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-phthaldehyde
- 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 handle 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 discolor 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 re-cleaned, 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 cannot be repeatedly sterilised without degradation and destruction, except for single use disposable products that are not intended to be re-sterilised.

- 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 cannot tolerate steam. To overcome this problem, many devices are pre-sterilised, 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 |
| % contamination if 10 units are tested          | 50%                  | 5%                  | 0.5%                |
| % contamination if 20 units are tested          | 6.7%                 | 25.9%               | 41.1%               |
| % contamination if 20 units are tested          | 3.4%                 | 13.9%               | 23.3%               |
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Sterilisation must be differentiated from lesser means of destroying or removing microbes. Terms and techniques such as antisepsis, disinfection, use of germicides, commercial sterilisation, sanitation, pasteurisation, decontamination and cleaning, preservation, antimicrobials, fungicides, viricides, 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

| 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   | $10^x$ |

*where x is variable based upon barriers, environment, and sterilant*

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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, 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:

\[ D_v = \frac{\text{time}}{\log N_o - \log N_b} \]

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>
<thead>
<tr>
<th>Exposure time, min</th>
<th>Microbial population</th>
<th>No. of D values</th>
<th>No. of Log-survivors</th>
<th>Log killed</th>
<th>% Survival population</th>
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<td>0.00000001</td>
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<tr>
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<td>0.000001</td>
<td>12</td>
<td>-6</td>
<td>12</td>
<td>0.000000001</td>
</tr>
</tbody>
</table>
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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 stearothermophilis*, 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 stearothermophilis* 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].
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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 if 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 \( \log Y_o / \log N_o \):

\[
\log N = -\text{exposure time}/D_v + \log N_o (\text{IR})
\]

Where \( Y_o \) is the \( Y \) intercept and \( N_o \) 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:

\[
\log N = -\text{exposure time}/D_v + \log N_o (\text{IR})
\]
### Theory and Kinetics of Destruction

A theoretical example of the order of death of a bacteria population (applicable for either physical or chemical treatment)

<table>
<thead>
<tr>
<th>Time increment</th>
<th>Bacteria living at beginning of time increment</th>
<th>Bacteria killed during one time increment</th>
<th>Bacteria surviving at end of time increment</th>
<th>Logarithm of survivors</th>
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<tbody>
<tr>
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<td>900,000</td>
<td>100,000</td>
<td>5</td>
</tr>
<tr>
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</table>

#### Survival Curves

- **Logarithmic death:** Homogeneous population, uniform cell distribution.
- **Retarded initial rate:** Clumped cells.
- **Accelerated initial rate:** Cells of unequal resistance present.

**Figure 1.2 Theory and Kinetics of Destruction**
Probability of a non-sterile unit (PNSU) or a sterility assurance level (SAL) = \( \log^{-1} \left( \frac{-\text{exposure time}}{D_v} + \log N_o \right) \) (IR).

When \( \log Y_o/\log N_o \) is > 1, the curve is a downwards concave shape; when \( \log Y_o/\log N_o \) is <1, the curve is a downwards convex shape and when \( \log Y_o/\log N_o = 1 \), 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.
Sterilisation of Polymer Healthcare Products

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., r² = 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:

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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.
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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.

References


15. C. Chamberland, Comtes Rendues, 1879, 88, 659.

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