial uses like chlorine dioxide for eliminating anthrax contamination within buildings. It may be used with EO on polyphenylene oxides (PPO) to make them non-flammable and non-explosive. Together they may prove to be synergistic.

Iodomethane may be more beneficial than EO and irradiation in some specific applications, (e.g., allograft tissue). EO and irradiation used to eliminate spores have associated technical problems that limit their use in processing these tissues for transplantation. Iodomethane requires further investigation for this application as well as for other potential sterilisation applications.

10.5 Radiation – Diversifying and Improving

For its first 50 years (1896-1946), ionising radiation was not considered for use as a sterilant but it was then found that it could inactivate microbes. But in the last 45 years use of radiation has developed, primarily in the medical device industry, with the advent of plastics that can be single process sterilised as most plastics cannot be sterilised with radiation. There is plenty of radiation source material as well as improved electron beams available. More and more polymer materials are being irradiated [10-13].

It is being used to sanitise mail that may be contaminated with anthrax. Currently, radiation is being used for processing of foods such as spices, meats, fruits, etc.

Although not proven, there is a suspicion that certain ionising radiation at different frequencies from low-level electron beam machines rather than from high-energy machines may be more bactericidal than at other frequencies.

X-rays were first used for irradiation sterilisation in 1896.

Current modes of gamma and electron beam sterilisation are the main forms of irradiation sterilisation of healthcare products, polymers and materials, but with X rays the penetration of gamma rays can be achieved without the need for the speed of electrons.

Currently the mail is sterilised with electron beams, but it is limited in the thickness of materials that can be penetrated, and also fires have been created. X-rays could penetrate platforms and carts of mail that electron beams cannot. However, post aeration is required to get rid of the noxious odours. Irradiation can also be synergised with heat [14, 15]. Radiation may be able to sterilise drugs without damage or destruction [16] by improvements and modifications (lower dose) in irradiation processing with heat.

X-ray sterilisation with Rhodotrons has the potential of deep penetration for sterilising entire pallets of a product so that the handling/reloading of the product that is required in electron beam and gamma facilities would potentially be eliminated, improving just-in-time (JIT) scenarios. It has low operating costs, and is easy to validate, operate and maintain.

10.6 Some Other Alternative High Level Disinfectants or Sterilants

Ortho-phthalaldehyde is a liquid sterilant that has a weak odour, with an exposure time of 12 minutes compared to 45 minutes for 2% glutaraldehyde. It stains protein grey.

Superoxidised water, a new disinfectant, is prepared by passing saline over electrodes to create reactive species, (e.g., hypochlorous acid, free chlorine). Microbial inactivation is significantly reduced in the presence of organic load.

There is an equipment reprocessor, (e.g., endoscopes) that uses performic acid at 1800 to 2300 ppm concentration, which inactivates spores in ten minutes exposure as per Association of Official Analytical Chemists (AOAC) sporicidal tests. Its reprocessor cleans first with enzymic detergent.

10.7 Other Possibilities

Formic acid in methanol may kill spores better than in hydrocarbon solvents or in water. In methanol it may be less corrosive. Methanol is compatible with many plastics. Formic acid can be easily neutralised in the presence of ozone; formic vapour will likely be synergised to a chemical radical.

Gaseous ozone sterilisation penetration and efficacy may be improved by applying gaseous ozone before and after humidification. Ozone stability and material compatibility may be enhanced by reducing temperature, e.g., cold storage.

Microwave sterilisation may be an option in the future. The Japanese and Belgians are already working successfully in its development. Use of microwave wavelengths (e.g., 915 MHz) rather than conventional ovens (2450 MHz) is another possibility. The US Army is looking at broad range microwave wavelengths to determine if microwave sterilisation occurs by another lethal mechanism rather than just merely generation of heat. Steaming with microwave may potentially inactivate anthrax spores in letters, followed by drying of letters after steaming of 10 or more minutes. Steaming occurs in a container that minimises loss of microwaved moisture vapour, but allows escape of air, and no build up of pressure. The use of microwave ovens with a steaming container is an interesting alternative for generating and maintaining steam without autoclave conditions. Microwaving of letters with moisture/steaming does not inactivate anthrax spores.

Great possibilities for future improvement of EO sterilisation still exist (device/drug combinations, sterilant combinations, reduced concentrations, non explosive mixtures, JIT, potentiation, synergy).

No sterilisation techniques, materials, environmental condition of micro-organisms listed in this manuscript should be accepted without adequate assessment, evaluation, verification or validation.

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11

Summary of Sterilisation for Hospital Products, Polymers and Materials

Sterilisation agents that predictably and reproducibly kill all micro-organisms from viruses to spores, are amazing magic bullets but their use is not without complications, limitations, and precautions. Many alternatives that are recommended may not penetrate certain plastics and mated surfaces, and even fail to demonstrate good microbiocidal kinetics.

New or alternative sterilisation processes like hydrogen peroxide, chlorine dioxide, sodium hypochlorite, peracetic acid, ozone, microwaves, pulsed light, and plasma and sporicide, performic acid can have limitations. Oxidising processes such as hydrogen peroxide or peracetic acid/plasma, ozone, sodium hypochlorite, chlorine dioxide for example, can't even sterilise paper or cellulosic materials without preferential absorption and reaction, (e.g., bleaching, deterioration), and they also oxidise aluminum. Many single use devices need paper for directons for use (DFU) inserts. Not every hospital has alternative sterilisers for reprocessing, but they do have steam sterilisers and possibly dry heat ovens. Also some devices and packages may not be penetrated by the alternatives. For example, 6 mg/l of H_2O_2 at a concentration of 58% can be used because the exposed surfaces remain treated. This peroxide vapour cannot sterilise all long lumens or cellulosic materials.

Others sterilants, (e.g., glutaraldehyde), cannot be considered for terminal sterilisation processes because they deviate from normal sterilisation kinetics or they do not typically sterilise devices within a barrier to protect them from post sterilisation contamination. One exception is the use of terminal end filters as a barrier that allows the liquid aldehyde to be flushed out without allowing microbes in.

In this case the glutaraldehyde is one of the few sterilants that will not inactivate enzymes used in monitoring and other uses. In other situations, it is used in crosslinking and sterilisation (it has been used efficaciously with porcine heat valves).

Ionising radiation has excellent penetration in plastics but this is not adaptable for typical hospital reprocessing because of the high equipment cost, repeated material deterioration, but dry and/or moist heat at lower temperatures may be good candidate. Dry heat can be used as a sterilising agent through dense heat conductive metal materials, whereas electron beam radiation cannot be used. Steam can sterilise liquids that radiation and

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dry heat may not.

Conventional methods like ethylene oxide (EO) or formaldehyde sterilisation may be useful for sterilising electronic devices, but are toxic and leave toxic residuals.

Glutaraldehyde is a liquid sterilant that does not always leave devices with a sterile barrier without aseptic handling, and may not achieve such normal sterilisation kinetics, as vapour sterilants or radiation.

While, most terminal sterilisation methods are preferred, dry heat remains typically unused and unrecognised as a possible alternative in the medical device field.

Not all terminal sterilisation methods survive. For example, the use of a peracetic/plasma system was recently discontinued at one medical site, because of injury to corneas due to the effects of this process on the brass in the ophthalmic instruments. These ophthalmic instruments were subsequently sterilised by steam autoclave, with no problems. A sterilisation method must be compatible with devices, their materials and not cause injury to the end user or the healthcare products it treats as well as causing inactivation of all microbes.

Autoclave, Chemiclave and dry heat are all effective methods of sterilisation of orthodontic instruments, however, sterilisation can result in formation of degradation problems which may present toxicological risk, or hydrolysis, softening or degradation of many biomedical polymers. Good quality instruments will withstand ultrasonic cleaning and sterilisation. Instrument corrosion problems, should be checked with the instrument manufacturer. Problems typically originate with the instrument and not the sterilisation process. An example of instrument corrosion can be demonstrated with metal plated pliers. As the plating gets chipped, the chipped area becomes a point of corrosion. In this case, it may not have been the steriliser that caused the corrosion, but the chip from rough handling or weakness in the metal plating. Quality, well-maintained orthodontic instruments will not corrode in a steam steriliser. Use of well maintained autoclaves for the last 10 years, which use good quality water for steam have resulted in no corrosion of good quality, well-maintained instruments.

Control of the quality and quantity of bioburden and devices are essential for reliable sterility assurance level (SAL), of all sterilisation methods. Therefore, cleanliness, sterilisability in product design should be considered an appropriate step in design control of medical devices and diagnostics for this method and all methods, where the device or items are to be reprocessed. It is recognised that high levels of bioburden and organic/inorganic encrustations about the bioburden, can lead to non logarithmic, non statistical inactivation of microbes, so that standard sterilisation statistical and lethality cannot be reliably relied on. Ethylene oxide while compatible with the most numbers of polymers and materials, has disadvantages of a long treatment time (because of its

gentleness), residuals, toxicity and need for careful handling. However, it continues to be an acceptable sterilisation method for many healthcare products, including combination products and custom device packs.

An abbreviated bioburden-based heat (dry or moist) sterilisation approach, similar in manner to that applied to radiation qualification and spacecraft sterilisation, may be an added adjunct for further assurance for all methods of sterilisation. At high bioburden levels (>1,000 colony forming units (cfu)), in the presence of soil, dirt or crystals, bacterial kinetics may deviate from its logarithmic order of death and become more sigmoidal in shape. Consequently, in the manufacture of devices it is very prudent to make sure bioburden levels are not at high levels, but below 500 cfu and clean. For reprocessing of hospital products, used devices and materials must be pre-cleaned thoroughly. Reprocessed products, parts and components should be designed so that they can adequately pre-cleaned after use, if they are to be sold to be reprocessed.

There are many bioburden, sterility results and formulae that demonstrate variations from simple statistical, straight line, logarithmic curves such as activation slopes, which may be smoothed with ratios. There are statistics that can deal with exceptions such as modifying factors and degree of sterilisation values for sterilisation processes. In the food industry, where microbes can multiply and regenerate, some pathogens such as *Clostridium botulinum* may require inactivation factors as high as 12 death value (12 D) or 10^{12} inactivation. But what must be recognised is that dirty, large, heterogeneous populations of micro-organisms can defeat sterilisation, and mess up statistics, so that straight statistics may no longer work, or even apply. For example in dry heat and EO sterilisation it has often been observed that bacterial populations over 1000 cfu can result in deviations from first-order kinetics, and failure to sterilise. Spores occluded in water insoluble crystals or within oils can prevent diffusion of steam, gases such as ozone, chlorine dioxide, hydrogen peroxide, and even EO, to reach the killing target in the bacteria, so that it survives. At times, those in the custom packaging industry have found micro-organisms surviving even beyond pretreatment with ionising irradiation, followed by EO sterilisation. Large quantities of anaerobic spores, e.g., *Clostridium*, have been shown to deviate from the straight line of the logarithmic curve with what is called tailing.

Bacteria spores submerged in water or moisture, nitrogen, creating an anoxic condition, may double the resistance of these spores and microbes to irradiation.

Consequently, it is very important to know, reduce, mitigate and environmentally control the bioburden characteristics and resistance for health care sterilisation applications.

Many of today's sophisticated, delicate medical devices cannot withstand sterilisation with high temperatures and high moisture environments. Arthroscopes, laparoscopes,

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cystoscopes, other rigid endoscopes, light cables and laserscopes are a few of the examples of devices with optic and electrical connections that limit the type of sterilisation procedures that can successfully be used without damaging the device.

Low temperature heat (dry and moist) sterilisation has the potential to expand the use of sterilisation in the next 2, 5 and 7 years. It is anticipated that there will be more investigation of lower heat processes, and more heat stable plastics. New packaging is available for low temperature dry heat sterilisation, (e.g., 105 °C and 120 °C), with Tyvec, paper, foils, and newer plastics (polyolefins with metallocene and co-extrusion), instead of the conventional metal and glass containers and trays used now. Many of the devices that could be designed for reprocessing could be made of materials that are compatible with low heat sterilisation temperatures. Device manufacturers can benefit from the adoption of these sterilisation methods with an innovative outlook. Manufacturers have already demonstrated this innovative outlook by using radiation sterilisation as materials have changed over the past 20-40 years.

All sterilisation processes, products, polymers, material characterisation and changes need to be assessed and evaluated. Thermal, chemical, physical or irradiation response of products, polymers materials must be considered in the design of all sterilisation cycles. Material changes for radiation, heat or other sterilisation processes could be made using approaches and techniques provided in AAMI's recent TIR 15, '*Material Qualification*' [1]. For guidance on sterilisation 'in general', consult the recent ISO standard – ISO 14937 [2]. For details specifically for Validation of Dry Heat, consult PDA's Technical Report No. 3 [3], because there is no ISO standard for dry heat.

This is relatively unknown, but steam and dry heat have been demonstrated to be effective in deactivating prions. Formic acid has been shown to be a decontaminant, too. Most standard sterilisation methods have not been able to deactivate prions, the agent that causes Creutzfeldt-Jakob Disease (CJD). Prion inactivation may become more important in the future. On the other hand most sterilisation methods including heat are not able to sterilise biomaterials without causing their inactivity or loss of function. Investigation of methods for sterilising biomaterials is needed for future applications. Improvements in polymer compatibility will continue to enhance the use of all sterilisation methods.

11.1 Decontamination and Sterilisation of Prions

Smaller than a viral particle and more resistant than anthrax spores or Nile Valley virus, prions are capable of reproducing without genetic material, but just protein. They are an alternative life form, an Anomeda Strain phenomenon of sorts. Prions cause scrapie, (CJD), kuru, mad cow disease and possibly other brain diseases. In some cases, we may

not know of their presence, e.g., prions, until an autopsy is performed. Prions have been found to be ultra resistant to traditional and contemporary sterilisation methods from heat, and even radiation. Prions would pass through standard microbial filters, and electron beam irradiation of mail. Incineration and burial of cattle with mad cow disease has been found to be ineffective. Prions are claimed to resist 327 °C dry heat. Ultra high and lengthy steam decontamination is effective, but at the expense of damage of most electronics, and polymeric plastics.

Modified decontamination and steam sterilisation cycles are necessary for effective disease control of items, particularly devices that are potential sources of prions. Prions are a disease control challenge and a real non-fictional Andromeda phenomena to study. Medical instruments that come in contact with neurological matter that is potentially contaminated require steam for decontamination or sterilisation. High temperature and increased steam sterilisation time is currently the commonly recognised procedure for sterilisation or decontamination of prions. Other methods or agents have been suggested as inactivating (reducing effectivity) prions, (e.g., hydrogen peroxide, ozone, and formic acid)

Further research and modification of all sterilisation methods, biomaterials and polymers can create more sterilisation opportunities for a growing number of needs, healthcare products, medical devices and diagnostics required for reprocessing as well as single use sterilisation and for other reasons and in other applications.

Most traditional methods will continue to work for healthcare products, but in some cases newer methods present an opportunity, particularly for new combinations of drugs and medical devices. All sterilisation methods have their limitations. Heat will distort or melt plastics and may adversely affect drugs. Irradiation and EO commonly react with drugs; but without moisture, some may be more compatible. Steam, EO, chemicals with moisture, and radiation may adversely affect electronics.

Given these concerns, it is suggested that a number of alternative methods be considered by medical device firms. For example, a diagnostic material/device with the use of a liquid sterilant and filter was created because the diagnostic material was adversely affected by EO, hydrogen peroxide, radiation, and heat.

Dry heat is another option. The use of low-temperature dry-heat methods to sterilise materials and surfaces has proven effective for medical prostheses and implants. The process involves exposing the product to hot air circulated in a chamber, The effectiveness of the dry heat process is based on both temperature and duration of exposure, so parametric-release process control is possible. The method has been shown to be well suited for electronic materials that are heat stable, but are sensitive to moisture, resistant to penetration by steam heat, or prone to radiation damage.

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Dry heat can be used to treat products with less heat than is traditionally recommended, i.e., below 160°C (for example 120–160 °C), when adequately developed, qualified, and validated as a new process. Reducing the sterilising temperature allows many more polymers, materials, and electronics to be processed and sterilised than can be by using more traditional methods. Silicone prostheses have long been dry-heat sterilised at low temperatures, because radiation crosslinks the silicone, and silicone retains high levels of EO residuals if it is EO sterilised.

One device firm has designed a sophisticated medical electronics that cannot withstand EO, steam, peroxide plasma, or irradiation, so they are using considering dry heat. But before it can be used, they will try to modify the radiation approach and see if they can make the electronics more compatible, and then provide dry heat in sequence to come up with a synergistic process.

Though not a new method, steam sterilisation is another option for combination drug and medical device healthcare products . For example, by designing and validating a sterilisation process for a prefilled syringe with a drug using steam. This is a modification of a very old method so that the device and drug are sterilisable.

Microbial inactivation by x-rays has been known since the 1890s with the beginnings of steam, dry heat, and formaldehyde sterilisation, but before the advent of glutaraldehyde, chlorine dioxide sterilisation, EO, hydrogen peroxide, plasma, peracetic, and conventional radiation methods (gamma and electron beam)

Only recently because of difficulties in sterilising/sanitising mail because of the presence of deadly anthrax spores have x-rays been properly recognised as another possible sterilant.

Consequently, like EO sterilisation (WWII) and gamma irradiation (cold war) conflict and war has stimulated the use of x-rays as a sterilisation method.

Further the FDA has increased the Mev energy allowed to be used in x-ray machines from 5 to 7 Mev.

The advantage of using x-rays is that its penetration is similar to gamma irradiation but without its ozone build up, and its speed is like electron beams but without generation of heat or double sided irradiation. Logistically, high energy x-rays allow for pallets of product to be sterilised, so that additional handling (unloading, loading) is not required as with conventional sterilisation methods. This could result in improved 'just in time' processing.

Validation requirements should be the same as those required for gamma and electron

beams. It is believed that the same and a few more materials compatible with current radiation should become available. However, reduction in presterilisation bioburden and synergism of x-rays may be accomplished logistically with pallet irradiation of product with the application of a heated preconditioning room and a post heated aeration room. Heat as well as other agents have been demonstrated to be synergistic with x-rays.

With improved conversion of electrons to x-rays from 6 to 12%, and synergised.

sterilisation with a 25-50% reduction of x-ray energy requirements, great possibilities of additional material and product compatibilities exist for x-ray irradiation in the future, that are limited under conventional sterilisation methods.

Further future possibilities exist. For example, use of synergised iodomethane that can create iodine upon exposure to sterilising ultraviolet light, has recently been proposed for safe buildings and accepted as a pesticide *in lieu* of methyl bromide, is not only potentially very fast but potentially capable of preserving the item it treats with antiseptic or antimicrobial protection properties.

Sterilisation processes that are capable of inactivating all micro-organisms, including resistant spores without adversely affect product quality, or polymer integrity are few. Only a few processes are capable of sterilising devices and healthcare products to a low risk of contamination (10^{-3} to 10^{-6} SAL). Steam and dry heat, radiation, EO, chlorine dioxide, hydrogen peroxide (with plasma sterilisation) are among a few of them, and should not be overlooked for sterilising a variety of products and polymers (see Table 11.1). This list of sterilisation methods, devices, components and polymers is only a beginning but not without potential complications, limitations and precautions if the sterilisation method and process selected is not designed, developed and controlled properly. Note: listed materials, devices, components must be qualified before use, because changes in moulding, construction, additives, and changes in sterilisation methods can alter compatibilites and quality.

Table 11.1 Major product and polymer categories and associated sterilisation applications		
Polymers	Healthcare products	Some applicable sterilisation processes
Thermoplastics		
Acrylonitrile butadiene styrene (ABS)	IV spikes, luer, Y-connector, roller clamp, filter cases, blood dialysis units	Limited steam, EO, radiation, H ₂ O ₂ , ozone
Acetals (Delrin)	Stopcocks, structural keel for a prosthetic device, engineering plastic, others	Dry heat, EO
Acrylics	Tubing connectors, needle adapters, blood set components, contact lenses, trays	EO, limited radiation, chlorine dioxide, H ₂ O ₂
Cellulosics - cellophane, cuprophane, etc.	Haemodialysis membranes, haemo-filters, structural members of medical devices, IV burette chambers, medical packaging	EO, radiation (limited H ₂ O ₂)
Hydrogel polymers (one example - a copolymer of 2-hydroxyethyl methacrylate and ethylene dimethacrylate)	Contact lenses, stents	EO sterilisation
Parylene-poly-xylene	Coating, used in catheters, stents, needles, cardiac assist devices, prosthetics, cannulae	Steam, dry heat, EO, radiation, H ₂ O ₂ , ozone
Poly etherethketone (PEEK)	Cardiovascular, orthopaedic, and dental implants, and tubing	Dry heat, EO, steam, radiation, H ₂ O ₂
Polyamide and copolymers (Nylon)	Catheters used in cardiovascular procedures, sutures, epidural catheters, laparoscopy devices, blood sets, joints, kidney dialysis, used in composite materials and films, and special packages	Steam, limited dry heat, EO, limited radiation, limited H ₂ O ₂

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Polycarbonate	Blood sets (oxygenators), tubing connectors, injection sites, valve occluders, general structural, syringes, rigid containers, cases, safety syringes, haemodialysers, cardiectomy trocars, reservoirs, surgical instruments, perfusion devices, blood centrifuge bowls, stopcocks, needleless syringes, drug delivery devices, IV connectors, part of positive expiratory pressure (PEP) system.	Steam (watch out for vacuums), limited dry heat, EO, limited radiation, H ₂ O ₂ , ozone
Poly(butylene terephthalate) (PBT)	Prosthetic devices and artificial skin	EO
Polyetherimide (PEI)	Surgical probes, part of instrument for impactor, heads, mallet heads and tissue separation, container lids for containers commonly used in trauma surgery, dental surgery and spinal surgery, reusable medical device material.	Steam, dry heat, EO, H ₂ O ₂
Polyethylene oxide (PEO)	Prosthetic devices and artificial skin, blood compatibility	EO
Polyethylene (PE)	Packaging, plastic containers, joint replacement components, also in shoulder, elbow, wrist, ankle and toe, replacements, polyethylene tubing	EO, radiation, steam (high density PE or some copolymers), some H ₂ O ₂ , oxone
Polyethylene vinyl acetate (EVA)	Packaging, hydrogel, gloves, drug delivery, films	EO, radiation
Poly(ethylene glycol terephthalate) (PET)	Packaging	EO, radiation H ₂ O ₂
Polyethylene terephthalate (PET)	Woven vascular prostheses, sutures, catheters and tubing, angioplasty balloons, blood collection tubes, specialty syringes, irrigation and wound drainage systems	EO, steam, dry heat, radiation

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Polyglycolide (PGA), polylactide (PLA) includes <i>co</i> -polymers, polycaprolactone (PCL)	Sutures, biodegradeables, hollow fibre, controlled release of medicinals, antibiotics, etc., orthopaedics	EO, limited H ₂ O ₂
Polymethyl methacrylate (PMMA)	Ophthalmology lenses and contact lenses, grout for artificial joints, orthopaedics, bone cement, cranioplasty, neurosurgery, membrane oxygenators, corneal prosthesis, contact lenses.	Limited chlorine dioxide, EO, limited radiation, H ₂ O ₂
Polymethylpentene	Containers, TPX film, medical instrument covers	Dry heat, steam
Polyolefins, polyethylene (LDPE<LLDPE, HDPE, UHMWPE) and polypropylene (PP), also PP/PE <i>co</i> -polymers	Films, tubing, and containers for packaging applications, container – sterile water, urine bags, blood oxygenators – polypropylene membrane and hollow fibres, syringes, dialysis membranes, heart valve occluders, sutures, catheters, protective tubes, hip joints, packaging, PP contact lens casting cup, part of positive expiratory pressure (PEP) system	EO, limited radiation (on polypropylene), limited steam (PP and some high density polyethylene), low temperature dry heat limited (polypropylene), H ₂ O ₂
Polyolefin <i>co</i> -polymers (PE/PP, PPCO)	Parenteral solution containers	Steam, EO, H ₂ O ₂
Polystyrene	Sputum cups, containers, tubes, petri dishes	EO, radiation, H ₂ O ₂
Polysulfone, polyphenylsulfone, polyethersulfone	Dialysis membrane, filter membrane, surgical instruments, hospital trays, dishes, medical cases, part of positive expiratory pressure (PEP) system, container lids for containers commonly used in trauma surgery, dental surgery and spinal surgery	Limited steam (limited but reprocessible), dry heat, EO, radiation, H ₂ O ₂

Polyurethane	IV containers, enteral feeding tubes, blood pumps, artificial heart ventricles, catheters and tubing, leads, catheters and components, valves, needleless syringes, drug delivery devices, diagnostic components, lipid resistant stopcocks, vials, connectors, components connected to PVC tubing, surgical instruments, facial prostheses, cuffs, transdermal patches, drug delivery devices, angioplasty balloons, blood device interfaces	EO, radiation, limited dry heat, steam, H ₂ O ₂
Polyvinyl chloride	IV therapy: glucose solutions and saline solutions, drug mixtures, nutrients, flexible PVC containers, rigid/semi rigid containers collection, storage and administration of blood and blood components (blood bags), tubing and catheters, oxygenators and dialysers, IV drip chambers, gastrointestinal grafts, shrink tubing, urine bags, endotracheal tubes, drip chambers, blood vessels, gastrointestinal grafts, hearing aid components	Steam, limited dry heat, EO, limited radiation, H ₂ O ₂
Styrene - acrylonitriles (SAN)	Dialysis, intravenous connectors	EO, radiation, H ₂ O ₂
Teflon - PTFE and fluorinated ethylene propylene (FEP), polychlorotrifluoroethylene (PCTFE), polyethylene tetrafluoroethylene (PETFE)	Surface treatment, orthopaedics, coating stem prostheses, aneurysm clips, neurosurgery, endoscopic sheaths, fibre optic upjacket, stopcocks, drainage tubes, coating for sutures, membranes for artificial lungs, cannulae and artificial bone joints, artificial vasculature	Heat sterilisation, EO, limited radiation (not PTFE), H ₂ O ₂ , ozone

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Thermoset Polymers		
Natural rubber (synthetic <i>cis</i> , 1-4, polyisoprene)	Foley catheters for urological use, needle sites, donor needle covers, surgeon's gloves	EO, steam, limited radiation
Silicone	Extruded tubing – small tubing, foley catheters for urinary drains, catheters used for infusion of IV solutions required for parenteral nutrition, silicone rubber membranes in blood oxygenators, nasogastric tubing, eustachian tube prosthetics (ear parts), other prosthetics (mammary glands, heart components, joints), reconstructive surgery, ear, bone and joints	Dry heat, limited steam (may not penetrate), EO (leaves residues), limited radiation (may crosslink), H ₂ O ₂ , ozone
Thermoset polyurethane	Heart valves; plastic surgery; oxygenators; catheters	Limited dry heat, EO, radiation
H₂O₂ typically means low temperature hydrogen peroxide with plasma, which reduces H₂O₂ residuals. Note: the examples in this table vary with formulations and sterilisation of items that need to be evaluated, qualified and validated before use. Contact polymer supplier for specific polymer type and formulation suited best for your application. Ozone sterilising material compatibility may be similar to H₂O₂/plasma sterilisation material compatibility.		

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12 Definitions

Bioburden: is a population of viable presterilisation micro-organisms in the finished product, raw material, component and/packaging.

Biocompatibility: is the state of the biomaterial within a physiological environment without the material adversely affecting the tissue or the tissue adversely affecting the material.

Biological Indicator (BI): a solution or carrier consisting of a known concentration of spores that are highly resistant to moist heat sterilisation, which can be used to predict lethality to the presterilisation bioburden by use of the BI system.

Calibrated or Certified BI: A BI spore population either in a spore strip or a suspension that has been laboratory tested for its D-value in a specialised chamber, e.g., Bier Unit, against a known ethylene oxide concentration, temperature, percentage relative humidity and time conditions.

Certification: is a documented review and approval of a process carried out as final step in the validation programme to permit product release.

Chain Scission: is the breaking of a molecular bond causing the loss of a side group or shortening of the overall chain. This results in a loss of strength and elongation, with irradiation of some polymers.

Commissioning (Installation Qualification): Obtaining and documenting evidence that equipment has been provided and installed in accordance with its specification and that it functions within predetermined limits when operated in accordance with operational instructions.

Computer Control: Control system that uses computer(s) to control time and initiate the electrical control system.

Crosslinkage: is a side bond that links two adjacent chains of atoms in a complex molecule which can result in an increase in strength and a decrease in elongation of some polymers with irradiation.

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Cycle Process Development: Documented programme or studies which are performed to define the sterilisation process based upon product/packaging or other considerations, and/or equipment limitation.

D-value: Exposure time required under a defined set of conditions [e.g., ethylene oxide (mg/l), % relative humidity and time] to cause a one-logarithm or 90% reduction in the population of a particular micro-organism. D_{121} is a D-value obtained at 121 °C for steam sterilisation.

D_{eo} is a D-value obtained in ethylene oxide sterilisation.

Design Control: a term describing the early development of a product prior to transfer to manufacturing and validation.

Development: activities performed in preparation for validation

Disposable: refers to a single use sterilised product. One that should not be reused.

Dose: a measurement of the amount of radiation absorbed or provided. A sterilisation dose is a quantity that delivers a minimum sterility assurance level.

Electromechanical Control: control system that uses mechanical means, e.g., cams or punch cards, to time and initiate the electrical control signals.

Environmental Control: Systems organised to maintain environmental conditions and contamination below specified levels In manufacturing areas of healthcare products.

Exposure Time: a period in which process parameters are controlled within specified tolerances.

F-value: measures the microbiological lethality (inactivation) capability of a heat sterilisation process.

F_0 **Value** is calculated at 121.1 °C with a D-value of 1.0 minute and a typical z-value of 10 °C. $F_0 = \sum L (\Delta t) \times 1 \text{ minute}$ where L (lethality) = $\log -1 (T - 121.1 \text{ °C}/10 \text{ °C})$.

Heat (temperature) Penetration Distribution: A study or series of studies to gather time-temperature data necessary to measure the temperature received by the large volume product during the sterilisation process. The heat penetration is performed inside the product within a loaded vessel, to determine thermal transfer characteristics and uniformity during critical points in the process.

Heat Penetration: a study or series of studies to gather time temperature data necessary to calculate the F_0 received by the large volume product during the sterilisation process.

Installation Qualification: *See commissioning.*

Microbiological (Biological) Challenge: biological indicator spore strips, inoculated with a product that contains a known population of micro-organisms and can be used in testing sterilisation cycle.

Moist Heat: heat that is derived from water either as a liquid (water) or as steam under pressure.

Operational Qualification: Obtaining data and documentary evidence that commissioned/ installed equipment performs with predetermined limits in accordance to protocol, manufacturing operational manual, etc.

Parametric (Process Control) Release: declaring a product as sterile based upon physical, chemical and/or biological process data rather than on basis of finished product sampling testing or biological indicator results.

Probability of Non Sterile Unit (PNSU): Probability of a viable (bioburden) micro-organism being present on or in the product unit after sterilisation. PNSU is normally expressed as 10^{-6} .

Process Lethality (L): capability of the sterilisation process to destroy micro-organisms at different sterilising temperatures. Lethality is typically equated to microbial inactivation at 121.1 °C with the following equation: $L = \log_{-1} (T - 121.1 \text{ °C} / 10 \text{ °C})$.

Process Challenge Device (PCD): Biological indicator spore strips placed into difficult to sterilise product or item(s) that contain known populations of micro-organisms and can be used in routine testing of sterilisation cycle. Capability of the sterilisation process to destroy micro-organisms in the PCD at different sterilising locations relative to the product indicates adequate inactivation typically equated to microbial inactivation of product. This is performed in a relative resistivity study under sub-process ethylene oxide conditions.

Protocol: The document containing a description of the programme to be followed in the evaluation of the process. The Procedure and Sterilisation Validation Document (SVD) can serve as the protocol for validation(s).

Qualification: A demonstration of reproducibility, uniformity, and effectiveness of a process, used to specifically qualify or test a steriliser or process.

Relative Humidity: Ratio of moisture to saturated conditions that is derived from water vapour either as a vapourised liquid (water) or as steam under pressure. The ratio is multiplied by 100.

Relative Humidity Distribution: is a study or series of studies to gather time-temperature data necessary to measure the temperature received by the large volume product during the sterilisation process, preconditioning and conditioning. The RH distribution is

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performed inside the product within a loaded vessel, to determine moisture transfer characteristics and uniformity during critical points (during preconditioning and conditioning) in the process.

Requalification: a repetition of a validation or part of a validation to assure continued sterilising success. Requalification can occur whenever a significant change occurs, or a period of time has elapsed (e.g., quarterly, annually, biannually).

Reuseable: a healthcare product that can be resterilised and reused, for a specified number of times or periods.

Sterility Assurance Level (SAL): Probability of a viable biological indicator microorganism being present on or in the product unit after sterilisation. SAL is normally expressed as 10^{-6} .

Temperature Distribution: A study or series of studies to ascertain the temperature profile of the sterilising medium in the vessel during the sterilisation cycle. A means to assess the existence of a vessel slow to heat or load cold location. Temperature distribution is typically, a study performed in an empty vessel to determine temperature uniformity around the vessel or process.

Thermal Chemical Death Time: Measures the microbiological lethality (inactivation) capability of a ethylene oxide sterilisation process, as it relates to exposure time, cycle temperature and gas concentration.

Thermal Resistance: the microbial resistance, particularly bioburden, to moist heat. Relative thermal resistance of bioburden survivors to heat are typically characterised and related to a D-121 value.

Tote weight scale: It is the weight of the carrier system, carrying the product. It is used to measure density.

Useable Steriliser Chamber Volume: the space inside the steriliser chamber generally recognised as occupied by the steriliser pallet load.

Validation: Documented procedure for obtaining, recording, and interpreting the results required to establish that a process will consistently yield a product complying with predetermined specifications. Process Validation generally covers four activities: commissioning (installation), physical (operational) process qualification, performance qualification and certification.

z-value: the slope of the thermal resistance curve or the thermal death time expressed as the number of degrees (temperature) required for a one log change in the D-value or F-value or the temperature change required to change a D-value one log or reduce it 1/10th; typically the z-value is considered to be 10 °C.

Abbreviations

12-D	12 Death values - an overkill approach
AAMI	Association for the Advancement of Medical Instrumentation
ABS	Acrylonitrile-butadiene-styrene
ACE	A cross evaluation of dose delivered in a dose mapping procedure
ACGIH	American Conference of Governmental Industrial Hygienists
AMC	Airbourne molecular contamination
AMES	A bacterial mutagenic test developed by Dr Ames
ANSI	American National Standards Institute
AOAC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Materials
ATSDR	The Agency for Toxic Substances and Disease Registry
A_w	Water activity
Bax	Bax is a member of Bcl2 protein family and induces growth arrest
Bcl2	Bcl2 is a protein family in apoptosis. The ratio of Bcl2/Bax is an expression used for cell death or programmed cell death.
BI	Biological indicator(s)
BSI	British Standards Institute
CDC	Center for Disease Control
CDRH	Food and Drug Administration's Center for Devices and Radiological Health
CEN	European Committee for Standardisation
CERCLA	Comprehensive Environmental Response Compensation and Liability Act
CFC	Chloroflurorocarbon(s)
cfu	Colony forming unit
CSO	Cotton seed oil
DEHP	Diethylhexyl phthalate
DEO	D-value obtained in EO sterilisation
DFU	Directions for use

Sterilisation of Polymer Healthcare Products

DIFF	Passive diffusive sampling
DIS	Draft International Standard
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DOE	Design of experiments
DOP	Dioctyl phthalate
DOT	Department of Transportation
D-value	Decimal reduction value
EC	European Community
ECTFE	Ethylene-chlorotrifluoroethyl copolymer
EHS	Environmental Health Services
EO	Ethylene oxide
EPA	Environmental Protection Agency
EPDM	Ethylene propylene rubber
ETFE	Ethylene tetrafluoroethylene
FDA	Food and Drugs Administration
FDA-GMP	Food and Drug Administration – Good Manufacturing Practice
FDIS	Final Draft - International Standard
FEP	Fluorinated ethylene propylene
FMEA	Failure modes and effects analysis
FO	Time of heat lethality equivalent at 121 °C
FTA	Fault tree analysis
FTM	Fluid thioglycollate medium
F-Value	Time of heat lethality equivalent to a specified sterilising temperature
FWT	Far West Technology dosimeters
GC-MS	Gas chromatography - mass spectroscopy
GLP	Good laboratory practice(s)
GMP	Good manufacturing practice(s)
GPC	Gel permeation chromatography
HACCP	Hazard analysis and critical control point
HEPA	High efficiency particulate air
HIMA	Health Industry Medical Association (formally) now AdvaMed
IARC	International Agency for Research on Cancer
IC	Ion chromatography

IDLH	Immediately Dangerous Levels to Health
IF	Inactivation factors
IMP	Impinger
IP	Intraperitoneal
IPA	Isopropyl alcohol
IR	Intercept ratio
ISO	International Standards Organisation
IUD	Intrauterine device
IV	Intravenous
JIT	Just-in-time
kb	Kilobyte
L	Lethality
LRV	Log reduction value
MEM	Minimum essential media
NASA	National Aeronautics and Space Administration
NDA	New drug application
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Science and Technology
NLT	Not less than
OSHA	Occupational Safety and Health Administration
PA	Polyamide
PAN	Polyacrylonitrile
PC	Polycarbonate
PCD	Process challenge device
PCTFE	Polychlorotrifluoroethylene
PDA	Parenteral drug administration
PE	Polyethylene
PEEK	Polyether ether ketone(s)
PEG	Polyethylene glycol
PEK	Polyether ketone(s)
PES	Polyethersulfone
PETFE	Polyethylene tetrafluoroethylene
PETG	Glycol modified polyethylene terephthalate
PFA	Perfluoroalkyl

Sterilisation of Polymer Healthcare Products

PLC	Programmable logic computer
PMA	Polymethacrylate
PMMA	Polymethyl methacrylate
PMP	Polymethyl pentene
PNSU	Probability of a non-sterile unit
PP	Polypropylene(s)
PPCO	Polypropylene co polymer
PPO	Polyphenylene oxide
PS	Polystyrene
PSF	Polysulfone
PTFE	Polytetrafluoroethylene
PU	Polyurethane(s)
PVC	Polyvinyl chloride
PVDF	Polyvinylidene fluoride
QC	Quality control
RF	Radiofrequency
RH	Relative humidity
RQ	Reportable quantities
SAL	Sterility assurance level
SAN	Styrene acrylonitrile
SARA	Superfund Amendments and Reauthorisation Act
SD	Standard deviation
SEBS	Styrene-ethylene-butadiene-styrene elastomer
SIP	Sample item proportion
SLR	Spore log reduction
SMC	Surface molecular contamination
SOP	Standard operation procedure
SOR	Sorbent tube
STEL	Short-term exposure limit
SVD	Sterilisation validation document
TAG	An American group working on specified ISO standards
TC	Technical committee
TLV-C	Ceiling threshold limit value
TPE	Thermal polyurethane elastomer

TPO	Thermoplastic polyolefin
TSB	Trypticase soy broth
TUV	TUV Rheinland
TWA	Time weighted average
USP	United States Pharmacopeia
UV	Ultra violet
WFI	Water for injection
Z-Value	The temperature change to cause one log change in D-value

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