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7 | The compendial sterility tests

Scott V.W. Sutton

BACKGROUND

The compendial sterility test is frequently presented as a flawed test for its stated purpose. This statement, of course, begs the question as to what exactly is the purpose of the sterility test as described in the compendia? The test first appeared in 1932 (1) and included the basic features of the modern test two media, prescribed dilution scheme (for bacteriostasis/fungistasis or method suitability) and a defined incubation time. The original test differed from the contemporary method in that it had the media incubated for five days rather than 14 and allowed two retests (all three had to fail to fail the test). However, the basic structure of the test is present.

This test has generated controversy as to its role in product quality testing for decades. Part of the problem is in understanding the role of the compendial tests. Those chapters in USP numbered less than 1000 (for example, the Sterility Test is USP chapter <71>) are referee tests in other words they are in place solely to demonstrate conformance to qualities specified in the product monograph as described in the current National Formulary (the other part of the book). A rigid interpretation would have it that if the product is not described by NF monograph, the test does not directly apply. In fact, the preface to the internationally harmonized sterility tests reads:

> The following procedures are applicable for determining whether a Pharmacopeial article purporting to be sterile complies with the requirements set forth in the individual monograph with respect to the test for sterility.

In a similar vein, sterile finished dosage forms have the following requirement in USP (from <1> *Injections*):

> "**Sterility tests:** Preparations for injection meet the requirements under *Sterility Tests* <71>"

This has a nice symmetry the test states that it is applicable for meeting the requirements set forth in the monograph, the requirement being that the material meets the requirements of the test.

So, one would have to conclude that the test is not flawed for its intended purpose, that purpose being to show that the material tested meets the requirements of the test. How did we come to think that this test was designed to show the sterility of the product?

We need something to demonstrate product sterility. 21 CFR 211 states the requirement:

> "211.167 Special testing requirements.
> (a) For each batch of drug product purporting to be sterile and/or pyrogen free, there shall be appropriate laboratory testing to determine conformance to such requirements. The test procedures shall be in writing and shall be followed."

The difficulty, of course, is that there is really no way, given current technology, to demonstrate sterility of a batch. This imposes significant validation issues as the most direct and persuasive documentation of product sterility.

However, there is an expectation in the GMP that a sterile finished product will have a release test. How are we to determine a suitable, "validated" release test for a characteristic that cannot be measured? A way to satisfy this requirement is provided in:

> "211.194 Laboratory records.
> (a) Laboratory records shall include complete data derived from all tests necessary to assure compliance with established specifications and standards, including examinations and assays, as follows: . . .
> (2) A statement of each method used in the testing of the sample. The statement shall indicate the location of data that establish that the methods used in the testing of the sample meet proper standards of accuracy and reliability as applied to the product tested. (If the method employed is in the current revision of the United States Pharmacopeia, National Formulary, AOAC INTERNATIONAL, Book of Methods,{1} or in other recognized standard references, or

is detailed in an approved new drug application and the referenced method is not modified, a statement indicating the method and reference will suffice). The suitability of all testing methods used shall be verified under actual conditions of use."

So if we can cite a "validated" test we do not need to develop one ourselves. Thus, the internationally harmonized Sterility Test is pressed into service as a product quality test, even though that is not its design nor its purpose.

THE STERILITY TESTS

There are two different GMPs describing sterility in the United States. The first is 21 CFR 211 and the second is the "Biologics" 21 CFR 610 and 612. By common consensus, the 21 CFR 211 cGMP looks to the compendial sterility tests, while 21 CFR 610 describes a separate test in 21 CFR 610.12. The Biologics test is similar in fundamental aspects to the compendial sterility tests. There is a finite (and small) sample size and two recovery media are used, each with specified incubation conditions. So both types (compendial and Biologics) share some common limitations (see the following text).

The compendial sterility tests describe two separate types of tests, the membrane filtration and the direct transfer methods. In the first, solution from a specified number of containers (volume and number determined by batch size and unit fill volume) is filtered through a filter of nominal pore size 0.45 um. Recovery of viable cells from the filter(s) is performed by submerging the filter in one of two recovery media followed by incubation as specified temperatures for 14 days. The second test is a direct immersion of the product or suspensions into a suitable volume of the two media to allow growth. The media are designed to support growth in aerobic, or growth in an environment of limited oxygen availability. Both types of tests require demonstration that the specific method used is suitable for that product.

As early as 1956 Bryce published an article describing the two critical limitations of this test. He put forward that the test was limited in that it can only recognize organisms able to grow under the conditions of the test, and that the sample size is so restricted that it provides only a gross estimate of the state of "sterility" of the product lot (2). Other concerns about the Sterility Test (e.g., choice of sample size, choice of media, time and temperature of incubation) were extensively reviewed in an article by Bowman (3).

There have been several changes in the compendial Sterility Test since that time, culminating in the internationally harmonized test (4). However, the two basic problems outlined in 1956 by Bryce remain today.

Limitations to the Sterility Tests

Sample Size

The sample size is set arbitrarily and does not provide a statistically significant population to estimate sterility (5). This is indisputable and unavoidable with a test of this type, which is destructive in nature. Let's look at some of the numbers:

Let the likelihood of a contaminated unit λ

By the Poisson distribution, the probability of picking a sterile unit from the fill (denoted P) is $e^{-\lambda}$, or $2.7182818^{-\lambda}$

Then, if you are picking 20 samples from an infinite supply (or for this discussion, from a pharmaceutical batch),The probability of passing the sterility test is P^{20}.

Conversely, the probability of failing the sterility test is $1 - P^{20}$

Therefore, given a known frequency of contaminated units in the batch:

Frequency of contaminated units in the batch	Probability of failing sterility test with the current sample size
0.001	0.0198 2%
0.005	0.0952 9.5%
0.01	0.1813 18%
0.05	0.6321 63.2%
0.1	0.8647 86.5%
0.5	1.0000 100%

The only way to modify this limitation would be to degrade the media (resulting in lesser recovery and therefore false negatives) or to increase the number of samples. Changes of this sort seem unlikely in the compendial sterility tests at this point in time. A discussion of different sampling plans that might be used is presented in Bryce (2), and a more full discussion of the controversy over the final resolution of the current procedure is provided in Bowman (3). After extensive review, all of the proposed sampling plans were found wanting for one reason or another.

One frequently overlooked aspect of discussions of sampling plans is that the statistical analyses all assume that the test system would recover even a single microorganism if it were present in the sample. In other words, one contaminating cell would result in media turbidity. This (unverified and unlikely) assumption leads us to the next topic.

Recovery Conditions

The harmonized test utilizes Trypticase Soy Casein Digest Broth and Fluid Thyioglycollate Medium. These media and their corresponding incubation temperatures were chosen to maximize recovery of potential contaminants early in the development of the tests. However, some authors have questioned the choice of media (6), while others have suggested the use of solid media rather than liquid media would be appropriate (7). The choices in the current harmonized procedure reflect those media to which all parties in the harmonization process could agree.

Then there was a concern about incubation duration. USP 23 (8) allowed a 7-day incubation period for products tested by membrane filtration; 14 days for those tested by the direct transfer method. This requirement changed in USP 24 (9) to include a 14-day incubation period for both types of tests with the exception of products sterilized by terminal sterilization (this exception was removed by USP 27 (10)). Similarly, the *Pharm Eur* 3rd Edition (1997) allowed a 7-day incubation period (unless mandated by local authorities). This allowance was amended in 1998 with the 4th edition to 14-day incubation. This extension was the result of concerns that the methodology might not be able to detect "slow-growing" microorganisms.

The incubation period was identified as a concern by Ernst et al. (11) who recommended a longer period of incubation time than 7 days might be necessary, perhaps as long as 30 days. More recently this position was repeated with retrospective data provided by German and Australian workers who wished to ensure that a harmonized procedure included an incubation period of at least 14 days (12,13).

However, even with the longer incubation period there is no assurance that all microorganisms can grow under these conditions, but are metabolically active. In fact a growing body of evidence suggests that there are a large number of microorganisms that are unable to replicate under standard laboratory conditions (viable but not culturable VBNC) (14 16).

CLARIFICATIONS AND ENHANCEMENTS TO THE HARMONIZED STERILITY TEST

There have been quite a few clarifications offered by different regulatory agencies to the compendial sterility tests. This section will not be a review of the genesis of the sterility tests; that discussion is outside the scope of this chapter. We will, however, take a look at a few of the clarifications offered by different regulatory agencies on the implementation of the harmonized test.

US FDA/CBER

US FDA/CBER (the Center for Biologics Evaluation and Research) has a section of the GMP under section 21 CFR 610. In this section, 610.12 describes a separate sterility test to be used with those products under CBER purview. There are several differences in the test from the internationally harmonized tests that include controls, method suitability requirements, media growth promotion procedures, etc. A major difference between the tests is that the CBER test allows a retest if the original sterility test fails. This retest must also fail for the product lot to be out of specification. While the manufacturer is urged not to attempt this approach by the author of this chapter, this is still technically allowed in the Biologics sterility test.

As an aside, the pharmacopeias and 21 CFR 610.12 do not reference or provide sterility guidelines for unprocessed bulk samples for protein and virus products, although the FDA guidance documents "Points to Consider in the Manufacture and Testing of Monoclonal Antibody Products for Human Use" (17) and "Points to Consider in the Characterization of Cell lines Used to Produce Biologicals" (18) require this testing. Common practice is to use 10 mL/media (for a total of 20 mL) for this testing.

USP

The USP introduced clarification in 2007 with a new chapter <1208> "Sterility Testing Validation of Isolator Systems" (19). This informational chapter provides background in isolator design and construction, the equipment qualification considerations for the isolator, validation of the decontamination cycle (this would include the internal environment, the exterior of the product containers entering for testing and the protection of the product from the decontamination cycle), and the maintenance of asepsis within the isolator environment. The reader is also instructed that the sterility test performed in a properly functioning isolator is very unlikely to result in a false-positive result. Finally, instruction is provided on the training and safety aspects of the isolator operation.

Pharm Eur

The European Pharmacopeia have published a nonmandatory chapter "5.1.9 Guidelines for Using the Test for Sterility" (20) in which further information on the sterility tests is provided. The user is instructed that the test can be performed in a class A laminar air flow cabinet located in a class B room, or an isolator. The reader is also reminded that this test cannot demonstrate sterility of a batch, and that it is the manufacturer's responsibility to adopt a representative sampling plan. Finally, elaboration is provided on "Observation and Interpretation of Results" in that during an investigation,

> "... if a manufacturer wishes to use condition (d) as the sole criterion for invalidating a sterility test, it may be necessary to employ sensitive typing techniques to demonstrate that a microorganism isolated from the product test is identical to a microorganism isolated from the test materials and/or the testing environment. While routine microbiological/biochemical identification techniques can demonstrate that 2 isolates are not identical, these methods may not be sufficiently sensitive or reliable enough to provide unequivocal evidence that 2 isolates are from the same source. More sensitive tests, for example, molecular typing with RNA/DNA homology, may be necessary to determine that microorganisms are clonally related and have a common origin."

TGA

The Australian Therapeutic Goods Administration (TGA) has published a 33-page document entitled *TGA Guidelines on Sterility Testing of Therapeutic Goods* (21) to explain how the harmonized sterility tests are to be interpreted when submitting a product into Australia while noting that the *British Pharmacopeia* (and therefore *Pharm Eur*) is the official test. This document is extensive and expands the details provided on controls recommended in the harmonized Sterility Test.

The Stasis Test is an additional control recommended here. In this test, spent media from a negative Sterility Test (media that has seen the membrane that filtered product and 14 days of incubation) is subjected to an additional growth promotion test to demonstrate its continuing nutritive properties.

There is also a great deal of discussion in this document on the interpretation of the test results and on how to investigate Sterility Test failures (see below).

PIC/S

The Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme (jointly referred to as PIC/S) has as its mission, "... to lead the international development, implementation and maintenance of harmonized Good Manufacturing Practice

(GMP) standards and quality systems of inspectorates in the field of medicinal products." There are currently 37 Participating Authorities in PIC/S (as of October 2009 see http://www.picsscheme.org for current information). The US FDA has applied for membership several years ago and awaits disposition of its application (22).

PI 012 2 "Recommendations on Sterility Testing"

PI 012-2 "Recommendations on Sterility Testing" provides a great deal of additional information that the inspectors are instructed to ask about. This includes direction on acceptable training of personnel, the sterility test facilities (including clean room design, airlocks, aseptic gowning, and clean room fittings), cleaning and sanitization, as well as environmental monitoring of the sterility test area. Additional detail is also provided on the test method.

The Sterility Test controls are also provided some attention in this document. In addition to their execution, the inspector is instructed to require a table of negative control failures and positive control failures.

The instruction provided for "validation" (or bacteriostasis/fungistasis) by PIC/S in this document is in conflict with the harmonized chapter. Where the harmonized chapter informs the user to add the inoculum to the final rinse, the PIC/S document states that the product should be inoculated unless it is not practical due to product interference (such interference, presumably, would have to be documented). In addition, the PIC/S document asserts that it is good pharmaceutical practice to revalidate all products every 12 months. The author is unaware of this practice outside this document for the pharmaceutical industry. The Stasis Test is also recommended in the PIC/S document. This test is also recommended to be repeated at least every 12 months.

Finally, there is a good deal of discussion on investigations (as in the TGA guidance). This will be discussed below.

PI 014 3 "Recommendation: Isolators Used for Aseptic Processing and Sterility Testing"

This guidance document covers the same basic material as described in the preceding text for USP chapter <1208> with some significant expansion on validation considerations, the nature of the sporicidal decontaminant, and the logistics of the isolator's operation. While this guidance is directed primarily to the use of isolators in manufacturing, it also claims sterility testing to be within its scope.

RMM AND THE STERILITY TESTS

A frequently discussed option for the sterility testing of finished dosage forms is to use a "rapid" method (23). Currently marketed rapid microbiological methods (RMM) can be grouped into two types those that require amplification (growth) to show low-level contamination and those that do not. In the first group would be technologies such as ATP bioluminescence, head-space analysis, and others. Examples of the second type might be technologies such as PCR and vital dye/chromatography methods. Why is this distinction important?

The concern with recovery conditions is that we do not know how to grow all microorganisms that might contaminate pharmaceutical products. Applying an alternate technology that requires growth does not result in an improvement in the sterility test method, since organisms that currently do not grow would not grow in the new method either (24). In addition, there is the continuing concern about the duration of the incubation period.

The currently required 14-day incubation period imposes a significant burden on the manufacturer who must quarantine product until successful completion of the test. Can this be shortened in an alternate test? The time required for microbial growth to turbidity can be thought of as the sum of two stages: a lag phase where the microorganism prepares to grow and the generation time requirements for a low level of microorganisms to grow to a concentration where they are visible using human vision, that is, approximately 10^7 cfu/mL. This separation of stages is important, as it seems that the lag phase is the most significant portion of time required for turbidity (25). Therefore, any alternate methodology that requires growth to amplify the microorganism will likely be required to incorporate a lengthy incubation period to ensure the recovery of "slow-growing" microorganisms.

Duguid and du Moulin (26) describe one approach to overcoming this issue. Using an amplification stage for an ATP bioluminescence technology, they started in 1999 to validate a sterility test for an autologous cell therapy product. This sterility test, which provided for product release in 72 hours with confirmatory results at the standard 14 days, was approved by FDA/CBER in 2004. In the time since they report almost 6000 sterility test results (samples included primary, expansion, and final product from this process) were collected including four positives detected. The alternate method detected them, on average, approximately 35 hours earlier than the confirmatory test (19 vs. 54 hours incubation).

Interestingly, US FDA/CBER (the Biologics group) has issued a draft guidance document on the validation of growth-based rapid methods for use in sterility testing (27). This CBER document is remarkable in its complete avoidance of any mention or consideration of the previous work done in validation of RMM by FDA/CDER, Pharm Eur, USP, or PDA.

The limiting aspects of growth-based methods as an alternative for the sterility test can be avoided by use of a rapid microbiological method (RMM) technique that does not require growth (24). The use of a method that avoids growth requirements offers an additional advantage in that the question of VBNC organisms is completely side-stepped. As no culturing is required, the recovery phase of the sterility tests can be optimized to all microorganisms regardless of growth requirements. This approach is described by Gressett et al. (28).

INVESTIGATIONS IN THE STERILITY TEST

There is a significant amount of literature written on OOS and investigations. Most of this concern, of course, stems from the 1993 Barr Decision (29). Barr Laboratories had a history of repeated current good manufacturing practice (cGMP) deficiencies, including repeated retesting and resampling of product as well as reprocessing of defective product without adequate justification in a practice that has come to be known as "testing to compliance." This is not good practice the out-of-specification (OOS) data is telling the manufacturer important information about the product and must be resolved. Unfortunately for the microbiology community, this initial situation, as well as most of the subsequent writing on this topic, has focused on OOS from an analytical chemistry perspective. The Food and Drug Administration (FDA) has provided guidance following the Barr decision, and drafted the "Guidance for Industry Investigating Out of Specification (OOS) Test Results for Pharmaceutical Production" (30). Interestingly, this guidance document only briefly touches upon microbiological data, stating that "the USP prefers the use of averages because of the innate variability of the biological test system." In addition, this guidance document specifically excludes microbiology from its scope in footnote 3.

A PDA task force that was assembled to look into this issue recommended the use of the phrase "Microbial Data Deviation" (MDD) in the investigation of issues in microbiology, at least until it is clear that the issue is a true product specification failure, as opposed to a lab error or process monitoring concern (reviewed in Ref. 31).

The harmonized Sterility Tests provide some guidance on MDD investigations:

> If evidence of microbial growth is found, the product to be examined does not comply with the test for sterility, unless it can be clearly demonstrated that the test was invalid for causes unrelated to the product to be examined. The test may be considered invalid only if one or more of the following conditions are fulfilled:
>
> a. The data of the microbiological monitoring of the sterility testing facility show a fault.
> b. A review of the testing procedure used during the test in question reveals a fault.
> c. Microbial growth is found in the negative controls.
> d. After determination of the identity of the microorganisms isolated from the test, the growth of this species (or these species) may be ascribed unequivocally to faults with respect to the material and or the technique used in conducting the sterility test procedure.
>
> If the test is declared to be invalid, it is repeated with the same number of units as in the original test. If no evidence of microbial growth is found in the repeat test, the product examined complies with the test for sterility."

Conditions "a" and "b" basically refer to a catastrophic failure of control. If it can be demonstrated that either the technique or the environment was not in control at the time of the test, the test can be declared invalid.

Condition "c" is interesting in its own right. The assumption when running a control is that the effort to run that control is justified by the information provided by the test. However, many labs will only consider the results from the negative control if the test fails. In other words, although the negative control is supposed to demonstrate the adequacy of the test conditions and performance, if the test samples pass, then a failing negative control is ignored. If the test samples fail, a failing negative control is used to invalidate the test. The author of this chapter urges that a consistent interpretation of controls be used in all testing.

Condition "d" is one that has received a great deal of attention. Additional detail is provided the previously cited Pharm Eur 5.1.6, the PIC/S guidance on sterility test, and the TGA document. This topic is also discussed in FDA's Aseptic Manufacturing Guide (32). Reduced to its essentials, the user is urged in these documents to use methods sensitive enough to demonstrate that the microorganism is not only of the same species, but also of the same strain or substrain of that species. It should be noted that even with this detail the best that can be done is to show a correlation between the presence of the strain from the two sources rather than a causal relationship. In other words, finding the same strain of *Staphylococcus aureus* on the testing technician and in the sterility test does not prove that the only possible source of that was the technician (the strain could also be present in the aseptic core), but it is accepted as sufficient proof in regulatory guidance that the test was compromised and so invalid.

The pharmaceutical literature provides some examples of Sterility test investigations that can be used as guides. Lee (33) described a detailed sterility investigation that included the identification of the contaminant, reviews of documents, training records, gowning practices, environmental monitoring records, lab procedures, and other critical controls. It should be stressed here that most of the work in an investigation occurs reviewing records. The practice of complete proactive documentation is critical to the success of any investigation. The likelihood of an inconclusive investigation (and therefore surety of failing product) is assured if the associated records do not support a definitive finding.

Schroeder (34) published a thoughtful review of considerations for a sterility failure investigation. He argues that for products sterilized by filtration filter failure must also be considered in addition to the other commonly cited areas of investigation.

CONCLUSIONS

The current, harmonized Sterility Test has two fundamental weaknesses, both of which have been obvious from its inception. The first is that the sampling plan is insufficient to meet the requirements implied by the title of the test. This weakness is not solvable in the current regulatory climate (nor has it been for over 70 years). The second weakness of the test involves recovery and recognition of microbial contamination in the sample, should it exist. There are several different varieties of the Sterility Test, and even when citing the harmonized test the user must be sensitive to regional expectations for that test. While there is great promise in finding a rapid method for conducting sterility tests, few examples exist of this having been successfully accomplished. Finally, there are clear expectations on the investigations to conduct into a failed Sterility Test, and the user is urged to be familiar with these expectations.

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8 Industrial sterilization technologies: principles and overview

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INTRODUCTION

A sterile medical product is one that is free of viable microorganisms. Most medical products produced under standard manufacturing conditions according to the FDA requirements (21 CFR 820 and 21 CFR Part 210/211) (1) have microorganisms on them, even though the numbers may be low. Such medical products are nonsterile. The purpose of sterilization then is to inactivate the microbiological contaminants and thereby transform the nonsterile medical products into sterile one. Also, the sterilization treatment must not render the medical product materials or functions unacceptable. Basic to the comprehension of a sterilization process is an understanding that the kinetics of inactivation of a pure culture of microorganisms by physical and/or chemical can be expressed by an exponential relationship between the numbers of microorganisms surviving and the extent of treatment with the sterilant. This means that there is always a finite probability that a microorganism may survive regardless of the extent of treatment. Therefore, for a given treatment, the probability of survival is determined by the number and resistance of microorganisms on or in the product and by the conditions used during the sterilization treatment. It follows that the sterility of any one medical product in a population subjected to sterilization processing cannot be guaranteed and the sterility of a processed population is defined in terms of the probability of there being a viable microorganism present on a medical product.

The requirements for the validation and routine operation of sterilization methods are given in a series of ISO (International Standards Organization) sterilization standards and guidelines published in the United States by the Association of the Advancement of Medical Instrumentation (AAMI). The requirements are the normative parts of the standard with which compliance is claimed. The guidance given in the informative annexes is not normative and is not provided as a checklist for auditors. The guidance provides explanations and methods that are regarded as being suitable means for complying with the requirements. Other methods may be used if they are effective in achieving compliance with the requirements of the standard. The development, validation, and routine control of a sterilization process comprises a number of discrete but interrelated activities; for example, calibration, maintenance, product definition, process definition, installation qualification, operational qualification, and performance qualification. There is generally a prescribed sequence of events outlined in each applicable standard that will expedite the validation process.

The standards for quality management systems recognize that, for certain processes used in manufacturing, the effectiveness of the process cannot be fully verified by subsequent inspection and testing of the product. Sterilization is an example of such a process. For this reason, sterilization processes are validated prior to use, the performance of the sterilization process monitored routinely, and the equipment maintained. Exposure to a properly validated, accurately controlled sterilization process is not the only factor associated with the provision of reliable assurance that the product is sterile and, in this regard, suitable for its intended use. Attention is therefore given to a number of considerations including

1. the microbiological status of incoming raw materials and/or components;
2. the validation and routine control of any cleaning and disinfection procedures used on the product;
3. the control of the environment in which the product is manufactured or reprocessed, assembled, and packaged;
4. the equipment and processes validated, calibrated, and controlled;
5. the control of personnel and their hygiene;

6. the packaging process and materials;
7. the product storage conditions.

There are three (3) main industrial sterilization technologies that are used to sterilize medical products: ethylene oxide (EO) gas, irradiation (either gamma or E-beam), and moist steam and three (3) less commonly used methods: dry heat, filtration, and liquid chemical. The most efficient of these sterilants is moist steam under pressure or autoclaving. The next most effective are dry heat and ionizing radiation. Radiation sterilization requires close monitoring with chemical and/or physical dosimeters. Next in efficacy are gaseous sterilants, such as EO, that are usually monitored with biological indicators (BIs) in every lot. The least effective sterilants are liquid chemicals that cannot penetrate to as many sites inside the product. Filtration procedures also fall in this last category. However, they all share two common characteristics:

- Providing sterility, as defined by compendial tests
- Require validation and monitoring to prove their effectiveness

The selection of the appropriate method depends on the product materials, design features, and contamination levels. The type of contamination on a product varies and this ultimately impacts the effectiveness of a sterilization process. So during this process of defining the sterilizing agent (Table 1), one must also demonstrate its microbicidal effectiveness, identify the factors that influence microbicidal effectiveness, assess the effects that exposure to the sterilizing agent have on materials, and identify requirements for safety of personnel and protection of the environment. This activity may be undertaken in a test or prototype system; the final equipment specification should be capable of being related to the experimental studies. Table 1 contains some significant considerations, but certainly not all, that will help with this decision.

The basic requirement to validate manufacturing processes, of which sterilization is one, is defined in the Food and Drug Administrations' Quality System Regulation 21 CFR Part 820, Sec. 820.75 (2), "Where the results of a process cannot be fully verified by subsequent inspection and test, the process shall be validated with a high degree if assurance and approved according to established procedures. The validation activities and results, including

Table 1 Considerations in the Selection of Appropriate Sterilization Method

Consideration	Ethylene oxide	Radiation	Moist steam
Product materials	Compatible with most materials; maximum temperature tolerance of 130°F; can use 100 120°F, but less effective (cycle time will be longer)	Selection of suitable grades of plastics to prevent degradation over time after exposure to maximum dose ranges	Very high heat (121°C) may destroy plastics or fabrics
Product design	Must allow penetration of gas and humidity into interior spaces	No restrictions	Few restrictions
Product package	Must be permeable to gas and humidity and allow aeration after cycle completion	No restrictions	Must be permeable and withstand high heat
Time from start to product release	9 days for BI release 4 7 days with BI incubation reduction 1 2 day with parametric release	2 3 days for E beam 2 5 days with gamma	1 2 days
Post sterile time	3 7 day quarantine for BI release and EO gas dissipation Parametric release is possible but requires additional validation testing	Dosimetric release No hold time	Dry time may be needed Parametric release
Typical products	Custom trays with multiple components	Liquids in impermeable package	Metal products Contacts

the date and signature of the individual(s) approving the validation and where appropriate the major equipment validated, shall be documented." Recent revision of 21 CFR Part 211 has included the following language in section 211.113(b) "...Such procedures shall include validation of all aseptic and sterilization processes."

While following this mandate in the validation of a sterilization process, additional assurance that the product is sterile and suitable for its intended use is obtained during the process by

- establishing, documenting, and following procedures to prevent microbiological contamination of products purported to be sterile;
- Defining, documenting, and validating the hardware and software used in the process and the operating characteristics of each piece of equipment;
- verifying the microbial kill (sterility assurance level, SAL) in the production vessel;
- ensuring by extra monitoring and sampling locations that the process is uniform and reproducible from cycle to cycle;
- confirming that the routine monitoring positions and the data obtained from these locations is sufficient to control the process.

Exposure to a properly validated and controlled sterilization process is not the only factor that provides a reliable assurance that the product is sterile. Medical products should be manufactured under conditions in agreement with requirements of a defined quality system defined in approved procedures.

As such, attention should also be given to several other factors including

- the microbiological status (bioburden) of raw materials;
- the resistance of the bioburden to the sterilizing agent;
- validation and control of any cleaning or disinfection methods used in the manufacture of the product;
- control of the manufacturing environment and personnel working therein;
- packaging of the product and configuration of the load;
- maintenance and calibration of the equipment;
- appropriateness of the cycle.

The validation process must be documented, monitored at a higher level than routine production cycles, and repeated to show consistency of operation and microbial kill. The validation will serve to define the limits of routine processing.

The entire sterilization system outlined in Figure 1 consists of multiple components, all of which require application of quality procedures, operator training, continuous monitoring, and failure investigation when necessary. The elements of the system are illustrated and will be discussed in this chapter.

MICROBIOLOGICAL CONSIDERATIONS

Microbiologists are familiar with the concept that a homogeneous population of bacteria subjected to a sterilizing agent will, in theory, die exponentially with time at a uniform rate. A constant percentage of the microbial population is inactivated with each successive time interval. The exposure time required to destroy 90%, or one (1) log, of the microbial population is defined as the *D* value, or decimal reduction value. Therefore, a semi-log plot (Fig. 2) will yield a straight-line relationship. Note that when the line crosses below 10^0, resulting in less than one survivor, it is expressed as a probability of survival. Thus, the 10^{-6} survivor level or SAL or a 12-spore log reduction (SLR) represents a one-in-one million probability of one microorganism surviving the process. Products intended to come in contact with compromised tissue, those with a sterile fluid pathway claim or those that are surgically implanted generally are validated to a 10^{-6} SAL. For other products not intended to come in contact with compromised tissue, for topicals, mucosal contact products or nonfluid pathway surfaces of sterile products an SAL of 10^{-3} can be used. It should be pointed out, however, that for sale of product labeled "sterile" in Europe an SAL of 10^{-6} is required used.

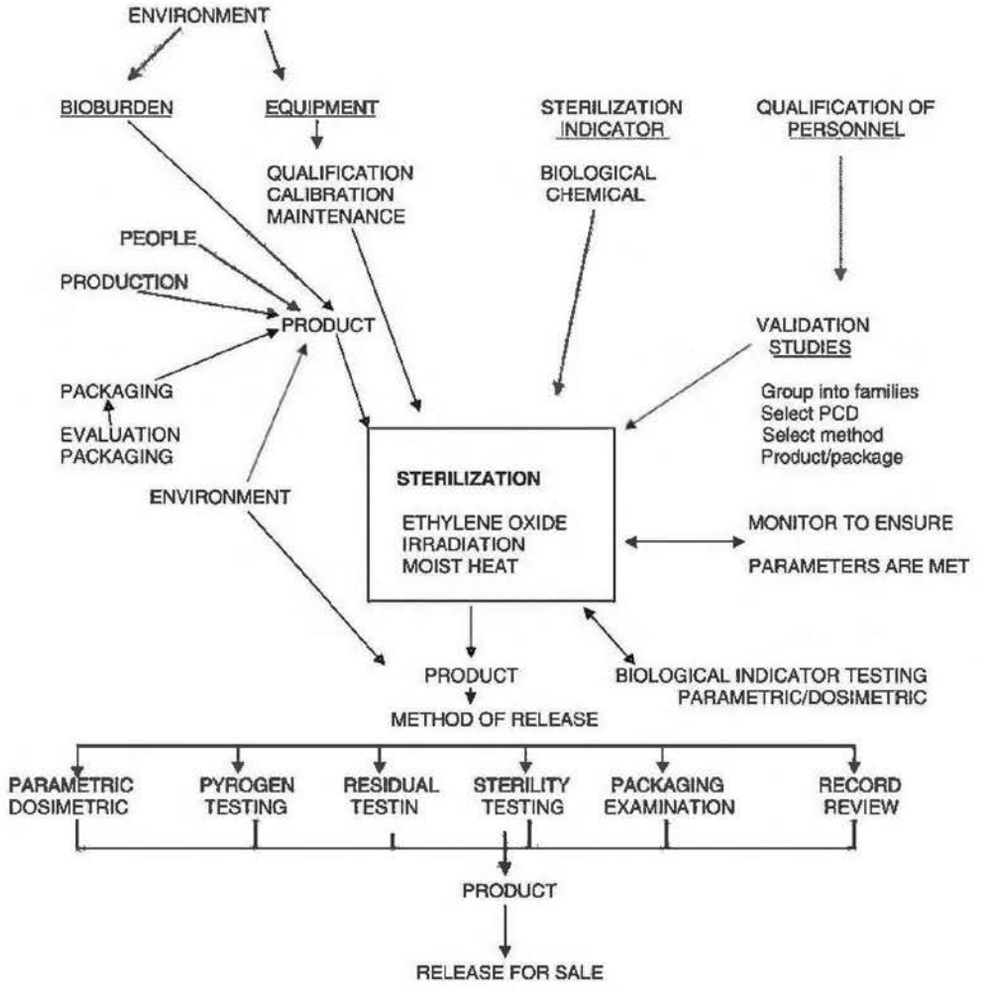

Figure 1 The sterilization system.

PRODUCT BIOBURDEN

An understanding of the viable microorganisms on a finished product is necessary and required to support the validation process. Recently, FDA has added bioburden testing in the list of control procedures in section 21 CFR Part 211.110. Bioburden data are important because the extent of the treatment of a sterilization process is a function of the bioburden on the product, the resistant of the bioburden, and the SAL required. The assessment of the bioburden needs to include the number of microorganisms with their identities. The identification need not be exhaustive, but confirmation of Gram stain characteristics and genus provide useful information and can be used to monitor changes over time and as a comparison to organisms recovered during environmental monitoring. In fact, by combining the simple information of cell arrangements (e.g., single, in clumps, chains), cell shape (e.g., sphere, rod), and Gram stain reaction, much can be deduced about the source and thereby, the control of the specific organism (Table 2). Some bacteria (*Bacillus* sp.) can form spores, a dormant form that is very

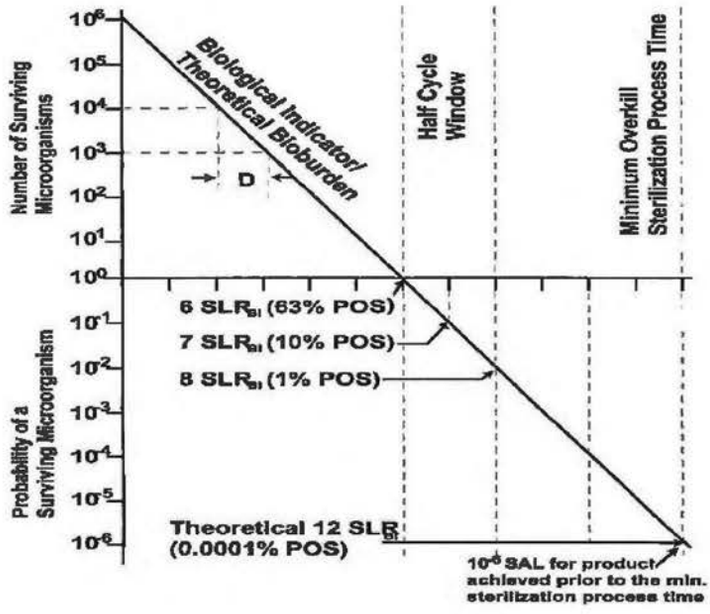

Figure 2 Semi log plot of theoretical microbial inactivation. *Source*: Courtesy of PDA/DHI Publishing.

Table 2 Characterization of Organisms Extracted from Medical Products

Microorganism	Characterization	Source
Acidovorax	Gram ve rod	Soil
Acinetobacter	Gram ve rod	Skin
Arthrobacter	Gram +ve rod, nonspore	Soil
Aspergillus	Mold	Soil, packaging
Geobacillus	Gram +ve spore former	Soil, water
Brevibacterium	Gram +ve rod, nonspore	Skin
Burkholderia	Gram ve rod	Water
Candida	yeast	Environment
Cellulomonas	Gram +ve rod, nonspore	Soil
Cladosporium	Mold	Soil, packaging
Chrysosporium		
Cochliobolus	Fungi	Packaging
Clostridium sp.	Gram ve rod, anaerobe	Environment
Corynebacterium	Gram +ve, nonspore rod	Mucous membrane, skin
Cryptococcus	Mold	Soil, packaging
Deinococcus	Cocci	Human
E. coli	Cocci, Gram ve rod	Human and animal colon
Flavimonas	Gram ve rod, nonspore	Human
Fusarium	Mold	Soil
Kocuria/Micrococcus	Gram +ve cocci	Human
Microbacterium	Gram +ve	Skin
Micrococcus	Gram +ve cocci	Water, dust, soil
Moraxella	Gram ve rod	Human
Paenibacillus	Gram +ve rod	Soil
Penicillium	Mold	Soil, packaging
Propionibacteria	Gram +ve, nonspore	Human skin
Pseudomonas	Gram ve rod	Water, packaging
Staphylococcus	Gram +ve cocci	Skin, mucous membrane
Streptococcus	Gram +ve cocci	Human
Streptomycetes	Mold	Soil
Trichoderma	Mold	Soil

resistant to adverse conditions. This renders them more difficult to sterilize than organisms existing in the vegetative stat.

Bioburden data exhibits significant variability with a skewed distribution. Contributors to bioburden levels may include one or more of the following:

- Raw materials
- Manufacturing components
- Assembly process (especially manual cutting and assembly)
- Manufacturing environment
- Product handling by manufacturing and inspection personnel
- Assembly aids, such as compressed air, water, lubricants, etc.
- Residue from cleaning processes
- Packaging

Detection of bioburden on and/or in products is performed by selecting 3 to 10 packaged products randomly from one (1) lot of recently manufactured product. Sample size can depend on

- magnitude of change in bioburden to be detected (for early detection of small changes, a larger number is recommended),
- variations in estimates of numbers present on individual products

If products are costly, the number sampled can be reduced to three to five items. A simulated product can be used but must be made from the same materials and in the same manufacturing process. Products rejected during the manufacturing process can also be used as long as they were exposed to all process steps. Do not use expired or "old" product for bioburden evaluation because the organisms on such products may not represent those present on recently manufactured products.

The frequency of the bioburden estimations, supported by documented evidence or rationale, should be established on the basis of several factors including

- data from previous bioburden estimates if historical data is consistent, less frequent testing is indicated (e.g., shift from monthly to quarterly or semiannually);
- use to be made of the bioburden data;
- manufacturing processes;
- batch size;
- production frequency for the product;
- materials used change in materials may trigger new bioburden estimate;
- variations in the bioburden estimates spikes or swings in data could signal more frequent testing.

The test method used only produces an estimate of the number of microorganisms. The method can be validated to establish the relationship between the estimate and the true number of microorganisms on the product. Whatever method is used must be reproducible so that the results generated on one occasion can be compared to data generated subsequently. The method of extraction most effective for bioburden recovery varies according to the substrate; therefore individual products may require different extraction methods to optimize organism removal. All treatments should avoid conditions that are likely to affect the viability of microorganism, such as excessive cavitation, shear forces, temperature rises, or osmotic shock. Acceptable bioburden recovery methods are available in ANSI/AAMI/ISO 11737-1:2006 (3), *Sterilization of medical products Microbiological methods Part 1: Determination of a population of microorganisms on products*. In addition, ISO 11737-3:2004, *Sterilization of medical products Microbiological methods Part 3: Guidance on evaluation and interpretation of bioburden data*, provides guidance on evaluating and interpreting the data generated during routine monitoring of the microbiological quality of medical products.

The estimation of the bioburden can be divided into three phases, all of which may affect the final results and therefore should be considered in the validation:

1. The removal of the microorganism from the product extraction techniques could include use of ultrasonication, mechanical agitation with or without glass beads, vortex mixing, flushing, blending, swabbing, and contact plating and stomaching. A surfactant may be used in the extraction fluid to facilitate removal of organisms.
2. Transfer of the organisms to the cultural conditions quickly methods could include membrane filtration, pour plating, spread plates, and/or serial dilution if large numbers of organisms are expected. Use proper incubation conditions for aerobic bacteria at 30 to 35°C for two to five days; yeasts and molds at 20 to 25°C for five to seven days and anaerobic bacteria at 30 to 35°C for three to five days.
3. Enumeration of the microorganisms colony counting is most commonly used.

Bioburden should be evaluated at least annually prior to the validation or requalification; it is recommended, however, to test product quarterly or semiannually to trend history and help monitor the component suppliers and the manufacturing environment. The resistance of the bioburden must be equal to or less than that of the BI used during validation of a sterilization process (as in EO, moist steam, and dry heat).

The following methods have been used to evaluate the resistance of the bioburden:

1. When the bioburden estimate is accompanied by microbial identifications, the *D* values can be determined or obtained from the literature for the resistant portion of the population. The time required to inactivate the bioburden can be compared to that of the BI. If the bioburden population consists mainly of vegetative organisms, physical determination of the *D* value may be impossible due to the rapid death rate of these organism.
2. When microbial identifications are not performed and the bioburden is low (<100), the appropriateness of the BI can be shown by inspection, in that the entire bioburden population would need to have a *D* value which is 1.5 to 2 times that of the BI to present a greater challenge. Resistance of this magnitude for naturally occurring bioburden is not supported by the literature.
3. When the microbial identifications are not performed and the bioburden is high, the appropriateness of the BI should be determined by exposure to sublethal cycles, as described in Cycle Development.

PRODUCT STERILITY TESTING

Crucial to the validation of any radiation process is product sterility testing of products subjected to sublethal dosing. Guidance for appropriate sterility testing can be found in AAMI/ISO 11737-2:2000, *Sterilization of medical products Microbiological methods Part 2: Tests of sterility performed in the validation of a sterilization process.*

There are two (2) general approaches in the performance of product sterility tests. These are as follows:

1. Direct immersion of the product into growth medium or by placing growth medium into the product followed by incubation for 14 days.
   - The product may be disassembled prior to exposure to facilitate transfer or aseptically subdivided prior to transfer to medium container.
   - Sufficient growth media should be used to cover the product or to achieve contact between the growth medium and the whole product
   - Agitate after placement in growth medium
   - Maintain contact between medium and product for the duration of the incubation. If the product is large, medium can be swirled daily to contact all product surfaces

Table 3 Quantities of Product for Sterility Testing

Quantity per product container	Minimum quantity for test
Liquids	
<1 mL	Whole contents of container
1 40 mL	Half of contents, but not less than 1 mL
>40 mL, <100 mL	20 mL
>100 mL	10% of contents, but not less than 20%
Antibiotic	1 mL
Product soluble in water or isopropyl myristate	Whole contents of each container, not less than 200 mg
Insoluble products	Whole contents of each container, not less than 200 mg
Solids	
<50 mg	Whole contents
>50 mg, <300 mg	Half, but not less than 50 mg
>300 mg up to 5 g	150 mg
>5 g	500 mg

Removal of microorganisms from the product by elution and either filtration of or transfer of the removed microorganisms to culture conditions.

- Use elution techniques similar to those used in bioburden estimation
- Addition of a surfactant may be required to improve removal of organisms by moistening the product surfaces
- Membrane filter should be rated 0.45 μm
- Aseptically transfer filter to growth medium or use Steritest system and add media after filtration.

Generally, a single culture medium is used that is optimal for the culturing of aerobic and facultative microorganisms during radiation dose verification studies. Soybean-casein digest medium (tryptic soy broth, TSB) is commonly used and the test samples incubated at 30 to 35°C for 14 days. Samples should be checked daily and growth, if any, recorded. During validation of EtO processes or in conjunction with aseptic fill validations, sterility testing follows USP <71> requirements. For devices 40 product samples and 2 media are used: 20 products are immersed in TSB and incubated at 20 to 25°C and 20 products immersed in thioglucolate (THIO) and incubated at 30 to 35°C. These tests are both incubated for 14 days. To ensure the test results are not adversely affected by the product or any leachable substance from the product, a bacteriostasis/fungistasis test is performed by inoculating 10 to 100 selected organisms into test samples containing the product. Quantities for sterility testing of other types of medical products are listed below in Table 3 excerpted from USP <71>, Vol 30.

DEFINITION OF TERMS

The inactivation of microorganisms that occurs during a sterilization process can be described using the following terms:

D Value (decimal reduction value) is the time, or radiation dose, under a given set of sterilizing conditions required to kill 90% (or one log) of a homogenous microbial population (see example below).

Time minutes	Population at start of new minute	Population killed in one minute
First	1,000,000	900,000
Second	100,00	90,000
Third	10,000	9000
Fourth	1000	900
Fifth	100	90
Sixth	10	9

Table 4 F_0 Lethality Equivalents

Equivalent Minutes	
Temperature (°C)	At 121.1°C (F sub 0)
100	0.0077
110	0.079
115	0.251
116	0.316
117	0.398
118	0.501
119	0.631
120	0.794
121	0.977
121.1	1.0
122	1.23
123	1.59
124	2.0
125	2.82

F sub 0: In steam sterilization, the equivalent time in minutes (F value) to produce a given sterilization effect at 121.1°C (250°F) when $Z = 10$°C (18°F) and D value = 1 minute F_0 of 12 to 15 minutes is usually regarded as adequate (Table 4)

- 60′ at 115°C
- 15′ at 121°C
- 4′ at 127°C

SLR is the lethality observed in a full or fractional sterilization cycle. SLR can be calculated as the log of the initial population minus the log of the final population. SLR = $\log N_o$ $\log N_f$. If there are no survivors, the true SLR cannot be calculated. If one positive is assumed for the purposes of calculation, the SLR should be reported as "greater than." 10^{-6} for most terminally sterilized products.

SAL is the statistical probability that a microorganism will survive the sterilization process (see example below). At any givenminute, one log or 90% of the microbialpopulation is killed. Theoretically, complete kill is never achieved.

Organisms surviving at each minute	Time (min)	Logarithm survivors
1,000,000	0	
100,000	1	5
10,000	2	4
1000	3	3
100	4	2
10	5	1
1	6	0
0.1	7	1
0.01	8	2
0.001	9	3
0.0001	10	4
0.00001	11	5
0.000001	12	6

Most probable number (fractional negative) or Stumbo Cochran Murphy method is used to calculate the D value under specified conditions. At sterilization doses where a fraction of the samples may contain survivors, the most probable number (fractional negative) or

Stumbo-Cochran-Murphy method is used to calculate D value

$$D \text{ value} = \frac{U}{\text{Log } N_o \quad \text{Log } N_u}$$

where U is sterilant exposure time (or radiation dose); N_o, initial bacterial population; N_u = 2.303 (ln n/r); N, total number of tests; R, number of sterile tests.

For example, 20 BI test samples within load subjected to EtO sterilization dwell of 20 minutes. Eighteen sterility samples are negative. Use equation to determine D value and predict the dwell time required to produce a 10^{-6} SAL.

REVIEW OF STERILIZATION TECHNOLOGIES

Ethylene oxide is an organic compound with the formula C_2H_4O. This colorless flammable gas with a faintly sweet odor is the simplest epoxide, a three-membered ring consisting of two carbon and one oxygen atom. Ethylene oxide is an alkylating agent that disrupts the DNA of microorganisms, which prevents them from reproducing.

EO sterilization is a chemical process consisting of four primary variables: gas concentration, humidity, temperature, and time. The sterilization process consists of several distinct phases as described below.

Preconditioning—The Preliminary Portion of the Process

Humidity is the most complex and critical of the controllable variables. Levels of humidity above 30% have been shown to be necessary for effective EO sterilization. The purpose of prehumidification both prior to entrance into the sterilization chamber is to drive the moisture deep into and through the materials within the sterilization load The most commonly used level is 50% to 60% RH at 130°F. The effect of humidification of kill time is shown in Figure 3.

Conditioning

If used, in-chamber heating and humidification should be shown to achieve minimum required product humidity and temperature before the gas exposure time. It is important that the humidity be added before the gas, so that the moisture will be carried in front of and with the sterilant. This way, the moisture will be forced into the inner most areas of the products and will not be left behind by the faster-diffusing EO. Addition of steam during the conditioning phase can take place in several different ways (Fig. 4):

- Dynamic environmental conditioning (DEC)
- Incremental addition
- Pulsed addition

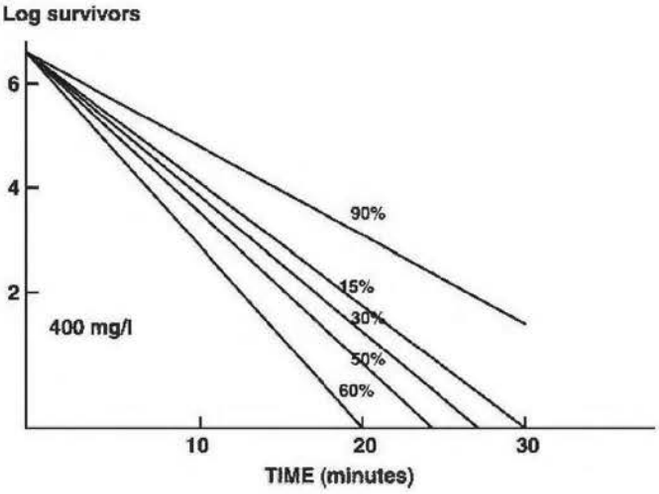

Figure 3 Effect of relative humidity of inactivation of *B. atropheous* spores. *Source*: Courtesy of PDA/DHI Publishing.

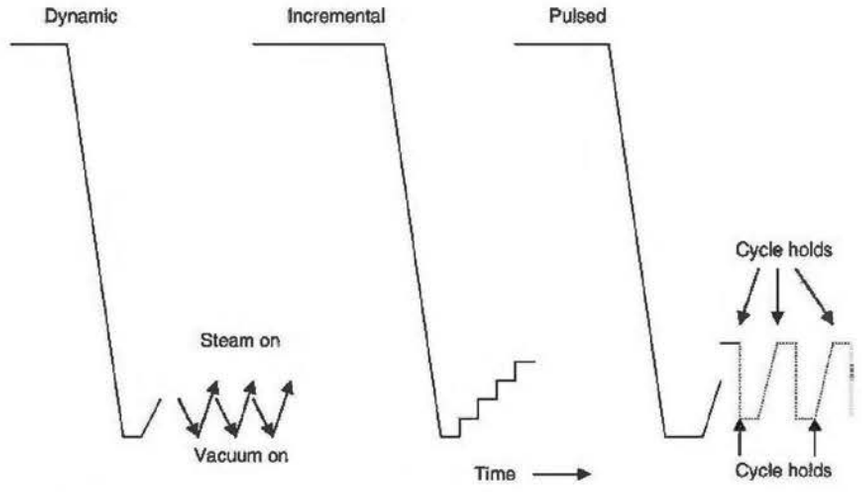

Figure 4 Three chamber conditioning methods for EO. *Source*: Courtesy of PDA/DHI Publishing.

The first method DEC consumes vast amounts of steam, which results in rapid and deep penetration of the steam into the product interiors. The second method incremental adds steam in increments until a preset vacuum point is reached. This can be repeated a number of times or can be the prelude to a static dwell period. The third method pulsed alternates between the deepest vacuum set point and steam rise set point, thereby pumping steam into packaging while maintaining the gentler dynamics. It can be used alone or with static dwell.

Sterilization—Addition of Ethylene Oxide

The temperature of the load influences microbial kill rate (Fig. 5). This effect is expressed as the Q_{10} value or the factor by which microbial death rates change as a result of a 10°C or 18°F change in temperature, and has been reported to range between 1.8 and 2.7 depending on the substrate. Thus for an 18°F difference below a designated sterilization temperature, the *D* value should approximately double. Thus, measures should be taken to minimize the temperature range within the sterilization load.

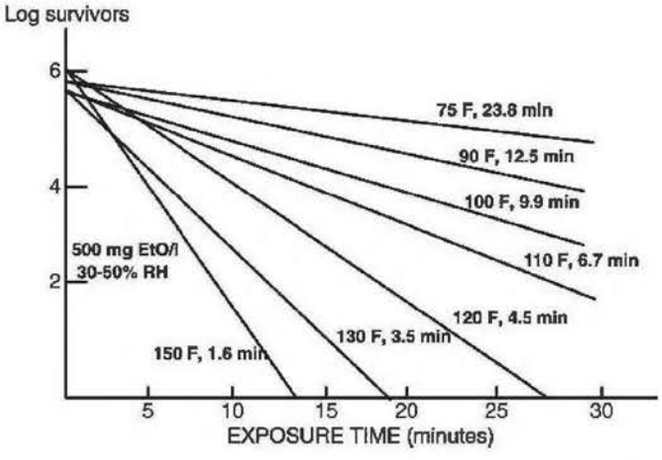

Figure 5 Effect of temperature on the *D* value of *Bacillus subtilis* var. *niger*. *Source*: From Ref. 5 (Courtesy of PDA/DHI Publishing).

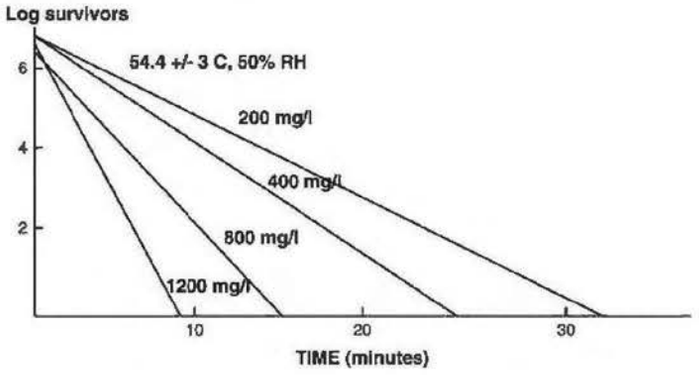

Figure 6 Effect of EO concentration on microbial inactivation rate.

The temperature range should be specified as the minimum range for routine sterilization. Product temperature should be measured during validation. It is common practice that the temperature range within a load during gas exposure be $\leq 10^{\circ}C$ ($18^{\circ}F$). If this cannot be achieved, a minimum temperature at the end of conditioning should be specified.

The sterilization chamber should have the following capabilities:

1. Independent systems for recording and controlling pressure, chamber temperature, and chamber humidity. Place at least one (2) probe at the coolest location,
2. Instrumentation for direct analysis of humidity during conditioning and EO concentration during exposure (parametric release requirement),
3. An adequate gas recirculation system to ensure uniformity of temperature, humidity, and gas concentration within the chamber,
4. Airflow detection alarms on the air recirculation system to ensure it operates within specification,
5. An instrument to monitor gas inlet temperature to ensure gaseous EO enters the chamber,
6. Recirculation system,
7. If software is used to run the cycle, it should be validated.

The EO concentration has a dramatic effect on microbial kill. As the EO concentration increases from 50 to 500 mg/L, there is a significant increase in the microbial death rate (Fig. 6). At concentrations above 800 mg/L, the rates do not increase significantly. Concentrations between 400 and 650 mg/L are recommended for effective microbial inactivation and more efficient gas removal from product at completion of the sterilant exposure. As EO is added to the chamber, it may be absorbed by the product and packaging materials in the load, and, subsequently, the pressure within the chamber will decline. Pressure can be maintained throughout the exposure phase by adding additional EO as the pressure drops. The use of inert gasses to maintain the pressure may result in reduction of EO concentration over time.

Aeration

Residuals of EO and its reaction products may be hazardous. Elevated temperature, dwell time, forced air circulation, and loading characteristics will all affect the rate at which gaseous EO diffuses out of the product load. Optimal aeration occurs at elevated temperatures in chambers or rooms (Fig. 7) with forced outside air circulation and product loading with adequate spacing between pallets. Also, some additional microbial kill can occur during aeration so it is recommended that the aeration time be minimized prior to removal of BI test samples during half-cycle performance runs.

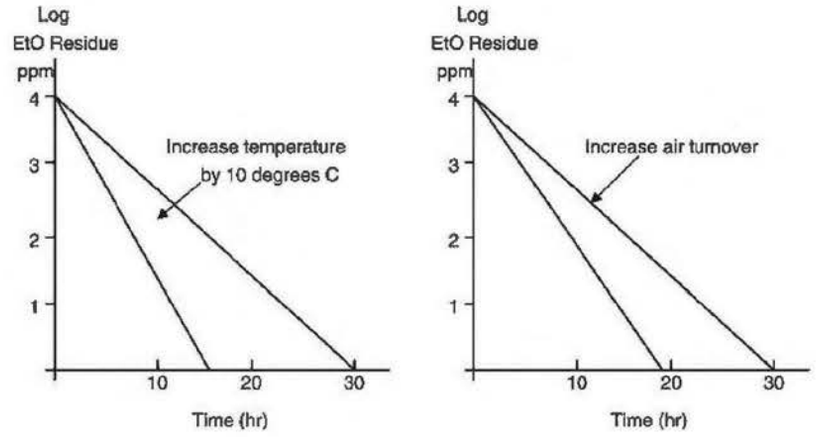

Figure 7 Effect of temperature and air exchange rates on reduction of EO.

The aeration areas should have the following capabilities:

- Airflow detection alarms or indicators on the air handling system to ensure continuous operation,
- Recirculation

Typical cycle parameters for an EO process is shown below:

Parameter	Fractional cycle	Full cycle
Preconditioning	90–125°F, 45–75% RH Minimum 18–20 hr	90–125°F, 45–75% RH 24–96 hr
Initial evacuation	2.0″ HgA ± 0.5″ HgA	2.0″ HgA ± 0.5" HgA
Humidity inject	Inject 1.0″ rise to 3.0″ HgA ± 0.5″ HgA	Inject 1.0″ rise to 3.0″ HgA ± 0.5″ HgA
Humidity dwell	40 min ±5 min	45 min 0, ±20 min
Gas inject	14.5″ HgA ± 0.5″ HgA	15″ HgA ± 0.5″ HgA
Nitrogen	27″ HgA ± 0.5″ HgA	27″ HgA ± 0.5″ HgA
Gas dwell	45 min 5, +0	4 hours, 0, +30 min
Evacuation	2.0″ HgA ± 0.5″ HgA	2.0″ HgA ± 0.5″ HgA
Nitrogen washes	HIGH 27.5″ HgA ± 0.5″ HgA	HIGH 27.5″ HgA ± 0.5″ HgA
(2 repeats)	LOW +2.0″ HgA ± 0.5″ HgA	LOW +2.0″ HgA ± 0.5″ HgA
Dwell temperature	120°F ± 5°F	125°F ± 5°F
Aeration	90–130°F ≥ 24 hr	90–130°F ≥ 24 hr

IRRADIATION TECHNOLOGIES

Two different types of irradiation processes are used in industrial radiation processing of medical products, that is, gamma rays and electron beam. A third type, X rays, have been shown to have microbicidal effects, but this method is not currently available for industrial sterilization. The microbial lethality of gamma rays and electrons is accomplished by ionization; electrons are direct ionizing radiation whereas photons are indirect ionizing radiation. The energy transferred by these radiations during the sterilization process produces chemical and/or physical changes at the molecular level resulting in chain scission, polymerization, cross-linking, sterilization, and disinfection.

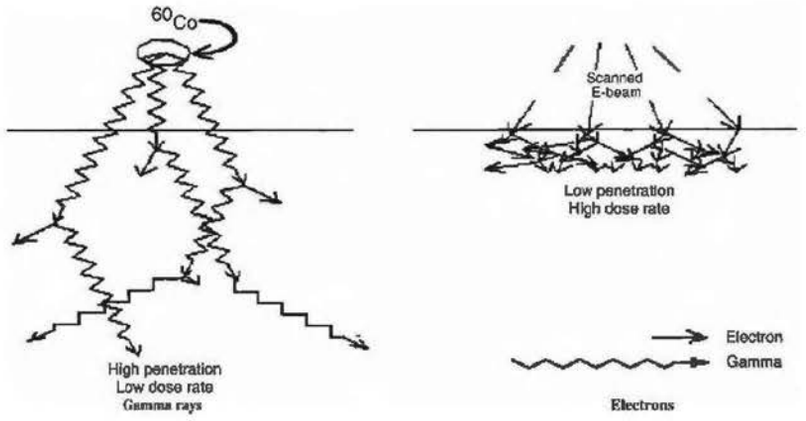

Figure 8 Penetration pattern of gamma and electron beam radiation.

PHYSICAL CHARACTERISTICS OF RADIATION

By far the most commonly used of the three methods is gamma radiation. Gamma rays are emitted from radioactive isotope source materials, the most common being cobalt 60 (^{60}Co). Gamma rays are electromagnetic waves frequently referred to as photons. Having no electric charge or mass, photons transfer energy to materials mainly through Compton scattering collisions with atomic electrons resulting in a uniform, exponentially decreasing depth dose distribution. The photon strikes free electrons in the material and pass part of their energy to the electron as kinetic energy. These displaced electrons continue on their way, deflected from their original path. The scattered gamma ray carries the balance of the energy as it moves off through the material, possibly to interact again with another electron. In the place of the incident photon, there are now a number of fast electrons and photons of reduced energy that may go on to take part in further reactions (Fig. 8).

It is the cascade of electrons that result in the physical and chemical changes in the material as well as the destruction of microorganisms. Because the probability of Compton scattering is low, the primary beam of gamma rays will penetrate long distances in material before the scattering occurs. This means that the gamma rays deposit energy over a relatively large area so that penetration is high (up to 50 cm) but the dose rate is low (Table 4).

By contrast to gamma, electrons focused into a beam generated by a linear accelerator with beam energies of 5 to 10 MeV have both mass and charge, so they interact readily with other charged particles, transferring their kinetic energy to materials by numerous elastic and inelastic collisions. In fact, as soon as charged particles penetrate solid materials, they are subject to the Coulomb force exerted by the atomic nuclei and are therefore in almost constant interaction with the material. These interactions result in many directional changes, ionizations, and radioactive processes that slow the electrons and ultimately limit their penetration to only 5 cm into material with a density of 1.0 g/cm^3 using a 10 MeV beam. E-beam energy is therefore deposited within materials over a short distance, making the dose rate very high (22,000 kGy/hr for a 50-kW beam) and allowing sterilization to take place in less than one minute.

The parameter measuring the energy transferred from the radiation source to the product is called the absorbed dose. The dose can be translated in terms of power requirements (i.e., intensity and energy of the beam) by taking into account the product characteristics (shape, size, and density) and the process parameters (i.e., throughput, scanning length). The penetration of gamma rays and electrons is inversely proportional to product density. The absorbed dose is the quantity of ionizing radiation energy imparted per unit mass of a specified material and is expressed as the gray (Gy) where 1 Gy = 100 rads or 1 kGy = 0.1 megarad. When a population of microbial cells is irradiated, the number of living units diminishes exponentially as the dose

increases, until no viable cells remain. Sterility is obtained in living organisms in two ways: directly through DNA strand rupture or through cell destruction related to chemical reactions in the organism or in its environment. Energy can be directly deposited in a bond of a macromolecule (protein, DNA, RNA) causing a rearrangement of its structure or free radicals generated from the water contained within the cell. The free radicals then react with the macromolecule altering its normal cellular metabolism that leads to loss of the reproductive capacity of the microorganism. In a nonaqueous environment as found in sterilization of most medical products, the principal sterilization mechanism is ionization of cellular material altering molecular structure or spatial configuration of biologically active molecules.

The parameters used to determine acceptable dose delivery of gamma sterilization are:

- Cycle time
- Product density
- Loading pattern
- Density mix

Process reliability and consistency are guaranteed by the well-known decay rate of the radioisotope. When the source and product are positioned correctly, small incremental changes are automatically programmed into the timer setting to account for the decay, thereby allowing products to be processed consistently. If product configurations remain the same as validated, the only difference in measured dose will be related to the variability in positioning product and uncertainties in dose measurement.

The parameters used to determine acceptable dose delivery of electron beam sterilization are:

- Beam energy
- Beam current
- Conveyor speed
- Scan width
- Product geometry
- Product density

Process reliability and consistency are guaranteed by control and monitoring of the beam, conveyor, and process parameters. Once parameters are established, products will receive the specified dose as long as product density, product packaging, and orientation are unchanged. The change from one product to another is relatively simple since the effect of the adjacent product are minimal.

STERILIZATION BY HEAT

Heat can be applied in either of the two forms: dry heat or moist heat. Dry heat kills the organisms by destructive oxidation of essential cell constituents. Inactivation of the most resistant spores by dry heat requires a temperature of about 160°C for 60 minutes. Dry heat is employed for glassware, syringes, metal instruments, and paper wrapped goods, which are not spoiled by high temperatures. It is also used for anhydrous fats, oils, and powders that are impermeable to moisture.

Moist heat kills organisms by coagulating and denaturing their enzymes and structural protein. Sterilization by moist heat of the most resistant spores generally requires 121°C for 15 to 30 minutes. Moist heat is used for the sterilization of culture media, and all other materials through which steam can penetrate. Moist heat is much more effective than dry heat. Sterilization can be done at lower temperatures in a given time at a shorter duration at the same temperature. Many sterilization cycles have been developed for use in a moist heat environment with calibrated equipment that has been properly installed and validated. Among the processes commonly used in industrial moist heat sterilization are the following:

1. Gravity air displacement: Sterilizers use gravity to remove air from their chambers. Steam introduced into the chamber creates a layer above the air, which increases until the air is pushed down through a drain at the bottom of the unit. After the air is

removed, steam temperature and pressure builds, and exposure time begins when the sterilization temperature is reached. Gravity sterilizers are used to sterilize surgical instrumentation, liquids, and linen.

2. Dynamic air removal (Prevac): This process is intended to sterilize products consisting of porous materials and/or items having cavities where air is difficult to remove. Prevacuum sterilizers use a pump to remove air from the chamber before steam is introduced. Dynamic air removal units are, therefore, more efficient than gravity air displacement sterilizers because air is pumped out before steam enters the chamber, so the steam can immediately penetrate packages.
3. Air pressure systems: Some product packaging cannot withstand the vapor pressure changes associated with moist heat sterilization. There are a number of available processes in which filtered compressed air is used to ensure that, for part or for the duration of the sterilization cycle, the pressure on the outside of the product equals or exceeds the inside pressure. These processes include cycles using air/steam mixtures, water spray, and water immersion.

Steam sterilization requires four critical parameters: steam, temperature, pressure, and time. Steam must be of high quality and contain no more than 3% moisture and a relative humidity (the amount of water vapor) of 97%. The appropriate temperature depends on the type of sterilizer being used. Gravity air displacement sterilizers require a temperature of 250°F (121°C). Dynamic air removal, washer sterilizers, and flash sterilizers require a temperature of 270 275°F (132 135°C). To achieve these temperatures, the pressure must reach 15 pounds per square inch (psi) for the 250°F (121°C) setting, and 27 psi to sterilize at 270°F. Note: Because the psi required to reach sterilization temperatures is related directly to the altitude, the exact psi required may vary slightly by geographical location. It is always best to consult the sterilizer's manufacturer for requirements in your area.

A typical steam cycle is outlined below:

Parameters for steam sterilization process (full cycle)

Phase	Parameter	Set point	Allowable tolerances
Pre-heat (Jacket temp. at 110°C)	Duration	35 min	±5 min
Conditioning	Vacuum pulses	3 with 6.50 psia delta	N/A
	Final pressure	2.0 psia reference (not a set point)	N/A
	Final temperature	114°C	±1°C
Steam injection	Rate (setting)	2°C/min after 112°C is reached	N/A
	Final pressure	31 psia (reference)	±3 psi
Exposure	Time	40 min	+1 min 0 min
	Temperature	122.0°C	+3.0°C 1.0°C
Exhaust	Exhaust rate	3 psi/min	±.2 psi/min
	Final pressure	2 psia	±1 psi
Drying time (under vacuum)	Time	20 min	+5 min 0 min

VALIDATION TECHNIQUES

A combination of biological and physical methods can be used to determine the optimal sterilization parameters for moist steam and EO. But for either gamma or E-beam irradiation, only the bioburden method is used. The selection of the appropriate approach is based on the nature of the product, bioburden, and packaging, manufacturing conditions, and type of sterilization equipment.

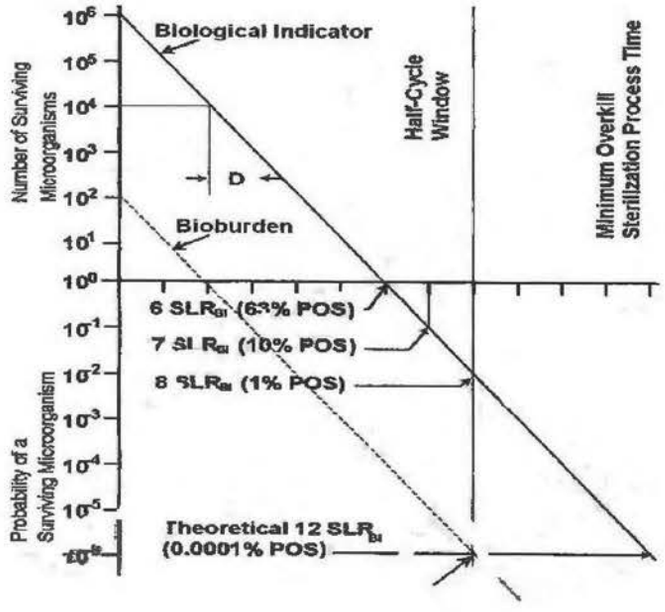

Figure 9 Example of an overkill cycle. *Source*: Adapted from ISO 11135.

Several methods can be used to develop effective cycles including:

- The overkill approach (Fig. 9) is the most widely used method for validation of EO or steam processes because it produces an overkill based on conditions causing inactivation of one million resistant bacterial spores that are more severe than those required to kill the bioburden. Three methods may be used in this approach:
  1. Minimally a 6-SLR at a half-cycle exposure time is demonstrated. This theoretically results in 10^0 survivors. When exposure is doubled, a 12 SLR is delivered and the product is considered sterile (has a sterility assurance level SAL-of 10^{-6}).
  2. Use a BI that has a greater resistance than required by a smaller and less resistant microbial population (Fig. 10).

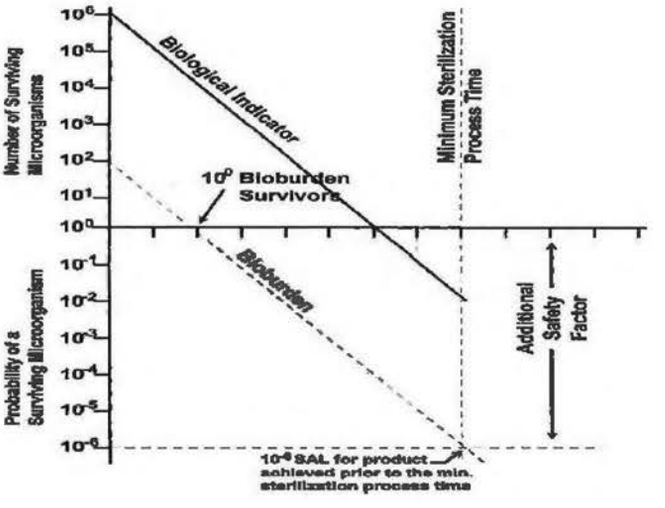

Figure 10 Relationship between bioburden and BI Survival.

For a steam process, this safety factor represents the inactivation of 12 logarithms of microorganism with a D121°C of approximately 1.0 minutes and a z value of 10°C. For challenge microorganisms having different D values, the population can be adjusted to achieve equivalent lethality; that is, the more resistant the challenge microorganism, the lower the population that can be used. For example, the challenge characteristics of a 10^3 population having a D value of 2.0 minutes, or a 10^5 population with a D value of 1.2 minutes are equivalent to those of a 10^6 population with a D value of 1.0 minute. It is important to remember that the D values and z values of microbiological challenges and product bioburden can vary in different environments (e.g., solutions and different manufacturing sites) and in different containers and closures.

- The bioburden approach this approach is used to validate either gamma or E-beam radiation processes. Sometimes natural bioburden may have a resistance greater than the BI system because of very high bioburden levels, high bioburden resistance to the sterilant, or the location of the bioburden on/in the product. Representative product samples should be subjected to incremental exposures (doses), sterility tested, or enumerated to generate a kill curve. Sometimes products may have very low bioburden or be made of temperature sensitive materials. Being able to validate a shorter exposure cycle or dose should be beneficial since the bioburden is usually much less resistant than a BI. Very tight control of bioburden is necessary for this approach.
- A combination BI/bioburden approach this approach is used when sufficient bioburden data is available to demonstrate that a BI challenge lower than 10^6 per carrier can be used. This method usually results in shorter cycle times and is gaining acceptance in efforts to optimize cycles.

VALIDATION OF A STEAM OR EO CYCLE

The sterilization validation program is conducted to demonstrate that the designed process can reproducibly sterilize specified products or product families to a defined SAL without damage to the product or package. The overkill method is commonly used. The BI for steam is usually 10^5 to 10^6 of heat-resistant spores of *Geobacillus stearothermophilus* with a D value of 1 to 1.5 minutes deposited on a carrier material or inoculated into a liquid-filled vial. For EO, the BI is a 10^6 population of *Bacillus aetrophaeus* spores with a minimum D value of three minutes inoculated on a carrier material, such as a filter strip, thread, or suture. A six (6) SLR of 10^6 BIs at a half-exposure cycle time is demonstrated, which correlates to 10^0 survivors. When doubled, the exposure time delivers a 12 SLR and an SAL of 10^{-6}. In addition, the process must demonstrate a microbial SAL appropriate for the product being sterilized. If products will be sold in Europe, an SAL of 10^{-6} is required for all products labeled "sterile." In the United States, some products that contact uncompromised tissue, such as drapes and gowns, can be validated to an SAL of 10^{-3}.

For efficient and cost-effective validation performance, prior product and process evaluation is suggested. If your company produces a wide range of sterile products, similar products can be grouped into families. A family of products can be considered to be all those products of similar design and materials of construction, but consisting of different sizes, that is, all Foley catheters, sized 8 French to 16 French, and similar bioburden levels. After family groups are determined, select the most difficult-to-sterilize representative product in the family to represent all the products in the group. Generally this product will have the highest and most resistant bioburden population (radiation) or have the most challenging design configuration and packaging that renders permeation a gas and steam into the product (EO and steam). If your evaluation results in multiple product families, it is advisable to select from the representative products, a single most-difficult-to-sterilize product that will be used as the master process challenge device (PCD).

SELECTION OF FAMILY REPRESENTATIVE

Each family of products will contain a number of products. From these products, the representative challenge product is selected. The selected product then will be the most

difficult to sterilize product in the family group and will be used in verification dose experiments. A simulated product not intended for sale can be used as long as it is made of similar materials and uses similar manufacturing processes as the actual product. The establishment and continued validity of the sterilization dose are related to both numbers and resistances of organisms on or in the product. This is the basic characteristic used to select the representative product. Other criteria that should be considered by a knowledgeable person to select the challenge product are:

- Number of microorganisms
- Types of microorganisms
- Size of product
- Number of components
- Complexity of product
- Degree of automation during manufacture (manually assembled products will generally have higher bioburden levels)
- Manufacturing environment

Modifications to products, such as raw materials, components or product design, changes to the manufacturing process, facility or environment should be formally evaluated and documented to assess their effects on bioburden levels and dose validation. Bioburden data should be collected on an establish timeframe for all products within the family to ensure that the selected representative product continues to be the most difficult to sterilize item in the group.

PREPARE THE PCDS BY PLACING THE BI STRIP (OR DOT OR THREAD) WITHIN THE PRODUCT

The BI should not occlude any passageway or limit the diffusion of the gas, but should be placed in the most interior location. Sometimes a simulated PCD is used, for example, a long length of tubing can be cut in half, the BI placed in a plastic connector and the two lengths of tubing attached to the connector. When making the simulated carrier be sure to seal the cut edges with adhesive to ensure that the gas cannot penetrate through the cut. This is usual done when the BI cannot physically be placed within the finished product. In steam validation, a small volume (10 μm) of a liquid suspension containing the challenge organism can be directly inoculated onto or in products. Sometimes the BI is placed within a product during manufacturing process before the product manufacturing process is completed. BIs of different physical shapes, including strips, dots, or threads, can be obtained from manufacturers to facilitate placement into small spaces.

The basic elements of the validation program are described below.

1. Installation qualification (commissioning): Equipment-oriented evaluation consisting of establishing and implementing the ancillary equipment programs and documenting the equipment present. The system must be defined by the operator (contract sterilizer or in-house) of the equipment and reviewed by the manufacturer.

   The minimum documentation required should include

   - As-built drawings and blueprints of the equipment and facility including sterilizer, all processing equipment, precondition rooms, aeration rooms and any ancillary systems for air, steam, EO, and water;
   - model and serial numbers of all individual components including gauges, timers, etc.;
   - calibration of all instrumentation used for monitoring, controlling, and recording;
   - operating instructions/procedures, calibration, and preventive maintenance procedures in place;
   - piping and electrical schematics and drawings;
   - copy of computer software and its validation;
   - utilities including adequacy and proper operation, proper materials of construction, sufficiency of supply, presence and location of filters, absence of dead legs in water and steam supply;

- safety procedures;
- operating procedures for all the equipment;
- step-by-step operating instructions.

2. Operation qualification: Cycle exposure tests necessary to assure the equipment operates as designed and is capable of delivering the specified process within tolerances. The tests are conducted without product.
3. Performance qualification: A series of planned runs with product, microbial challenge (PCD), and measurement of load temperatures (RH in EO) that confirm that the cycle parameters from the cycle development program produce the required microbial lethality and do not compromise product or package functionality. Any change to loading pattern, packaging, equipment or process parameters, or on the addition of new or altered products shall result in an evaluation of their effect on the validation. Loading patterns shall be specified and a representative load based on the most difficult to sterilize load shall be used. During design of a steam cycle, F_0 calculations can be considered because lethality is occurring at temperatures above 100°C (Table 4).

At a minimum, the following runs shall be performed in an EO validation only:

1. Fractional cycle: A minimum of one (1) run with all critical parameters at a minimum and the gas exposure time set at 1/4 or 1/6 of the predicted full cycle exposure time. This cycle should contain high bioburden product(s) of product family representatives and BIs placed within the process challenge device(s) (PCD) to demonstrate that the resistance of the product bioburden is less than or equal to that of the BI.
   - Sterility tests results should indicate total kill of product bioburden and survival of some or all of the BIs. If product bioburden is not entirely inactivated, but fewer tests are positive compared to the BI, the run is acceptable. Another fractional cycle with increased gas exposure time can be performed to demonstrate total kill of the bioburden.
   - An external PCD can also be included to determine the relationship to the internal PCD. If the BI growth from the external BI is equal to or greater than the BI growth from the internal PCD, then the external PCD can be used for monitoring routine loads.
2. Half cycles in both steam and EO: A minimum of three (3) consecutive acceptable runs with all critical parameters at a minimum and the dwell time set at 1/2 of the predicted full cycle time.
   - All BIs placed within the PCD shall be inactivated in these cycles. All process parameters shall operate with defined specifications and tolerances. If unacceptable data from any of the three (3) runs is found (e.g., BI positive, cycle parameter not met), an investigation is performed. A cause related to cycle lethality may result in restarting the validation.
   - BIs in the external PCD used only in EO can be all negative or some positive since this BI is merely an indicator of lethality and is shown to be more difficult than internal PCD.
3. Full cycles: A minimum of one (1), but three (3) suggested, with all critical parameters at nominal settings. One (1) additional run may be performed with critical parameters set at maximum for evaluation of product residuals and/or functionality.
   - Product samples are evaluated for residuals and functionality; packaging for maintenance of sterile barrier.
   - EO residuals are evaluated in one (1) or more products for EO and ECH.
   - If more than one resterilization is contemplated, some product samples can be exposed to more than one full cycle to ensure functionality when resterilized.
4. Certification: Formal review of the data and documentation with an approval by the appropriate organizations within the company (final report).

The PQ shall confirm for an EO validation:

a. At the end of preconditioning, the load is within the temperature and humidity ranges document in protocol cycle parameters;

b. The maximum transfer time of the load from preconditioning to the sterilization chamber is not exceeded;
c. Gaseous EO has been admitted to the chamber (pressure rise, loss of weight of the gas cylinders);
d. Quantity of gas used is within the specified range;
e. All BIs are inactivated in the half cycles
f. The temperature and humidity in the chamber, and other process parameters, are within ranges documented in the protocol;
g. During aeration, the load is within specified range.

The PQ shall confirm for a steam validation:

a. All BIs are inactivated in the half cycle
b. Product and packaging remain functional after a full cycle
c. Dry time is sufficient

5. Requalification timeframes: A statement on the frequency of the requalification; the industry average is annually.
   - It is acceptable to perform a paper work review at the first annual term. If this review shows that no changes have been made to the cycle or product that the cycles run during the previous year were without major deviations and the contractors equipment testing is acceptable, the cycle can be considered requalified upon acceptance of review documentation.
   - At the next annual review, a minimum of one (1) half cycle should be run under protocol to document the continued process lethality.

VALIDATION OF AN IRRADIATION PROCESS

Four approaches to selection of the dose can be used depending on the batch size and product bioburden level:

1. Method 1: Determination of the bioburden then used to select and test a 10^{-2} verification dose based on population C,
2. Method 2A and 2B: Incremental dosing of product samples,
3. Method VD_{max}: Substantiation of 25 kGy as a sterilization dose; appropriate for products with <1000 colony forming units (CFU)/product.

GROUPING INTO PRODUCT FAMILIES

Product families for radiation processing are based on bioburden. Bioburden histories for individual products should be maintained over time. In addition, assessment of individual products and their similarities should be considered as well as the impact of the variables shown below on the bioburden. Document the review and the rationale for placement of products into families and create a final family listing including the product name and catalog (part) number. This can become part of the protocol or incorporated into a standard operating procedure (SOP). Additional sterilization doses at 15, 17.5, 20.0, 22.5, 27.5, 30.0, and 32.5 kGy can be validated as outlined in AAMI TIR 33: 2005 (5).

After evaluation of bioburden populations, examples of product-related variables to consider are:

- Raw materials
- Components
- Product design and size
- Manufacturing process
- Manufacturing equipment
- Manufacturing environment
- Manufacturing location

DOSE SETTING USING METHOD 1 (BIOBURDEN METHOD)

The methods of selection of the sterilization dose use data derived from the inactivation of the microbial population in its natural state and are based on a probability model for the inactivation of microbial populations. The selection depends up experimental verification that the response to radiation of the product bioburden is greater than that of a microbial population having a standard resistance. Using computational methods and the standard distribution of resistances (SDR) shown below, individual doses required to achieve stipulated SALs have been calculated for levels of bioburden on product just prior to irradiation. These values are the basis of the dose table documented in ISO 11137-2:2006 (6).

Standard Distribution of Resistances D_{10} Values

D_{10} kGy	1	1.5	2	2.5	2.8	3.1	3.4	3.7	4	4.2
Probability	0.6549	0.2249	0.063	0.0318	0.0121	0.0079	0.0035	0.0011	0.0007	0.00007

This method depends on experimental verification that the response to radiation of the product bioburden is equal to or less than that based on historical data of microbial population having a standard resistance. In other words, the probability model used to develop Table 5 in ANSI/AAMI/ISO 11137-2:2006 assumes that the in situ bioburden is a mixture of homogeneous populations, each having its own unique susceptibility to radiation and its own rate of inactivation (Fig. 11), which presents a lesser challenge than the model. Testing is performed at a dose calculated to give an SAL of 10^{-2}. This is called the verification dose and represents the probability that a unit of product contains one or more viable organisms. Sterility testing of products subjected to the verification dose should produce 1% positives. If a larger than expected number of units test positive, then either the resistance of the bioburden is higher than expected or the bioburden has been underestimated. Method 1 is preferred in most situations because of its reasonable cost and study time. Sample requirements initially total 136 (100 for the dose experiment, 30 for bioburden determination, and 6 for bacteriostasis/fungistasis testing) and 110 (100 for the dose experiment and 10 for bioburden determination) thereafter on each quarterly dose audit.

The sequence of steps required to validate a radiation process using method 1 as follows:

1. Select the appropriate SAL and obtain samples of product units.
2. Determine the bioburden levels using 10 final packaged products from 3 different batches. Apply correction factor. Even though validation of bioburden recovery

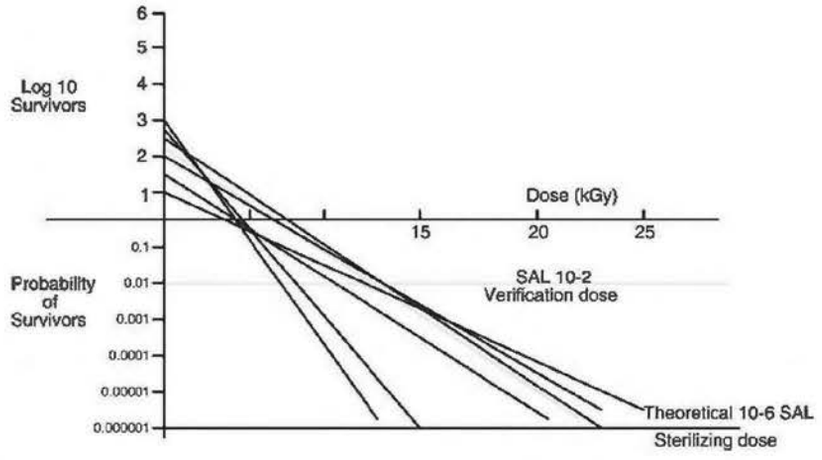

Figure 11 Theoretical survivor curves for method 1 population (1000 CFU). *Source*: Courtesy of PDA/DHI Publishing.

Table 5 Material Penetration Depth of Three Types of Radiation

		Penetration (cm)	
Type	Source	Irradiating 1 side	Irradiating 2 sides
Gamma	CO^{60}	10.2	40.6
X ray	50 KeV	<0.1	0.5
	10 MeV	12.7	61
E beam	5 MeV	1.8	4.3
	10 MeV	3.8	8.6

method is not required, it is recommended to have a better understanding of the actual numbers of organisms that will be subjected to the verification dose.

3. Determine the batch average of each of the three batches.
4. Calculate the overall batch average.
5. Select the verification dose from dose Table 5 of ISO 11137-2 using either the highest batch average (if one or more batch average is greater than the overall batch average) or the overall batch average.
6. Perform the verification dose using 100 final packaged products from a single batch. The samples can be selected from any of the three batches from which the bioburden samples were taken or from a fourth batch. Send the packaged samples to the irradiator and indicate the purpose and the dose. The actual dose delivered can vary by +10%. If the dose does not meet the specification, do not proceed to the sterility test. Repeat the verification dose using fresh samples.
7. Sterility test the 100 units by incubating the dosed products in soybean/casein broth at 30°C ± 2°C for 14 days. Bacteriostasis/fungistasis testing should also be performed if this is the first time the product has been subjected to a sterility test.
8. Review results to assess the acceptability of the experiments:
   - 1 or 2 positive tests = acceptable.
   - >2 positives with no deviations in the testing or dose delivery = dose method is not valid for the product and the alternative method should be used (method 2)
9. Establish sterilization dose if test is acceptable by finding the closest bioburden number in dose Table 5 equal to or greater than the average bioburden and the selected SAL level.

Recently, a new validation approach called VD_{max} was developed. This method based on the SDR of the method 1 population can be used for any size production batches with average bioburden of less than 1000 CFU per product. The method preserves the conservative aspects of the resistance characteristics of the SDR, but is more accurate for low bioburden products. It is not limited to batch size or production frequency and the number of product samples (10) needed for the verification experiment is constant. The VD_{max} method can be used for selected sterilization dose of 15 and 25 kGy as outlined in ISO 11137-2:2006, *Sterilization of health care products Radiation Part 2: Establishing the sterilization dose.*

The following steps are followed for substantiation of a 25 kGy sterilization dose:

1. Obtain at least 10 product units from each of three production batches immediately prior to sterilization.
2. Determine the average bioburden on each product as outlined in ISO 11737-1:2006 and average the bioburden values for each batch. Apply the correction factor on the basis of the validation of bioburden recovery. Compare the three batch averages and select the grand average or one average if two or more times the overall average.
3. Obtain verification dose. Find the closest bioburden value greater than or equal to the average in Table 9 in ISO 11137-2. Obtain the corresponding verification dose.
4. Irradiate 10 product units from a single batch at the VD_{max} obtained in Table 9. These may be selected from any one of the bioburden batches or a fourth batch. The actual

dose may vary from the calculated dose by not more than +10%. If the delivered dose is less than 90% of the verification dose, the experiment may be repeated.

5. Sterility test the product units according to ISO 11737-2 (7) using soybean-casein digest broth incubated at 30 ± 2°C for 14 days. Record the number of positive tests.
6. Interpretation of results. If no more than one positive test is observed in the 10 tests, 25 kGy is substantiated as the sterilization dose to achieve at least a 10^{-6} SAL. If 2+/10 tests are observed, a confirmatory verification dose experiment shall be conducted. If 3+/10 tests are observed, 25 kGy is NOT substantiated and another dose setting method must be used.
7. Confirmatory verification dose experiment (if required)

   Randomly select 10 product units from a single batch (can be from the batches previously sampled or from a new batch). Use the same dose as determined initially and irradiate the 10 product units at the confirmatory verification dose. The same dose tolerances apply. Sterility testing results are evaluated as follows:
   - 0+/10 25 kGy is substantiated
   - 1 10+/10 25 kGy is not substantiated.

ROUTINE MONITORING FOR EO AND STEAM

After successful completion of the sterilization validation, a process specification must be written, which explains the proper procedures to be followed routinely. The process specification must describe the aspects of the sterilization process necessary to assure conformance with the validated cycle and be maintained with an established change control procedure. All specified process parameter minimum values must be met or product cannot be released as sterile regardless of the microbial test results. All BIs must test sterile for the indicator organism and results of product sample (if used) testing must be acceptable.

The process specification should include

1. identity of equipment qualified for sterilization;
2. list of the items approved for sterilization in the process covered by the specification, that is, the product listing;
3. written procedures for sterilization process operations, or reference to specific operator manuals;
4. sterilizer loading configurations and pallet patterns (EO);
5. descriptions and diagram of the placement of BIs and other test samples;
6. list of all process parameters with set points and minimums and maximum tolerances, and reference to the recording and controlling instruments for each;
7. requirements for routine quality control tests and periodic audits related to sterilization;
8. written criteria for sterile product acceptance, reprocessing, rejection, and release for distribution, including instructions for selection, handling, and testing of samples.

Routinely, the process is monitored with the same resistant BI used to qualify the cycle. Routine use of product sterility testing is not required. A minimum number of BIs must be included in each cycle. The recommended number for EO is based on the load volume as defined in ISO 11135-2:2006. If an external PCD has been validated, no internal BIs are required. EtO cycles can be validated for parametric release as outlined in AAMI TIR 20: 2001. (8).

The quality function is usually responsible for reviewing sterilization documentation. Even if the process is performed by a contractor, the manufacturer is responsible for assuring that the appropriate cycle was performed and that the cycle parameters were within acceptable tolerances established during the validation, as follows:

1. Minimum product temperature was met before entering preconditioning
2. Temperature and humidity in preconditioning met specification
3. Transfer time from preconditioning to the sterilization chamber
4. Temperature and pressure throughout the cycle
5. Secondary record of gas admission to the chamber (usually cylinder weight)
6. Exposure time

7. BIs used were sterile and within expiration date
8. Time and temperature within aeration room

Failure to meet the physical specification or BI sterility should result in quarantine of the sterilization load and in an investigation. The investigation should be documented. If the physical process variables are below the minimum tolerances of the specification or growth of the test organisms is observed, the sterilization load should not be released; product should be either resterilized or scrapped. If one (1) or more of the BIs test positive and the growth is identified as the indicator organism, an investigation should be performed and the load resterilized. If the BI on a validated full routine cycle tests positive, then a major problem has occurred that just be identified and rectified.

ROUTINE MONITORING FOR RADIATION

After successful completion of the irradiation sterilization validation, a process specification must be written, which explains the proper procedures to be followed routinely. The process specification must describe the aspects of the sterilization process necessary to assure conformance with the validated dose and dose mapping and be maintained with an established change control procedure. All specified process parameter values must be met or product cannot be released as sterile. The process specification should include

1. identity of radiation modality qualified for sterilization;
2. list of the items approved for sterilization in the process covered by the specification, that is, the product listing;
3. the maximum dose allowed and the sterilization dose;
4. written procedures for sterilization process operations, or reference to specific operator manuals;
5. sterilizer tote loading configurations and dose mapping showing relationship between the reference point and the maximum and minimum dose positions;
6. descriptions and diagram of the placement of dosimeters and other test samples;
7. specified minimum dose and minimums and maximum tolerances, and reference to the dosimeter system used routinely;
8. requirements for routine quality control tests and periodic audits related to sterilization;
9. written criteria for sterile product acceptance, reprocessing, rejection, and release for distribution, including instructions for selection, handling and testing of samples.

Failure to meet the physical specification should result in quarantine of the sterilization load and in an investigation. The investigation should be documented. If the delivered dose is below the validated dose, the sterilization load should not be released; product should be either resterilized or scrapped. Since radiation effects on materials are cumulative, any decision to resterilize must be based on acceptable product aging test data after multiple sterilizations. Process interruptions or delays should be evaluated to determine the effect on the microbiological quality of the product and on the dosimetry systems.

In addition, to ensure the numbers and resistance of the bioburden remains steady, a verification dose audit is preformed each quarter. This is essentially a repeat of the initial validation dose experiment but only 10 bioburden samples are pulled from a single lot. The result of the bioburden test is for information only because an additional 10 products (for VDmax) or 100 products (Method 1) are dosed at the original validated verification dose no matter the current bioburden levels. If audit results fail (>1+/10 or >2+/100) augmentation or revalidation is required.

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9 | Steam sterilization

James Agalloco

INTRODUCTION

Moist heat sterilization in an autoclave is certainly the most widely utilized means of sterilization within the healthcare industry. It may also be the oldest method in use with the first practical sterilizer dating to the late 19th century (1). The vast industry experience with steam sterilization has resulted in the development of a variety of steam processes adapted for specific applications. In each of these, the sterilization process is accomplished by the presence of liquid water on the surface of the microorganism at elevated temperatures (2). The liquid water is necessary for coagulation of proteins within the microbe that result in its death and is implicit in "moist heat" sterilization.[a] In steam processes, the liquid water can be provided by saturated steam in contact with the surface (as utilized in hard goods sterilization) or via water content in a liquid filled container (as in media preparation or terminal sterilization of finished products). The requirement for liquid water must not be overlooked; sterilization with superheated steam (where no liquid is present) has far more in common with dry heat processes than it does with moist heat. A graph of the saturation curve for steam is shown in Figure 1.

Moist heat sterilization can be accomplished along the saturation curve itself where water is present both as liquid and as a gas (steam), or in the liquid region (above the saturation curve) where the pressure exceeds saturation and only the liquid phase is present. Sterilization with saturated steam is preferable to water, due to the additional heat available when the saturated steam condenses and releases its heat of condensation.[b] The rapid transfer of heat to the items to be sterilized by condensing steam is essential to rapid destruction of microorganisms and a major reason why moist heat is preferred over other sterilization methods.

MICROBIOLOGY OF STERILIZATION

The death of microorganisms by all sterilization methods shares a common phenomenon (3). The log number of surviving microbes when plotted against the exposure time provides an essentially straight line (Fig. 2).

The steeper the slope of this line, the less resistant the organism is to sterilization process. The inverse of this slope is known as the *D*-value and is usually expressed in minutes. The *D*-value can be influenced by a number of factors aside from the microbial identity including recovery media, age of the microbe, recovery methods, substrate on which the microbe is exposed, etc. (2). *D*-values are determined through the use of a specially designed sterilized vessel called a biological indicator evaluation resistometer (BIER) (4). BIER vessels provide precise control over exposure temperature and process time to allow for determination of the microbial death curve. Bioburden microorganisms are destroyed far more rapidly than the moist heat-resistant spore formers customarily utilized as biological indicators (Fig. 3). Nearly all sterilization processes are validated using biological indicator with higher resistance whose death in the validation exercise provides added assurance that the bioburden microorganisms with lower resistance are destroyed in routine usage of the sterilizer (when the biological indicator is not present).

The preferred biological indicators for steam sterilization are spore-forming microorganisms from the *Bacilli* or *Clostridia* sp. whose resistance is several orders of magnitude higher than vegetative cells under the typical moist heat process conditions. The body of

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[a]Sterilization in the absence of liquid water requires substantially higher temperatures (typically >150°C) and impacts the microorganisms differently.

[b]A 1°C drop in temperature for 1 g of liquid water releases 1 calorie. The condensation of 1 g of saturated steam to liquid water at 121°C releases approximately 525 calories.

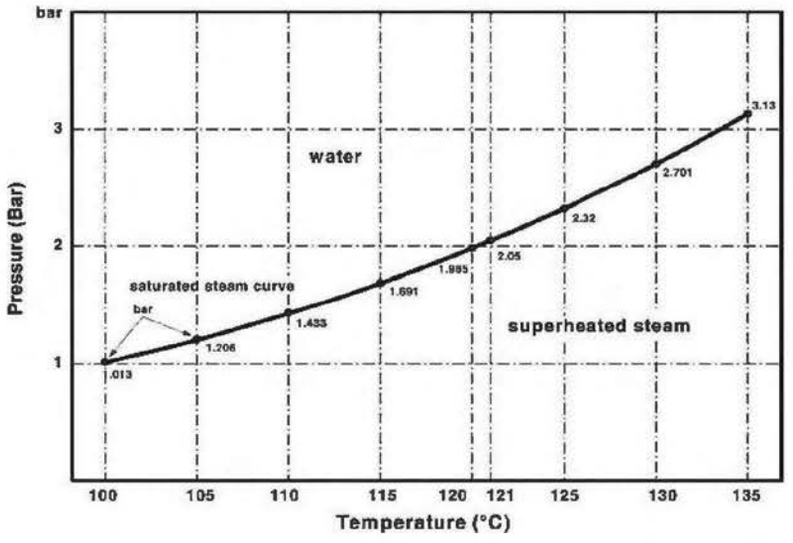

Figure 1 Saturated steam curve. *Source*: Courtesy of Fedegari Autoclavi.

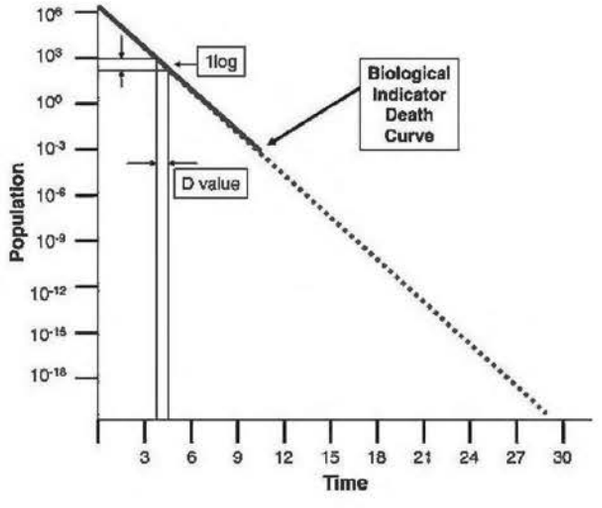

Figure 2 *D* value.

knowledge regarding steam sterilization and consistency of the results is such that mathematical correlations between the physical and microbial data are utilized to provide appropriate process control (2). These correlations are necessary to assure product safety (sterility) to the desired level. A closer look at Figure 3 provides some clarification as to why these correlations are necessary. In each death curve, determining the number of microorganisms is only possible when there are viable microorganisms present (the solid portion of the biological indicator death curve). The dotted line portion of the death curve represents the probability of surviving microbes where their number is too low to count. Where the desired level of confidence in the sterilization process on the vertical scale intersects the death curve defines the minimum process time required (Fig. 4).

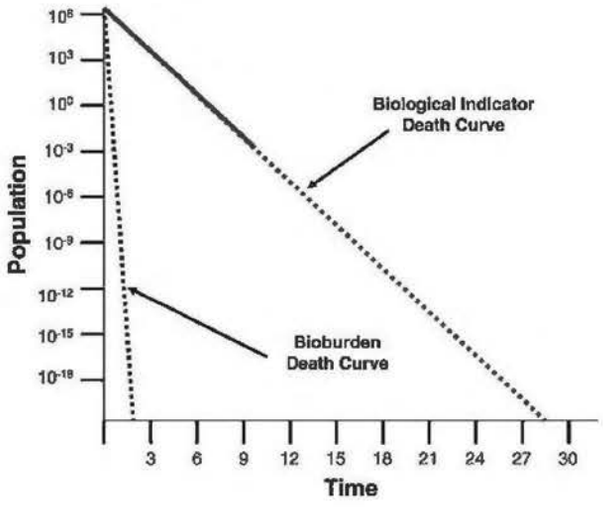

Figure 3 Microbial death curves relative resistance of bioindicator and bioburden organisms.

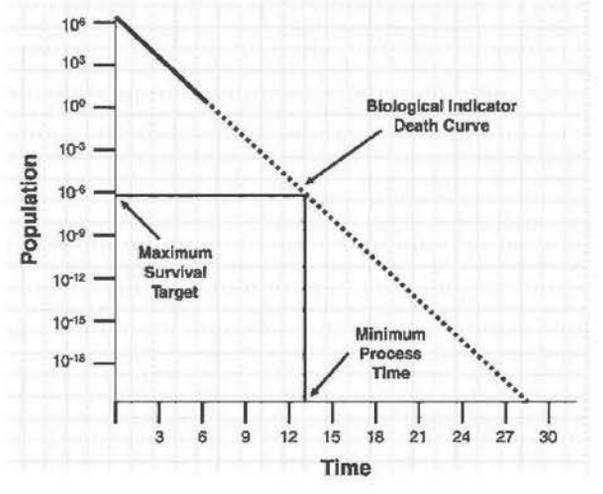

Figure 4 Setting process target for 12 log reduction of biological indicator population.

Were the process to be operated at precisely the conditions where the *D*-value has been determined, initial validation and day-to-day process control would be greatly simplified. Unfortunately, it is extremely difficult to provide essentially constant process conditions in routine sterilization process on a commercial scale. There are many real world factors that prevent constant conditions in production settings including chamber size, load size, item complexity, item wrapping materials, and item orientation. To accommodate these elements, a means for relating physical conditions at varying temperatures to microbial destruction is necessary. The *D*-value, which is essentially the rate at which microorganisms are killed, is largely a function of the temperature the higher the process temperature, the more rapid their destruction. The general method for sterilization process evaluation uses this temperature dependency to allow for the estimation of the lethal effect on microbes at a range of temperatures near the *D*-value (5,6). A plot of the *D*-value against process temperature provides a straight line for many organisms and the slope of the line is termed the *z*-value (Fig. 5).

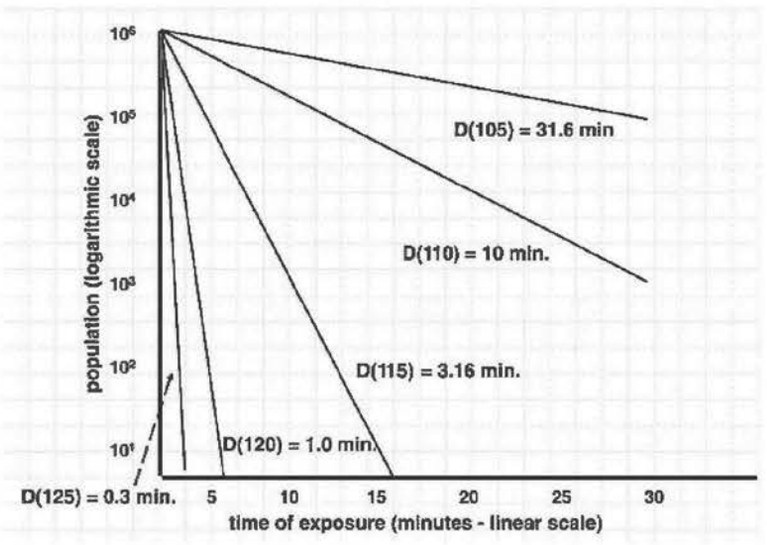

Figure 5 Effect of temperature on *D* value.

The general method estimates the lethality over the process duration by calculating the kill rate for microorganisms as the temperature progresses through the sterilization cycle. The shorter the time interval at which the temperature is measured the more accurate the estimate will be (Fig. 6).

To compare the effectiveness of sterilization processes, a standard means of lethality estimation was defined. When first developed, the concern was for food safety and the survival of *Clostridium botulinum* in canned foods. A process temperature of 250°F was found to be effective for this process, and this condition was established as the standard base temperature for estimation of sterilization process lethality. The Celsius temperature scale equivalent of 250°F is 121.1111...11°C.[c] To calculate lethality relative to a base temperature a correction is applied employing the *z*-value. At 121.1°C (250°F) a *z*-value of 10°C (18°F) is customarily assumed.[d] One minute at this temperature has been defined as F_0. Simple mathematics can be utilized to calculate estimated lethality at other temperatures using the lethality equation. For the specific reference temperature of 121°C and a *z* value of 10.0°C, the lethal-rate equation is:

$$L = 10^{\frac{T-121.1°C}{10.0°C}}.$$

Summing the instantaneous lethality over the sterilization process allows for the calculation of the overall process lethality delivered at those varying conditions. Table 1 indicates how this might be determined for an arbitrary steam sterilization process. The accumulation of lethality is only possible when the steam is saturated. For terminal sterilization, this occurs throughout the process. For parts sterilization it begins during the come-up and ends when evacuation of the chamber begins at the conclusion of the dwell period. It is customary to only consider lethality contributions at temperatures above 100°C because the contribution below that temperature is miniscule (7).

[c]For the sake of convenience, the temperature value is truncated to 121.1°C, but 121°C or even 120°C could be used, allowing for easier calculation, albeit with slightly different values. The arbitrary nature of the lethality standard must be recognized, given that the original choice of 250°F was equally arbitrary.

[d]The *z* value chosen 10°C is another arbitrary choice that facilitates calculation. The *z* values for moist heat resistance biological indicators are typically between 7 and 12 minutes at 121°C.

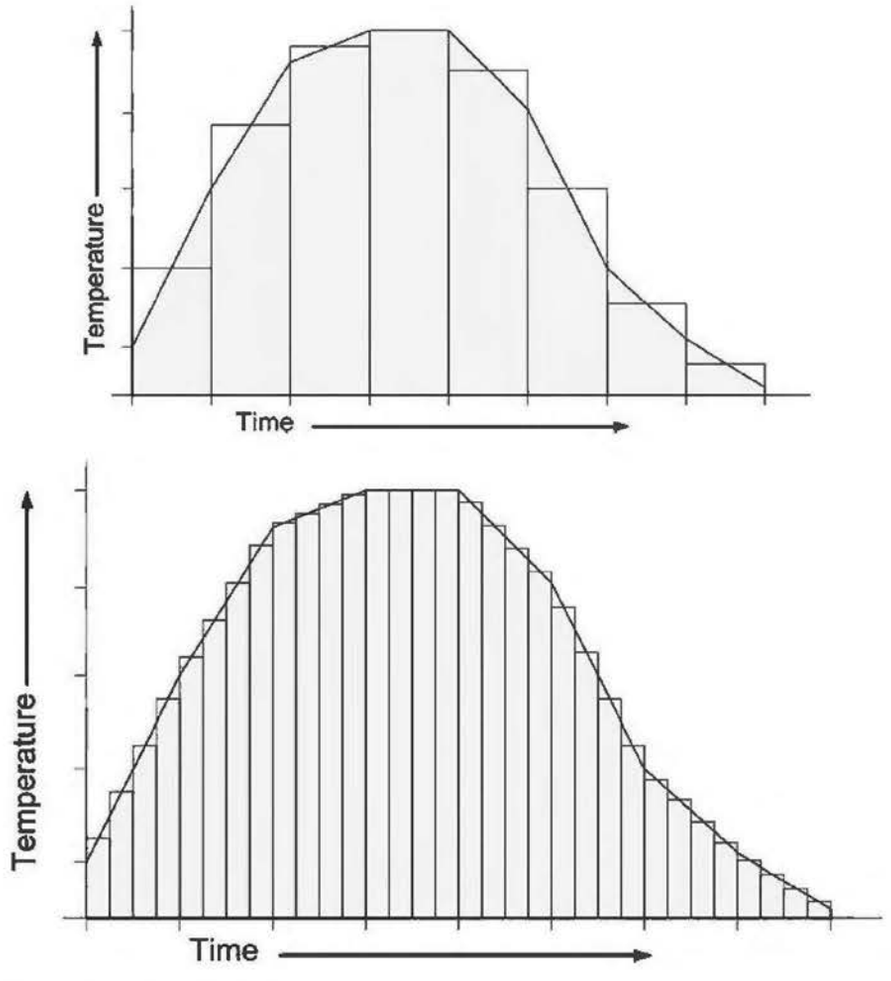

Figure 6 F_0 graphical calculation.

Table 1 Calculating Process Lethality

Calculating lethality $L = 10^{\frac{(T_0 \quad T_b)}{z}}$

T	$T \quad T_b$	$\frac{T \quad T_b}{z}$	Incremental lethality	Cumulative lethality
101	20	2	0.01	0.01
111	10	1	0.1	0.11
121	0	0	1	1.11
124	3	0.3	1.995	3.006
131	10	1	10	13.006
121	0	0	1	14.006
111	10	1	0.1	14.106

$T_b = 121°C$, $z = 10°C$, $\Sigma L = F_0$.

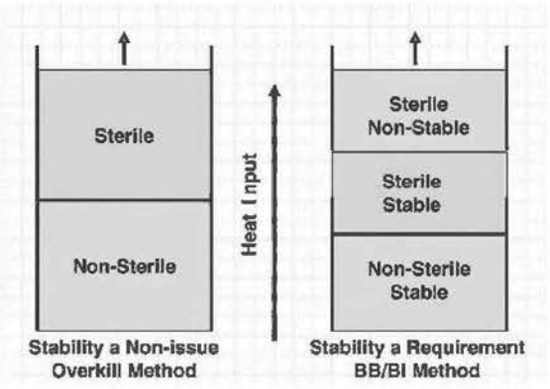

Figure 7 Comparison of parts and terminal sterilization.

STERILIZATION AND MATERIALS

The effects of the sterilization process must be considered on more than just the microorganisms present on or in the materials. Sterilization processes by their very nature incorporate conditions that have a deleterious impact on the materials being sterilized. The extreme temperature and moisture conditions that are microbiologically lethal can readily alter the chemical and physical properties of many materials. In the sterilization of stainless steel, glass or most other equipment items the adverse material effect is minor or nonexistent. The time-temperature conditions required for microbial inactivation can be substantially exceeded without concern for the material consequences. This can be compared to jumping over a hurdle; clearing the hurdle by an excessive height has no measurable adverse effect. Where the items being sterilized are subject to damage by the sterilization process, an upper limit to the process should also be defined. This might be considered equivalent to jumping through an open window (Fig. 7). Maximum conditions that must not be exceeded if material properties are not to be altered should be defined in addition to those required minimally to achieve sterilization. Having upper and lower constraints on the process may require changes in the process design, process equipment, and validation methodology. Each of these will be addressed later in this chapter.

The division between the simpler process requirements necessary for heat stable items and the more complex needs of materials that are potentially affected by heat has resulted in increasing differences in the sterilization cycles. Loads of heat-resistant items are typically called hard good loads or porous item loads in Europe and parts loads in North America. The items in these loads are sterilized by direct steam contact with the items on the surface. The removal of air (and condensate) from the sterilizer chamber assures a more consistent process across the chamber. For items that are sensitive to excessive heat such as finished products in their final product containers, in-process solutions, and laboratory media, care must be taken to avoid overprocessing. The internal water content of the materials in the containers provides the means for sterilization of the contents. The heat necessary to reach sterilizing conditions is provided by heat sources external to the product container by steam, steam-air mixtures, steam-water-air mixtures, or superheated water. In these systems the removal of air (and condensate) from the chamber may not be necessary. Loads for liquid filled containers are termed nonporous in Europe. Consistent with the differences in the sterilization cycle being performed, the sterilizer equipment may be adapted to better suit the specific process being executed.

STEAM STERILIZATION CYCLES AND EQUIPMENT

The first steam sterilizers originally built in the later 1800s were relatively simple pieces of equipment in which saturated steam was supplied to a pressure vessel (1). Air and condensate (formed by the steam as it heated the load items) left the chamber at the bottom through the actions of gravity (cold air and condensate are both denser than hot steam). This type of

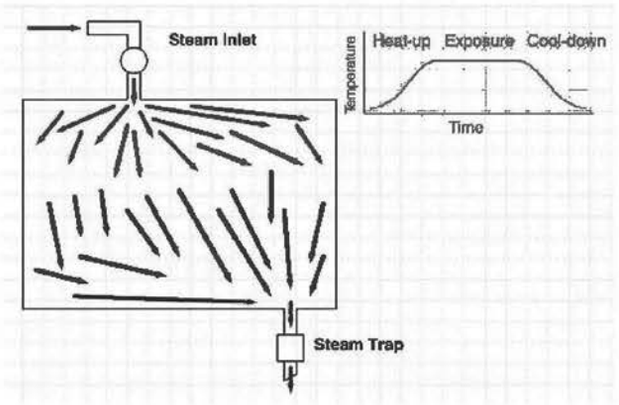

Figure 8 Gravity displacement cycle.

process was termed a gravity displacement cycle and is still in common use in smaller sterilizers utilized in medical and dental offices. The cycle profile is shown in Figure 8 and shows a slow ramp-up of temperature to the desired process condition.

One of the primary limitations of the gravity cycle is the time required for steam to penetrate all of the loads items. If sufficient time is not allowed during the dwell portion of the cycle, residual air and/or condensate could be retained that might prevent the cycle from achieving sterility of the load items. This cycle can be performed in the simplest of sterilizer designs: a simple chamber with a steam inlet at the top and a drain at the bottom.

The prevacuum cycle was developed as a means for improving process efficiency and effectiveness through the mechanical removal of air and condensate. Initially, only single prevacuum was drawn on the chamber, but it was quickly recognized that multiple alternating vacuum and steam pulses would be substantially more effective (Fig. 9).

Adapting the sterilizer design to accommodate the prevacuum cycles requires only the addition of a vacuum pump to the chamber (Fig. 10). The vacuum pump provides a means for improved drying of load items post exposure by lowering of the chamber pressure aiding in steam and condensate removal by evaporation.

The gravity displacement and multiple prevacuum cycles are commonly utilized for sterilization of hard goods, and while they can be utilized for the more complex processes needed for liquid filled containers, sterilizer designs specifically intended for nonporous loads are employed where those processes are in routine use. Steam-air (Fig. 11) and steam-water-air (Fig. 12) sterilizers are in common use for liquid containers in the pharmaceutical industry. The water utilized in steam-water-air units is customarily sterilized with the load, so its initial microbial quality is of little consequence. Air utilized for overpressure (or for breaking of vacuums at the conclusion of post-cycle drying) is 0.2 μm filtered just prior to entering the chamber. The cycle performed in these units are somewhat similar, and many employ an increase in air pressure during the latter stages of the cycle to prevent container damage due to pressure differences between the container interior and exterior (Fig. 13).

Firms producing larger volumes may employ continuous sterilizers in which a belt system moves containers through heating and cooling chamber in series. These types of designs are also commonplace in the food industry for canned goods. Immersion sterilizers where the load is sterilized by superheated water are utilized for smaller volumes in the food industry, but have not seen widespread use in the global healthcare industry.

Terminal sterilization is frequently associated with parametric release, especially for those firms that produce large volume parenterals. Parametric release replaces the end-product sterility test with controls that focus on successful execution of the sterilization process within restrictive requirements derived from the validation effort. Parametric release requirements are defined by the regulators to assure that the firm's product release approach adequately assures patient safety (8 11).

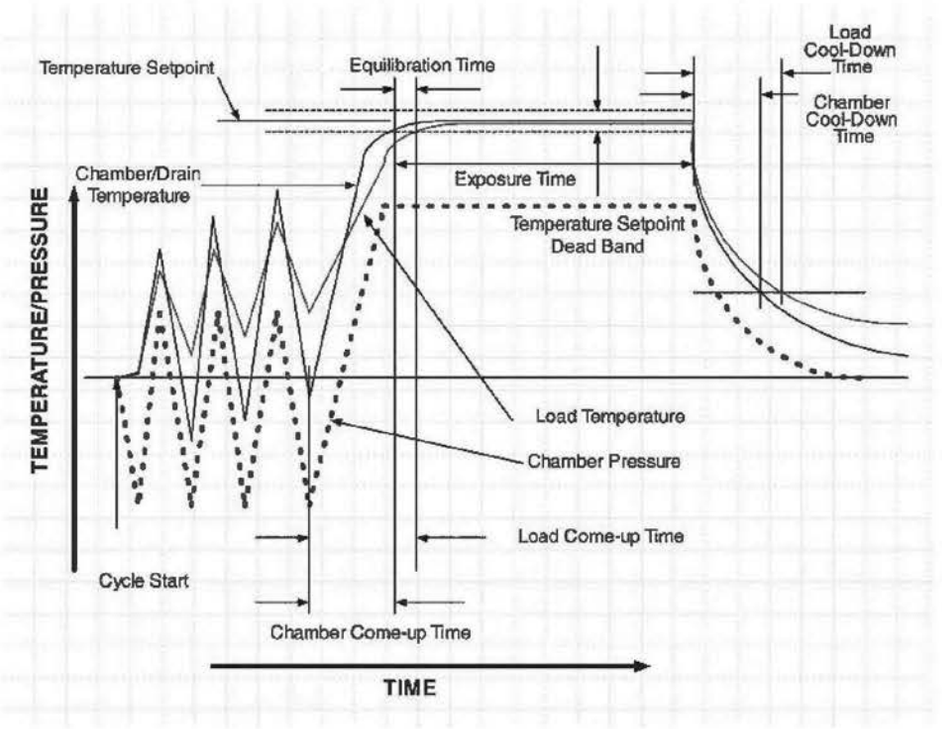

Figure 9 Multiple prevacuum cycle.

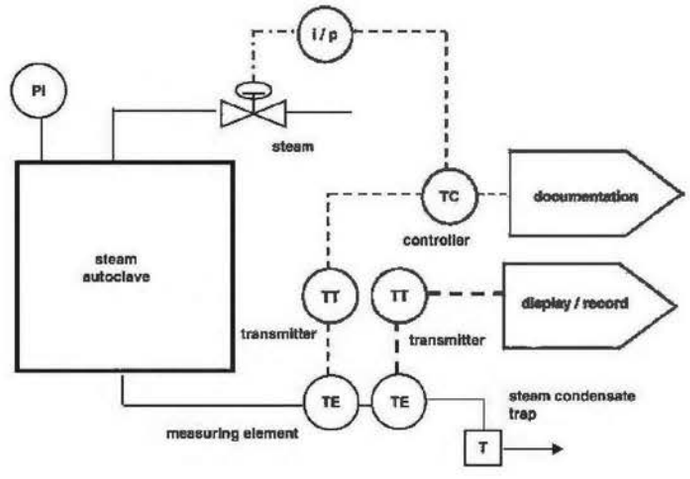

Figure 10 Typical sterilizer with vacuum pump.

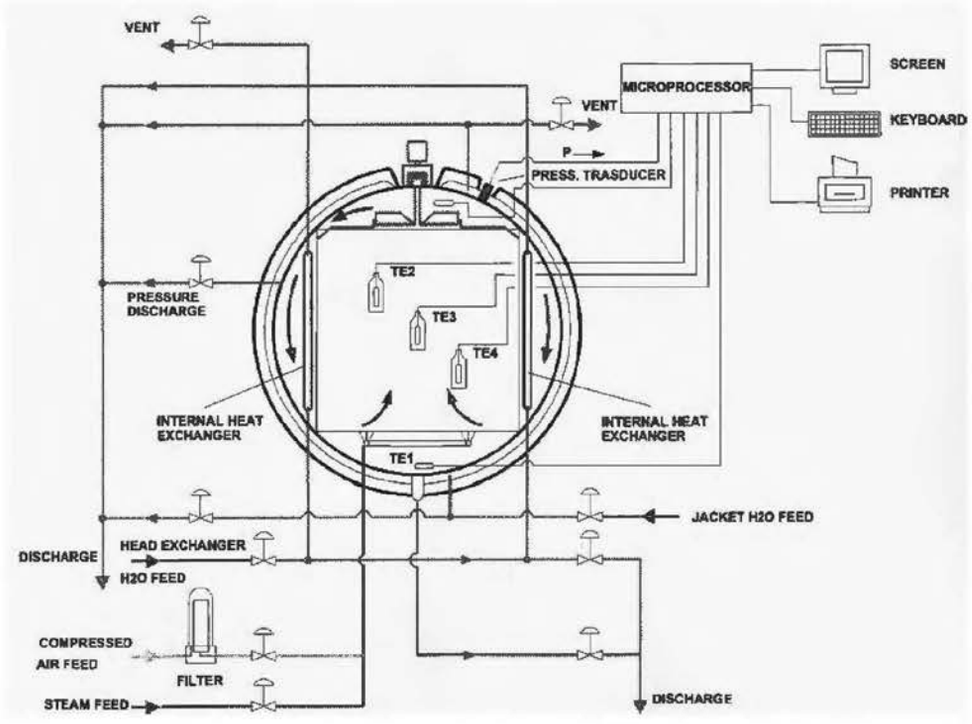

Figure 11 Steam air sterilizer. *Source*: Courtesy of Fedegari Autoclavi.

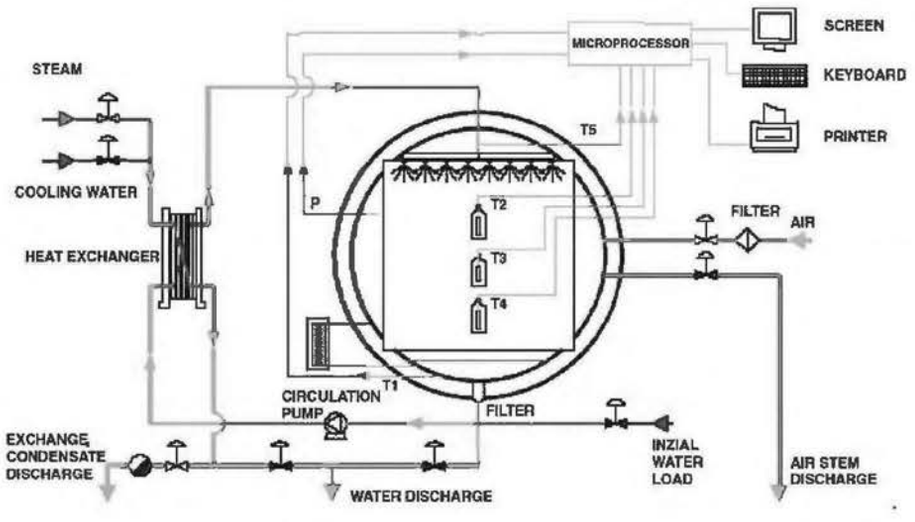

Figure 12 Steam air water sterilizer. *Source*: Courtesy of Fedegari Autoclavi.

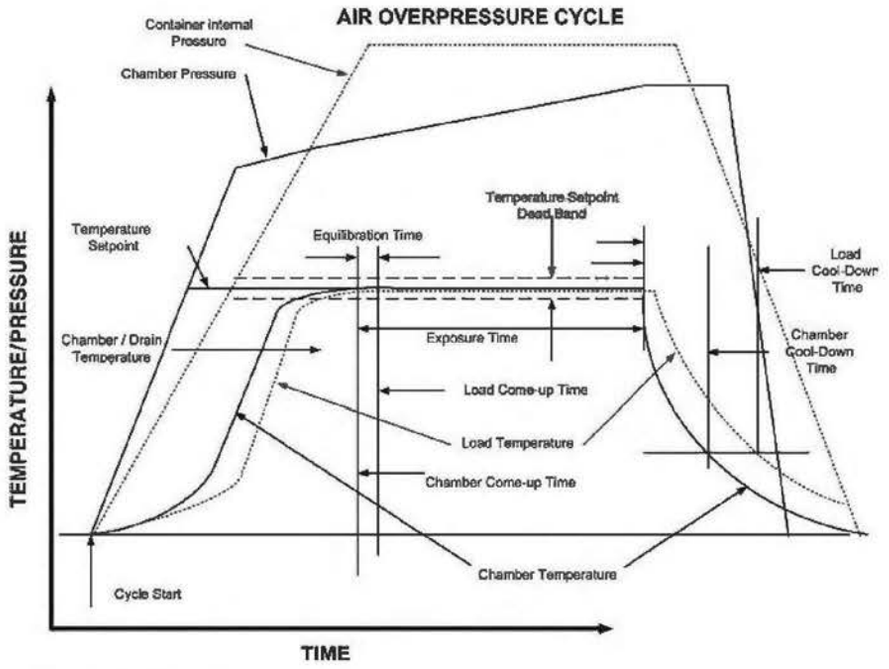

Figure 13 Air overpressure cycle.

APPROACHES TO STERILIZATION CYCLE DEVELOPMENT AND VALIDATION

There are three methods for the design/development and validation of a steam sterilization process, and it is essential the same approach be utilized for both activities (7). The different approaches exist in large part because of the differences in heat resistance of the items being sterilized. The overkill approach is the simplest, and inherent in its selection is the recognition that the load items will be subjected to a larger amount of heat than with the other methods (12). The bioburden approach requires the most effort initially and on an ongoing basis, but subjects the materials to the least amount of heat.[c] The bioburden/biological indicator [BB/BI or combination] method falls between these extremes with regard to both ease of development/validation and the amount of heat applied to the materials. Figure 14 provides a pictorial representation of how the various sterilization validation approaches compare.

The choice of sterilization approach is largely defined by the types of items being sterilized. While the overkill method is always the method of choice due to its relative ease of use, the BB/BI may be more appropriate for heat sensitive materials. The terminal sterilization of liquids in their final containers follows the BB/BI approach as it results in shorter cycles more conducive to maintaining product shelf life. Hard goods by virtue of their heat stability are almost always validated using the overkill method.

Overkill Method

This method despite its almost universal usage across the industry suffers from a lack of clarity. There are a number of conflicting definitions for this method. A recent definition was

[c]The bioburden method is little used for steam sterilization in industrial settings, as the bioburden/biological indicator method is substantially easier to manage and subjects the materials to only slightly more heat input.

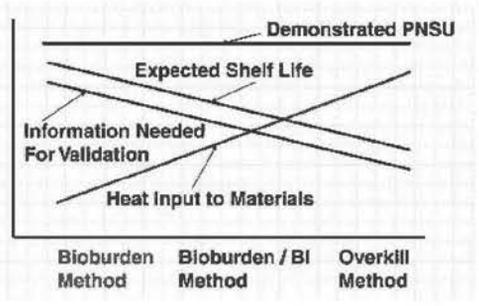

Figure 14 Sterilization approaches.

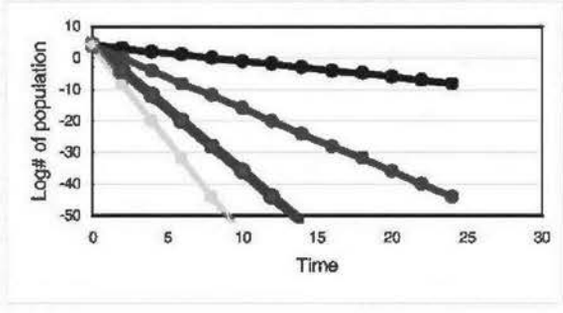

Figure 15 *(See color insert)* Overkill approach.

developed with the goal of reaffirming the original intent of the overkill treatment (overwhelming destruction of the bioburden):

> "Overkill sterilization is a process where the destruction of a high concentration of a resistant microorganism supports the elimination of bioburden that might be present in routine processing. That objective can be demonstrated by attaining any of the following: a defined minimum F_0; a defined time temperature condition or a defined log reduction of a biological indicator." (12)

The overkill approach relies on the substantial difference in the relative resistance of biological indicator as compared to the bioburden (as is the case for the other methods as well). Figure 15 shows how this might be accomplished in a real world validation study.

The universal assumption made when using the overkill method is that any bioburden present will have substantially less resistance than the biological indicator, and that destruction of the large numbers of the resistant indicator organism (customarily replicate studies with multiple biological indicators with a population of 10^4 or more per strip) supports a greater reduction (1,000,000 or more times given the differences in relative resistance) of the bioburden. Cycle times are established by estimating the time required to inactivate the bioindicator (typically 8 9 times its *D*-value) and adjusting the cycle dwell time accordingly. The biological indicator is placed within the load items at locations expected to be slow to heat, that is, center of filters, inside tubing, etc. In this approach, it is required that all of the indicators are killed during the cycle. Since the number of biological challenges placed in the load is at least 10, a 6 log reduction in the microbial population is obtained when all of the indicators are dead when a minimum population of 10^4 spores is used and greater reduction can be achieved with a higher challenge level. The kill of the biological indicator demonstrates a greater than 6 log reduction of spores of *Geobacillus stearothermophilus*. The reduction in bioburden population assuming a biological indicator D_{121} value of 1 minute and D_{121} value of the bioburden of 0.00001 minutes would be 100,000 times greater.

Clearly, this approach provides levels of sterility assurance for bioburden organisms that provide substantial confidence in the effectiveness of the cycle. The bioburden organisms are killed in such excess that the process is deemed an "overkill" process. Given this degree of lethality that is provided by the process, little consideration is given to identification, quantification, or resistance determination for the bioburden. Some inspectors have made absence of information on the bioburden an issue, but there is little rationale for this concern.

Bioburden/Biological Indicator Method

The BB/BI method also relies on the differences in relative resistance of the bioburden and biological indicator. Destroying even a modest population of the biological indicator requires heat input to the materials that may alter its properties adversely. The enormous difference in relative resistance means that a robust process can be defined in which the biological indicator used has a lower population or is not fully inactivated, but still confidently supports bioburden death in all instances.

The BB/BI process requires information on the population and moist heat resistance of the bioburden and ongoing monitoring/control over the bioburden (Figs. 16 and 17).

In this model, the initial biological indicator population of 10^6 is reduced over an 8-minute cycle to a population of 10^2 (a 4 log reduction). Over the same 8-minute period, the bioburden population is reduced from 10^4 to 10^{-12} (a 16 log reduction). In this example, the biological indicator D_{121} is 2 minutes while the bioburden D_{121} is 0.5 minutes (a resistance and population much higher than might be anticipated in a typical pharmaceutical solution). The only difficulty with this experiment is that the biological indicator population must be accurately determined at the 8-minute time interval.

The second example has an initial biological indicator population of 10^2 that is reduced over the same 8-minute cycle to a population of 10^{-2} (also a 4 log reduction). Over that same 8-minute period the bioburden population is reduced from 10^4 to 10^{-12} (a 16 log reduction). In this example, the biological indicator D_{121} is 2 minutes, while the bioburden D_{121} is 0.5 minutes (a resistance and population much higher than might be anticipated in a typical

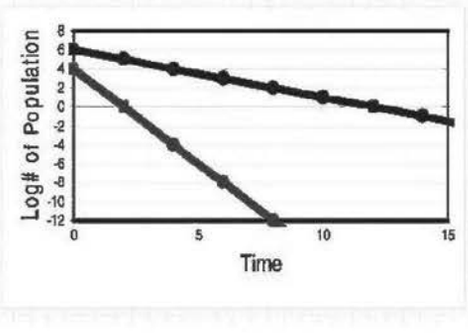

Figure 16 *(See color insert)* BB/BI method with survival count.

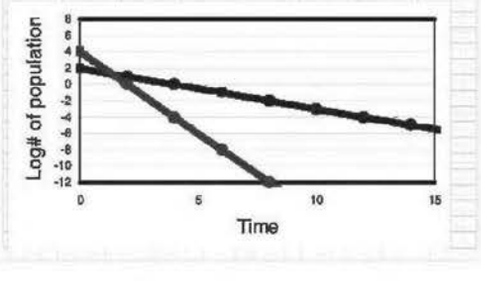

Figure 17 *(See color insert)* BB/BI method with total BI kill.

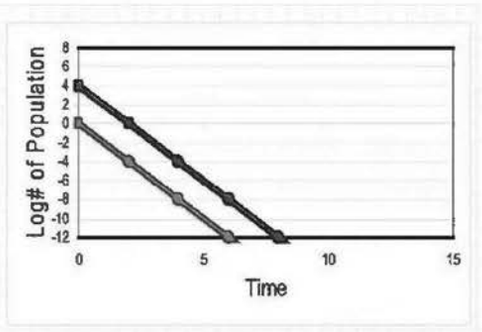

Figure 18 *(See color insert)* Bioburden approach.

pharmaceutical solution). In this experiment, the biological indicator is fully inactivated in the 8-minute process dwell easing execution of the study.

Bioburden Sterilization

For bioburden sterilization, a "worst case" bioburden isolate is utilized as the biological indicator. It requires knowledge of the bioburden present in every lot produced. Initial screening of the bioburden is made to identify the most resistant strain of microorganism present. Once determined this organism is used as the biological indicator, following methods similar to that described in the preceding text for the BB/BI method. If the proper organism is initially selected, then its destruction in the process confirms that any of the other bioburden organisms, presumably of lesser resistance and lower number [the chosen organism should be cultivated such that when used as a challenge the number present will be substantially higher than the lot bioburden] (Fig. 18). Monitoring of the bioburden for each load is required to confirm that the population and resistance have not increased to the point where the cycle is no longer effective. Inherent in the use of this method are defined limits for the bioburden number and resistance applied to each lot prior to sterilization. Any lot not meeting the limits cannot be accepted as adequately sterilized by the process cycle.

Obviously, a bioburden cycle will require substantially less time at temperature to inactivate the typical organism, which might be used as the challenge. It appears to be the ideal choice for the terminal sterilization of filled containers as a consequence of the reduced heat input that the filled units must receive to inactivate the nonspore forming organism used in this method. Less heat required to achieve sterilization should mean that products sterilized using this approach will have greater chemical stability post process as a result when compared to the same product sterilized by the other methods. This advantage is largely offset by the intensive bioburden monitoring required, initially to establish and on an ongoing basis to maintain confidence in the sterilization process. Appealing as this process might appear, it is the least widely used of all approaches because of the extensive microbial testing support required.[f]

QUALIFICATION/VALIDATION OF STEAM STERILIZATION

The terminal sterilization of liquid-filled LVP containers was perhaps the first process subjected to validation in the pharmaceutical industry. Global practices for all validation activities have their roots in the early 1970s, when microbial contamination in LVPs in the United States and hospital infections in the United Kingdom led to the introduction of a regulatory expectation for sterilization validation (13). The speed with which sterilization validation was introduced into the global industry led to some unfortunate simplifying assumptions that have had long-term consequence. Sterilization processes of all types, but

[f]The bioburden method forms the basis for all forms of radiation sterilization, and only occasionally with other sterilization methods.

most importantly in the context of this chapter, parts sterilization had their validation requirements defined by the more rigorous requirements of terminal sterilization. Much of what is considered essential for parts sterilization has never been evaluated objectively against the simpler needs of their sterilization.

The first efforts to codify the requirements of a validation program were found in the U.S. FDAs Proposed Good Manufacturing Practices for Large Volume Parenterals (14). An essentially parallel, but quite differently focused effort in the United Kingdom resulted in HTM-10, which did not appear in print until 1980 (15). These documents were the first regulatory efforts to outline validation practice for moist heat sterilization. Soon after FDA published its proposed regulation, PDA (at that time a predominantly U.S. Association) developed Technical Monograph #1, Validation of Steam Sterilization (16). PDA's effort focused on FDA's proposed regulation and thus the common practices of the U.S. LVP industry formed the basis for the steam sterilization validation across U.S. firms. The PDA's document relied heavily on biological indicators as the principal means to establish sterilization cycle efficacy. The practices outlined focus on biological challenges using resistant microorganisms as the most appropriate means to establish cycle effectiveness.

By the time UK's HTM-10 appeared, the global pharmaceutical industry had already begun to adopt practices following PDA's Monograph. It was not until the formation of the EU along with the emergence of ISO standards and establishment of EMEA that the precepts of HTM-10 were brought into greater prominence. The original HTM-10 and its many derivative standards have a completely different focus to steam sterilization validation that what was derived from FDA expectations and PDA's initial efforts (17,18). These standards place substantially greater emphasis on physical measurements of process parameters, especially those that relate to steam quality and equilibration time. The points of contention between European regulatory expectations and U.S. style validation practices persist; practitioners are forced to satisfy regulatory communities with quite different perspectives (19,20).

Over the years the differences in validation emphasis have endured to the point where the validation of steam sterilization, especially as it relates to parts sterilization, is one of the more contentious subjects within the global healthcare industry. The chapter will review the areas of agreement and difference with respect to the validation of both terminal and parts sterilization.

EXECUTION OF PERFORMANCE QUALIFICATION STUDIES

The validation of any process commences with the qualification of the process equipment and steam sterilization is no exception. This is a subject that has been treated extensively in the literature and is largely without any confusion or contention. The reader is encouraged to follow the well-documented practices in this area (21).

Empty Chamber Studies

Performance qualification of steam sterilization ordinarily begins with evaluation of empty chamber temperature distribution. This entails the placement of thermocouples (type T thermocouples are most commonly used) across the chamber, with the most important locations in the eight corners of the autoclave chamber, and at the location of the autoclave's controlling temperature sensor. Other locations can be monitored if additional probes are available. Thermocouple access for conducting these studies must not obstruct the steam inlet, drain valve, or any safety release access. The autoclave cart can be used as a support structure for this assessment to provide greater reproducibly of thermocouple location. The thermocouples should be positioned so that they do not contact any internal surface and are measuring steam temperature (Fig. 19).

The customary criterion (±0.5°C) for the empty chamber temperature distribution is derived from FDA's Proposed LVP CGMP's from 1976 (14). Originally established for heat sensitive materials where a tight control is required, it was adopted as an appropriate criterion for all steam sterilizers. Its application without alteration for parts sterilization is excessive, given that there is no reason to limit the temperature provided it exceeds the desired set point. For a comparatively simple requirement, it is subject to some interpretation. Consider that

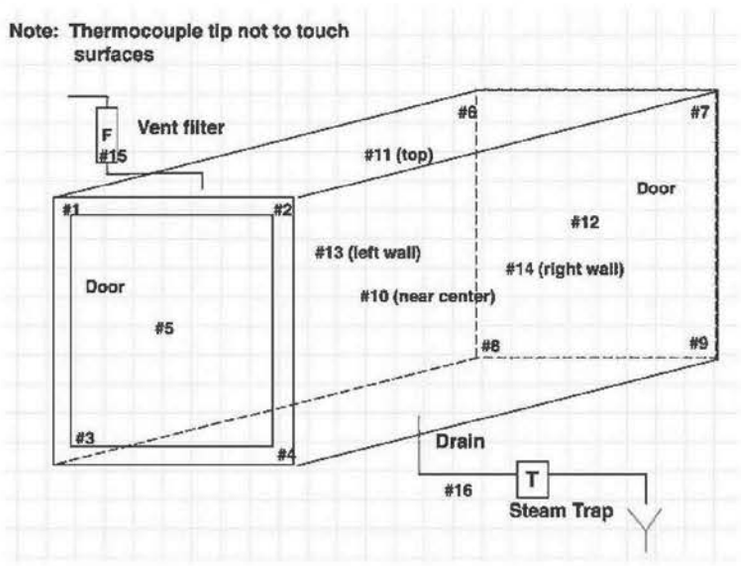

Figure 19 Empty chamber study thermocouple locations.

there is no defined method for interpreting the temperature data. The criterion can be applied in a variety of ways:[8]

- All thermocouples over entire cycle duration
- All thermocouples excluding the first few minutes
- All thermocouples over a shorter period
- All thermocouples over a single time period

Conducting the evaluation omitting the first few minutes of exposure is perhaps most appropriate; it ignores only the very beginning of the dwell when steady state might not have been reached at all locations.

Regardless of the criterion and data set utilized, the most important consideration is the frequency of execution. Empty chamber studies should be conducted on each cycle the sterilizer can execute (cycles differing only in the duration of the dwell period can be evaluated in only the shortest duration cycle) during initial qualification. It may also be useful in the evaluation of changes to the sterilizer that are primarily mechanical or control system related. Its utility for the periodic requalification of the sterilizer is extremely limited as it cannot evaluate steam penetration (the most important consideration in cycle effectiveness).

Container/Component Mapping

Before inserting any container or object into a sterilization load, it should be evaluated for its steam penetration. Complex items of hose, stainless steel parts and filters with wrappings, and containers larger than 50 mL may have a discernable cold spot where the temperature reaches the set point temperature last (7). Smaller containers and simple geometry hard good items can ordinarily be ignored in these studies as it will be virtually impossible to identify a discernable cold spot.

Mapping studies should be conducted to determine where in the item the temperature probe and biological challenge should be placed. These studies can be performed in a laboratory setting provided that prevacuums and steam introduction is comparable to that of

[8]There is no broadly accepted method for this test in the regulatory literature or compendia.

the sterilizer the item is being introduced into. Orientation and wrapping for these studies should be identical to that used in routine sterilization. Care must be taken in these studies not to impede or assist air/condensate removal and steam penetration as this will lead to location errors. Special fittings should be employed to provide thermocouple access without alteration of the results (these fitting are also necessary for steam penetration studies in the sterilizer). Once these locations with the items have been established, they should be monitored in all subsequent studies.

Loaded Chamber Temperature Distribution Studies

This activity is largely associated with terminal sterilization processes, where excessive variation in temperature across the chamber could result in localized under- or overprocessing. While the true demonstration of cycle effectiveness is the subsequent heat penetration studies, difficulties with temperature distribution may predict later problems with that activity. Where all of the items in the load are identical as is customary in terminal sterilization and may also be prevalent in component sterilization for stoppers and other items, these studies can be of some benefit in identifying whether uniform conditions can be attained. Difficulties with temperature distribution can ordinarily be resolved by altering load density, positioning, and/or arrangement. Other possible corrections would entail changes in process parameters, physical location of temperature probes, steam entry, cooling water introduction, etc. Criteria for this study are not defined; the only expectation is that conditions across the load be reasonably constant at steady state. In the course of these studies, load cool and hot zone or spots may be identified. This knowledge is essential for the subsequent steam penetration/biochallenge studies to follow. The objective of this study is to establish the uniformity of process conditions across the sterilization chamber that is essential to a consistently lethal sterilization process.

Where the load is composed of mixed items of differing size, dimension, and mass, heat distribution studies are of substantially less value. The difference in the items is of far greater consequence than any chamber variation and thus evaluation of loaded chamber temperature distribution can be omitted in the validation of parts sterilization loads (22).

Load Mapping

When sterilizing identical items, whether for part or terminal sterilization, a definable cold spot in the sterilizer can be located where probed items demonstrate the lowest overall F_0. Identification of this region is of greater importance than loaded chamber heat distribution as it focuses on the sterilizing effect. Load items in this area are those that are at greatest risk for underprocessing. In most loading patterns, this is usually a point near the bottom center of the load. When performing the biochallenge studies, the preponderance of challenge units should be in or near this zone.

In terminal sterilization efforts, it is also necessary to identify the hottest portions of the load where the maximum F_0 is delivered. Product stability at these conditions may be adversely affected and when collecting samples for stability studies, preference should be given to this region of the load.

Load mapping must address variable loads if that is the expected operational practice for sterilization. The "cold" and "hot" spots should be identified in both minimum and maximum loads. As noted in the prior section, where the load is comprised of mixed items, "cold spots" are ordinarily associated with the item and not with sterilizer performance. In these situations, the load should be rearranged between repetitive runs to support that cold spots are item dependent. This can be accomplished in separate load mapping studies specific to that purpose (and then repeated in the biochallenge runs) or directly in the biochallenge runs (22).

The process control of many terminal (and even a few parts) sterilizers may be supported by load temperature probes positioned with the load. It might seem appropriate to place these temperature sensors in the coldest parts of the load, and thus assure that minimum sterilizing conditions have been delivered. From an operations perspective, this has proved somewhat impractical. These probes are quite large and difficult to place and remove from the middle of the load where the coldest items are located, especially when the load arrives at the sterilizer as a complete pallet. These probes are best placed in convenient units near the top of the load,

with the lethality delivered there correlated to what is attained at the load cold spot. This practice accommodates such aspects as: container size; fill volume; viscosity, and heat capacity differences across the various products the sterilizer will process. In parts sterilization, load probes serve little purpose and they can be either removed or placed in a standard location in all cycles (22).

Loaded Chamber Heat/Steam Penetration and Biological Challenge Studies

The core of all sterilization validation efforts is the challenge study in which biological indicators are distributed throughout the load to confirm the lethality of the process. For steam sterilization, this is accomplished simultaneously with heat/steam penetration using temperature measurements within the load items.

Parts Sterilization

In part sterilization studies, these studies are relatively easy to perform. Biological indicators and thermocouples are placed within the load items and customarily exposed to conditions only slightly less lethal than the routine sterilization process (a 1°C set point reduction and a 1-minute shorter dwell period is sufficient) (22). Control of sterilization cycles for parts loads is customarily accomplished by temperature measurements in the drain line where temperatures are the coldest. Destruction of the biological indicator (ordinarily spores of *G. stearothermophilus*) coupled with comparable physical lethality (as established by the temperature probes) in this worst case cycle supports the efficacy of the sterilization process. In some firms the load arrangement for these studies is fixed, however more progressive efforts can support changes in load positioning, provided wrapping and orientation are maintained. This is accomplished by performing triplicate studies (as is customary in the validation of all loads in a new sterilizer or a new load in an existing sterilizer) in which the load is reconfigured between the individual runs.

The biological indicator is customarily a spore strip of *G. stearothermophilus* inserted in the item at the location previously determined to be slowest to heat. Custom biological indicators in the form of inoculated wires or strings can be used in smaller items. Inoculation of the spores on the surface of the item is the method of choice, as there is a regulatory belief that the resistance of the microorganism will change dramatically relative to a spore strip. While there is a change in resistance of spores on the surface relative to a spore strip, the difference is ordinarily within a single order of magnitude. As the confidence in the sterilization cycle is actually obtained from the difference in resistance between the biological indicator and any bioburden present on the item (which is minimally 6 7 orders of magnitude different), requiring inoculation of surfaces provides minimal additional confidence in the sterilization process.

Temperature measurements are typically performed using thermocouples positioned in slow to heat zones with the load items. The use of specialized fittings to permit thermocouple access without compromising the integrity of the item and any wrapping material is strongly recommended. Where this is not the case, the physical data should be considered suspect as air removal, and steam penetration may be improved relative to unprobed load items. If there is any question regarding the integrity of temperature probed units, biological indicators should be located in an adjacent identical item without penetrations for a temperature probe. In evaluating the physical data, the location with the lowest overall F_0 is considered of greatest concern. It represents the item(s) where the delivered lethality is the lowest. That knowledge is essential to understanding the sterilizers' performance. The load in which the lowest F_0 is demonstrated is conventionally utilized in annual reevaluation of the sterilizer.

In considering the loads to evaluate, the maximum load of mixed items is most appropriate as a worst case challenge for each unique sterilization process. The large mass of the maximum load will entail greater steam to bring the items to sterilizing conditions resulting in more condensate than would be encountered with smaller loads. The choice of the largest load would ordinarily include items where air removal might be difficult to accomplish. Where a smaller load includes a unique item with potential air removal issues, it should be validated as well. For loads comprised of many identical items such as stoppers or containers, the evaluation of both minimum and maximum loads affords the greatest flexibility in routine operation. Where air removal and steam introduction differ for loads in a sterilizer,

that is, gravity displacement and prevacuum cycles are both utilized, then the loads for each type should be considered separately. As noted above, rearrangement of the loads between repetitive runs is recommended to ease operational loading of the sterilizer.

Terminal Sterilization

Sterilization of products entails consideration of both sterility and stability; a two-sided concern that essentially doubles the work required relative to parts sterilization.[h] Biological challenges must consider the effect of the fluid on the moist heat resistance of the microorganism: the effect can be either protective or destructive and must be determined precisely in specifically designed laboratory studies. Once determined the *D*-value in the fluid will define the minimum lethality to be delivered across the sterilizer (usually a Probability of a Non-Sterile Unit (PNSU) of not greater than 1 in 1,000,000 units). The fluid must have available water content (values as low as 5% water appear to be adequate, but must be confirmed experimentally for any fluid with low aqueous content) that serves to sterilize the fluid and when converted to steam the headspace above the liquid. The biological challenge for terminal sterilization must be considered with some caution. *G. stearothermophilus*, the preferred challenge organism for steam sterilization of hard goods is often inappropriate for use with terminal sterilization. Its resistance to steam sterilization is such that the minimum F_0 with which it can be comfortably used (assuming a D_{121} of 2 minutes and a challenge level of 10^6 spores per container) is 18 minutes.[i] As that amount of heat input is excessive for many materials, alternative indicator spore forming microorganisms are often chosen. Among the organism that can be used as biological indicators are *B. coagulans*, *C. sporogenes*, and *B. subtilis*. Those organisms and others are appropriate choices provided the resistance of the chosen spore is evaluated in the product.

Where either the containers or closures are not sterilized prior to filling, a further complication ensues. The process must be able to demonstrate sterilization at the container-closure interface where steam from the fluid may not penetrate. This is accomplished by inoculation of the interface with spores of *B. atrophaeus* (a dry heat biological challenge indicator microorganism) and confirming their destruction in the intended process. The challenge level may be reduced provided bioburden controls on the components are in place. In some cases, the time-temperature conditions to inactivate the spores in the interface may exceed those necessary for the sterilization of the fluid in the container (23).

Temperature measure inside the liquid filled containers is accomplished by positioning thermocouples through the stopper in the container[j]. Syringes and ampoules are customarily monitored using thermocouples external to the container, which given their typically smaller size and thinner walls rarely creates significant difficulties in cycle confirmation. The use of self-contained probes that can individually record data can be used in very large sterilizers or continuous sterilizers where the use of wired thermocouples is problematic.

The studies should be conducted at the intended cycle conditions as the "window" for attaining sterilization while maintaining product stability rarely allows adjustment of parameters without adverse impact (Fig. 7). Biological challenge units in product-filled containers are positioned across the load pattern, with emphasis on the cool point determined during the load mapping studies. Thermocouples are positioned in separate containers next to those with the biological challenge. The entire sterilizer load for validation need not utilize product containers; the use of placebo filled containers is commonplace, provided that the placebo units approach the tested product in fill volume, viscosity, and heat capacity.

Minimum and maximum loads should be evaluated in triplicate studies. In each load size, consistency of minimum and maximum delivered F_0 is the key requirement. Biological challenge results must perform as intended.[k]

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[h]Laboratory media and in process fluid sterilization can be validated in a similar manner.

[i]Assumes a 9 log reduction is required to provide a 1 in 1000 chance of a survivor in the validation studies.

[j]The location should have been determined in the mapping studies described earlier.

[k]In cycles defined by the bioburden/biological indicator sterilization method, bioindicator count reduction rather than destruction may be the intent.

ONGOING CONTROL

Steam sterilizers share many considerations as other pieces of pharmaceutical process equipment. To be utilized they must adhere to common CGMP practices intended to support commercial use. These practices include requirements for instrument calibration, maintenance (preventive and corrective), review of records, and bioburden monitoring.

Proper calibration of the steam sterilizers' instrumentation on a periodic basis is essential for maintaining process effectiveness. As microbiological kill is logarithmically related to the sterilizing temperature, slight variations in temperature can have a substantial effect on process lethality. This must consider the entire control system from point of measurement to the process recorder (24). The pressure and any other instrumentation on the unit should also be calibrated. Calibration must include any instruments that do not record or display. Instrumentation utilized for the validation of the process must be calibrated as well.

Keeping the equipment in proper working order is an essential requirement. Preventive maintenance as defined by the sterilizer manufacturer is intended to keep the sterilizer in proper working condition. There should be a defined schedule for its execution using methods and parts provided by the vendor. This form of maintenance is presumed to have no adverse impact on the sterilization process, and while records of it must be maintained evaluation of the change is normally not indicated. Corrective change that repairs malfunctions of the equipment presents quite a different situation. Each repair whether planned or unplanned must be formally evaluated for its potential impact on the performance of the system. The review must consider the extent to which the repair and/or the condition prior to the repair could alter the effectiveness of the cycle. In some instance, there will be little or no impact from minor changes to the system, while more extensive changes will likely result in a formalized evaluation of the equipments performance. The evaluation might require a repetition of one of more of the elements of the equipment qualification, or, in extreme cases, the performance qualification of the sterilizer itself.

Record review is a requirement for the release of materials produced by any process. In steam sterilization, the records of individual cycles must be carefully reviewed to determine their conformance to process requirements. Many firms establish formalized review sheets defining the expected conditions to be attained and the tolerance around them for ease of record review.

Where the sterilizing approach mandates that the presterilization bioburden conforms to specified limits, it is essential that routine testing be performed. For parametric release this is an every lot requirement.

CONCLUSION

Steam sterilization is a relatively simple process; its criticality and universal use suggest that individuals working in this industry must have a thorough understanding of the principles associated with its use and validation. There is perhaps more information available on this process than any other in our industry. The reader is encouraged to explore that information if the information provided within this effort proves inadequate.

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10 | Gas, vapor, and liquid chemical sterilization

James Agalloco

INTRODUCTION

There are instances in the manufacture of pharmaceutical products and medical devices where an item must be sterilized, yet its properties eliminate methods of sterilization based on moist or dry heat or radiation. The simplicity and speed of heat and radiation sterilization makes them the methods of choice in most instances; however, the effects of these sterilization processes on many materials are detrimental to essential material properties. When faced with these circumstances, the practitioner often turns to chemical methods where microorganisms are destroyed by exposure to chemical agents in gas, vapor, or liquid form. This chapter will review the available processes, outline their development, describe suitable validation approaches, and delineate the necessary routine process control requirements.

While all of these processes rely on a chemical action against microorganisms, there are meaningful differences in their application that must be understood to use them effectively. The same chemical agent will likely require differing controls when delivered in a different manner. The processes for sterilization by the varying agents that operate in a particular phase are all similar and resemble each other more than the processes for a single agent applied in different phases. This can perhaps be better understood by a rapid review of the relevant aspects of physical chemistry. The basic definitions of a gas and liquid are presented below:

> Gas: Matter in a state intermediate between liquid and plasma that can be contained only if fully surrounded by a solid; it can condense to form a liquid (1).
> Liquid: A state of matter between solid and gaseous. A liquid can evaporate into a gas (1).

All materials in a liquid state have some tendency to evaporate into the gaseous form. At any fixed temperature of a liquid, there is a vapor pressure created by the gas in equilibrium with that liquid. As the temperature increases, so does the vapor pressure, corresponding to a higher concentration of the material in the gas phase above the liquid. As gases cool, they may reach their dew point at which temperature a portion of the gas reverts (condenses) to the liquid state. Chemical agents such as hydrogen peroxide and peracetic acid are utilized for sterilization in ways where both liquid and gas phase may be present simultaneously and is often called a vapor. Gaseous agents such as chlorine dioxide and ozone are also effective in aqueous solution under very different conditions from those used for gas sterilization.

> Vapor: "Diffused matter (as smoke or fog) suspended floating in air and impairing its transparency (2)." When large amounts of liquid are suspended in the gas it has the appearance of a fog or cloud (Fig. 1A).

The laws of physics mandate that both gases and liquids be uniform in the concentration of all components present in each. As a consequence these processes are relatively simple to develop, validate, and operate. The biphasic nature of vapor presents several challenges to the scientist. The premise behind most vapor sterilization processes is that by increasing the temperature of the liquid it can be converted into a gas and maintain the same high concentration despite the phase change. This may result in a meta-stable situation with localized condensation of the material at locations where the surface temperature is less than the dew point temperature of the material. Variations in temperature across a chamber will result in different amounts of condensation at each location. Locations where the temperature is higher may not have any condensation. All of this tends to make sterilization using vapors far more problematic than either gas or liquid sterilization. The situation is actually even more complex, as introducing a hot vapor into an ambient temperature chamber will result in a gradual temperature rise over the course of the process.

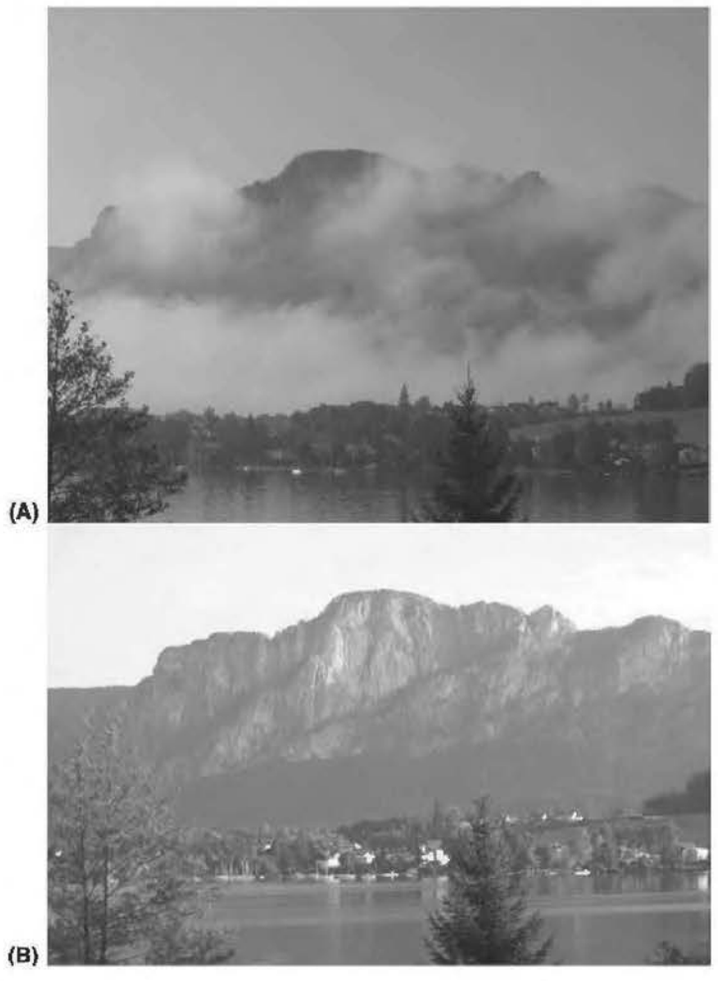

Figure 1 (**A**,**B**) The Mondsee & Schafberg with and without "vapor."

The chemical effect of the gas, liquid, and vapor agents against microorganisms is believed identical regardless of the phase in which the agent is present when exposed to the agent. The concentration of the chemical agent has the greatest impact on the effectiveness of the sterilization process regardless of the phase. Of course, substantially higher concentrations are possible in the liquid phase relative to the gas phase. Attaining the same concentration in each phase for a vapor agent is virtually impossible. Therefore in vapor processes, the sterilizing effect on the microorganisms will differ in the vapor and liquid phases due to localized differences in concentration, and adsorption potential of the agent from each phase to the microorganism. Further difficulties result from the solid nature of the target microorganisms (whether biological indicator or bioburden) and the potentially varying nature of the agent at the point of exposure to the microbe.

There are other important factors essential for effective sterilization of microorganisms by chemical agents. Moisture must be present as well for effective sterilization to assist in penetration of the agent through the spore coat (3). In liquid sterilization, the presence of liquid water is assured. In gas sterilization, moisture is provided by the humidity present in the gas phase. For vapor sterilization the moisture necessary for effective sterilization is present as

either a gas or liquid depending on the temperature at the location. Vapors present additional problems for determination of moisture levels as the amount of water will be different in each phase. Temperature for the gas and vapor process is important, predominantly as it influences the relative humidity level with gases and vapors.

In the context of gas, liquid, and vapor sterilization, the essential factors are comparatively easily determined in gases and liquids, whereas vapor processes present all manner of measurement uncertainties. The agent concentration and humidity levels are neither constant across the processing environment (and process cycle), and measurement of one phase to calculate the equilibrium concentration in the other is only useful where the entire process is isothermal given that the antimicrobial agent and water are present in both phases. As vapors are introduced as either hot gases or derived from hot liquids, temperature is rarely constant and thus concentration measurement is at best a locally correct number, and at worst near useless in establishing process conditions and relating them to lethality.

STERILIZATION BASICS

Sterilization is a process that completely destroys or removes microorganisms. In the context of this chapter, the emphasis is on completeness of the treatment. The agents described in this chapter when applied without adequate control measures should not be considered sterilizing. In sterilization processes, microbiological death curve can be graphically described by the logarithmic number of microorganisms remaining alive (4). When plotted against time, a straight line results. This line can be extrapolated to estimate the number of possible survivors in a large number of units (Fig. 2). This is termed the Probability of a Non-Sterile Unit (PNSU). An acceptable PNSU has been defined as 1 positive unit in 1,000,000 units (a risk value originally developed for food safety).

The slope (the inverse of which defines the *D*-value) of the microbial death curve is an inherent property of the microorganism and the conditions of the sterilization treatment itself. The slope of the curve is the time in minutes for the microbial population to be reduced by 90% (or 1 logarithm) and is commonly termed the *D*-value (4). Accurate determination of the *D*-value requires precise measurement of the lethal conditions to which the microorganism is exposed. As noted earlier, the determination of sterilizing conditions for gases and liquids is relatively simple. Establishing the conditions for vapors sterilization is problematic; however, the principles for establishing cycle efficacy for vapors are essentially identical to that for gases and liquids. Validating the physical destruction of microorganisms relies in part on differences in the relative resistance of a biological indicator and bioburden organisms (Fig. 3) (5).

The validation exercise supports the efficacy of the sterilization process against the microorganisms present during routine processing. Depending on whether the sterilization process is gaseous, liquid, or vapor, the details of the validation will vary; however, the basic principles described in the preceding text remain the same. Each of these will be discussed

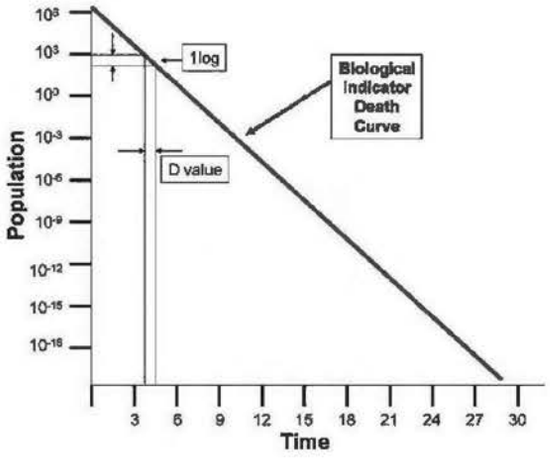

Figure 2 Microbial death curve.

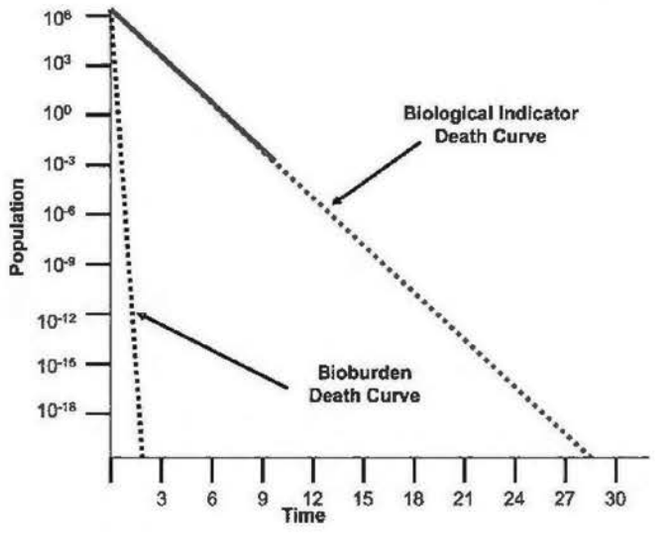

Figure 3 Relative resistance of bioburden and biological indicator organisms.

separately accommodating the differences in them. A sterilizing agent will require different instrumentation, equipment, and controls for effective usage depending on the phase(s) in which it is delivered. All of the validation methods exploit the difference in resistance of the biological indicator relative to that of the natural bioburden as depicted in Figure 3 regardless of whether the sterilizing agent is gaseous, liquid, or vapor.

GAS, VAPOR, AND LIQUID STERILIZATION FUNDAMENTALS

Material Effects

Sterilization processes are designed to kill microorganisms and as such they utilize conditions that may be destructive of essential material properties. Moist and dry heat sterilizers employ extremes of temperature, while radiation processes expose the materials to various forms of radioactivity. These processes have potentially adverse effects on the materials being processed, and the development of sterilization treatments must always consider that effect. Gas, vapor, and liquid sterilization processes are not exempt from this phenomenon and material evaluation required. The strong oxidative powers of many chemical agents, pH extremes of acids and bases, and the presence of substantial moisture can all lead to significant changes in the materials being sterilized. Some agents, especially ethylene oxide (ETO), are known for allowing degradants to remain on the materials post processing, presenting a different adverse effect, and the amounts of these residuals is closely regulated (6). Lastly, the effect of the agent on the processing equipment must be considered. The typical sterilizing chamber is comprised of many different materials all of which must be tolerant of the sterilizing conditions. Consideration of each of these possible adverse consequences must be an integral part of process selection, equipment design, cycle development, and process validation.

Process Equipment

Gas and vapor sterilizations are ordinarily carried out in jacketed chambers much like those utilized for steam sterilization. To assure greater process reliability, external and/or internal mixing is utilized to enhance uniformity of the lethal agent and relative humidity throughout the chamber. The jacket provides for temperature control, while the pressure (and vacuum rated) chamber serves to contain the potent chemicals employed for the sterilization process. The process is executed by a control system that provides sequencing, regulation of process parameters, and documentation on the process.

Contemporary control systems for sterilization systems are electronic, either programmable logic controllers (PLCs) or minicomputers. These systems include various features including operator interface, recipe management, process execution and control capability, documentation, and interfaces with surrounding systems. The control system is vital to sterilization success. A well-designed control system facilitates operation of the system and is essential to maintaining a compliant sterilization process. Its importance cannot be overstated. It is the critical for providing the control necessary to support and maintain a validated sterilization process.

There are vendors that supply stand-alone control systems that can be used to supply and, in some instances, exhaust simple vessels with gases or vapors for sterilization. In these instances, the end-user is responsible for interfacing their process equipment with the freestanding control system. Temperature regulation, pressure/vacuum capabilities, and other operational features must be provided independent of the vendor-provided controller. The process equipment that can be sterilized with these units varies from the complexity of a freeze dryer to the simplicity of a stirred tank.

Equipment for liquid sterilization varies with the scale of the operation. Large commercial systems might use a jacketed stirred tank, the liquid counterpart of the sterilizing chamber used for gases and vapors. Process control would be provided by a PLC, distributed control system (DCS), or minicomputer. For smaller scale processes, the equipment might be as simple as a nonpressure rated container where the items to be sterilized are submerged. Agitation, temperature control, and sequencing would be provided by the operator using laboratory apparatus and/or room environmental controls.

GAS STERILIZATION

Gas sterilization is widely used for materials and equipment liable to damage by moist heat, dry heat, or radiation processes. Many of the common polymeric materials used in medical devices are difficult to sterilize by any other means. When finally packaged for delivery into operating and other critical settings, the medical device packaging must be sterile as well. The most prevalent gas utilized for sterilization is ETO, and sterilization using other agents is based on methods used for ETO. Other commercially available gas agents for sterilization are ozone and chlorine dioxide. While their use is not widespread, they offer the user alternates to ETO. Other gases that have demonstrated sterilization capability but almost no commercial support include methyl bromide, propylene oxide, helium/oxygen plasma, and sulfur dioxide (7,8).

Gases will not condense under typical sterilizing conditions and are highly penetrating. The penetrating abilities of the more common gases vary: ETO is superior to ozone, which is in turn superior to chlorine dioxide (9). Sterilization efficacy is enhanced when prehumidification is provided prior to sterilization. Optimum temperatures vary: ozone and chlorine dioxide are typically performed at room temperature, while ETO can be effective from ambient temperature to 60°C (9).

Gas sterilization process equipment must properly control gas concentration, relative humidity, and temperature throughout the process to provide consistent process lethality, as changes in the essential process parameters can alter the effectiveness of the gases ability to penetrate and react with the microorganisms and thus lethality. Humidification is typically accomplished using clean steam injection directly to the sterilizing chamber. ETO is highly penetrating through corrugate, polymers, and paper materials, which make it well suited for sterilization of medical devices in their final packaging. Ozone and chlorine dioxide are less penetrating, and their application for medical devices must be considered with some caution. Because each of these agents is a gas and the chamber is well mixed, single-point monitoring of gas concentration and RH provides adequate process control over the sterilization process. Despite this seemingly minimal monitoring, regulatory approval for parametric release for ETO sterilization is widespread.

ETHYLENE OXIDE

ETO is a powerful oxidizing gas that kills microbes primarily by chemical reaction with various sites in microorganisms primarily those with NH2, SH, COOH, and CH_2OH groups (10). Microbial kill with ETO approximates first-order kinetics and is directly related to

gas concentration, relative humidity, and process temperature (10). ETO is widely used for terminal sterilization of medical devices in final packaging. Sterilization methods for ETO (and essentially all other sterilizing gases) for the pharmaceutical processes follow medical devices practices because of the extensive experience with ETO for that application. ETO sterilization is effective across a wide range of conditions: gas concentration (300 1000 mg/L); relative humidity (35 85%), and temperature (20 65°C), although the usual processing ranges are somewhat narrower (10). ETO is an extremely potent material, has been identified as a mutagenic, carcinogenic, neurotoxic, and highly explosive (11). Trace residuals from ETO sterilization are also associated with adverse effects, so effective aeration of this is essential for safe use. For these reasons, internal usage within pharmaceutical operating companies has decreased. There are a number of firms providing contract ETO sterilization that have invested in the necessary controls to assure both worker and patient safety, and these offer most of the available industrial capacity for ETO sterilization.

As ETO processes are so extensively utilized for medical devices, the typical process is largely tailored to the specific requirements of their sterilization. The typical ETO process sequence includes

- pre-humidification (to raise internal humidity and performed in a room dedicated for that purpose);
- transfer to the sterilizer (with minimal delay);
- reconditioning in the chamber (to replace humidity lost in transit);
- air removal (to enhance gas/humidity penetration);
- exposure to ETO with humidity adjustment;
- initial aeration in the sterilizing chamber;
- transfer to a post-exposure aeration location; and
- post-conditioning (final aeration to remove residual ETO, ethylene chlorhydrin, and ethylene glycol) (6).

The preprocess treatments ensure adequate moisture is present on the surface of the materials for effective kill. The use of pre-humidification chambers/rooms to raise the internal moisture content of medical devices is almost universal for ETO sterilization. Post-processing aeration chambers are utilized with ETO to reduce residuals to safe levels after exposure. ETO sterilization processes introduce essentially all of the gas at the start of the process and minor adjustment during the exposure may be performed to maintain pressure. Humidity is commonly introduced using clean steam to the chamber preexposure for reconditioning after transfer, and adjustment may be required through the end of the exposure period.

ETO process control, like all sterilization processes, relies on a combination of physical measurements and biological assessments. Biological indicator kill in conjunction with data from the sterilizer instrumentation are utilized in evaluating process effectiveness. Recently, a lethality model has been proposed that mimics those utilized for steam and dry heat (12). Its broader adoption by ETO practitioners is anticipated as it simplifies lethality confirmation.

The extensive experience with ETO in medical devices has allowed many firms to implement parametric release in lieu of sterility or biological indicator testing of ETO-sterilized materials. Parametric release replaces sterility testing with a defined set of requirements derived from the initial validation exercise that must be satisfied in conjunction with the execution of each subsequent sterilization cycle (13,14). Submission to regulatory agencies is required prior to implementation and must be supported by comprehensive data derived from prior practice. Once implemented, the user is obligated to utilize parameter evaluation exclusively.

OZONE

The simplest of all gas sterilization processes uses ozone. The electrical field generation (starting with pure oxygen) and destruction (using a platinum catalytic converter) of ozone requires no moving parts, and the only required utilities are oxygen and steam (for humidification of the load) (15). Ozone has a half-life of several hours in the gas phase at ambient temperature (16). Ozone is microbially lethal at concentrations ranging from 2% to

10% at humidity levels of approximately 80% at room temperature. Ozone is less penetrating than ETO, and due to its reasonably short half-life it does not require post-cycle aeration. Ozone sterilization processes follow a sequence of humidification, injection, exposure (without added O_3), and exhaust. Pre-humidification of the materials may be beneficial prior to introduction into the sterilizing chamber. The preferred biological indicator for ozone is *Geobacillus stearothermophilus*. Process control requirements are essentially identical to those indicated for ETO. There are no reports of parametric release for ozone sterilization.

CHLORINE DIOXIDE

Chlorine dioxide is one of the newer sterilization methods available. Chlorine dioxide is a relatively unstable gas and must be generated in situ. It has none of the safety or environmental limitations of ETO. It is less penetrating than ETO, and because of its limited penetration and low absorption, aeration is relatively easily accomplished. Chlorine dioxide cycles incorporate preconditioning (outside the chamber), humidity stabilization, ClO_2 injection, exposure dwell period, and aeration (17). Chlorine dioxide concentrations required for sterilization range from 5 to 30 mg/L with humidity levels in the 60% to 75% range at ambient temperature. Biological indicators utilize spores of *Bacillus atrophaeus*. Chlorine dioxide levels can be measured using UV sensors, facilitating routine process control. Process control mirrors the practices for ETO described previously with only minor adaptation. The limited industrial experience with chlorine dioxide is such that it is premature to consider parametric release.

VAPOR STERILIZATION

Sterilization using vapors presents a substantial difficulty to the practitioner because of potential condensation of the agent (and perhaps water vapor as well). The most commonly utilized vapor agent is hydrogen peroxide, although materials such as peracetic acid or formaldehyde can also be utilized. These materials are supplied in aqueous solution and are always introduced into the process with substantial amounts of water vapor. Vapors are delivered to the sterilizing chamber as either an elevated temperature gas (vapor) or an atomized mist of liquid. In either case, the injection will result in temperature and relative humidity variation across the chamber initially and throughout the process. Attaining a consistent uniform process with vapors is substantially more difficult than for gases or liquids and requires constant mixing.

The addition of heat converts the solution components to the vapor phase as it is introduced into the sterilization chamber. On entry into the chamber that is generally at a lower temperature than the inlet gas stream, some portion of the vaporous material will revert to the liquid (or solid in the case of formaldehyde) phase. Vapor processes differ from gas sterilization in that vapors always have two distinct phases present inside the sterilizing chamber. Vapor sterilization processes typically operate at or near room temperature and are thus appropriate for heat sensitive materials. Depending on the temperature within the chamber, agent concentration and humidity level within the sterilizing chamber, some quantity of the agent will revert to its initial liquid (or solid) state. As the water vapor is also subject to condensation, it too can be in either phase. The concentration of agent and water condensed at each location may be variable based on the temperature at that location. The concentration in the gas phase will be uniform to the extent that the internal chamber is well mixed. Penetration by vapor agents through permeable materials as gases is certainly possible; however it is unlikely to occur once they have condensed. As a consequence, these agents are rarely utilized where penetration through layers or wrapping is required.

Vapor sterilization requires appropriate agent concentration and relative humidity. The difficulty created by the presence of two phases in the sterilizing chamber is that concentrations of the agent and relative humidity will not be constant across the entire chamber. Concentration determinations in the gas phase (where concentration can typically be measured rather easily, if not inexpensively) may not correlate with concentration in the liquid phase. This substantially complicates precise control of the sterilization process, as the target microorganisms are solids, and presumably at a lower temperature than the vapor. Kill rates of microorganisms by these agents differs with concentration and the phase present (35% liquid

kills at a different rate than 35% gas), and is further complicated by temperature variation across the chamber that creates localized concentration and relative humidity differences. Nevertheless, provided the system maintains reasonable temperature control, and the vapor within the chamber is well mixed, the process uncertainties can be minimized and effective sterilization demonstrated across the entire chamber and load.

Biological indicators for vapor systems cannot have defined resistance in the form of *D*-values as the effective concentration of the agent in contact with the microorganism cannot be determined with precision because of the condensation potential. Gas phase concentration (which can be measured) cannot correlate directly to surface concentration (where condensation might be present) unless the temperature throughout the chamber is constant. Thus, while microbial destruction is certainly evidenced by vapor processes, the rate of kill is unfortunately inexact. As the process parameters cannot be accurately determined, *D*-value determination is problematic and reported values are likely inaccurate.

HYDROGEN PEROXIDE

Hydrogen peroxide effectiveness as a sterilizing agent is well established (18). Hydrogen peroxide is available commercially in aqueous mixtures. Solutions of hydrogen peroxide should be kept away from flammable materials and reducing agents for safety reasons. Solutions of H_2O_2 should also be protected from light. Delivery to sterilization chambers is accomplished by heating the solution (30 50% H_2O_2 in H_2O mixtures have been used) above the boiling point ($\sim 100^{\circ}C$), simultaneously supplying the sterilizing agent (H_2O_2) and required humidity (H_2O). The sterilization process may incorporate an evacuation (or drying) step to allow for increased H_2O_2 concentration without condensation. Thorough mixing of the chamber is recommended as it increases uniformity of all process variables. Penetration of H_2O_2 in the gas phase is likely comparable to that of H_2O, while liquid penetration is minimal. After exposure the chamber is aerated/evacuated to remove H_2O_2 from the materials. This portion of the cycle may be the longest as re-evaporation of any condensed H_2O_2 typically requires more time than the rest of the process. A slight modification of the more common process includes the addition of electrical energy to an H_2O_2-filled chamber, which increases the process effectiveness by the creation of short-lived free radicals (19).

PERACETIC ACID

Peracetic acid, which is typically supplied as a mixture with H_2O_2, is an effective sterilant because of its strong oxidizing potential (9). It is explosive at temperatures above $110^{\circ}C$, and thus is introduced into sterilization processes as a liquid mist at ambient temperature. A small amount of the peracetic acid may evaporate into the gas phase. Surfaces to be sterilized must be exposed directly to the liquid because concentration in the gas phase is generally low. It is a strong oxidizing agent and corrosive to many materials, and thus presents considerable handling/safety issues.

LIQUID STERILIZATION

There are many available liquid materials that are effective for microbial destruction by a variety of chemical reactions. These chemically active agents are capable of rapid kill of vegetative cells and spores. Acids, bases, aldehydes, halides, and strong oxidants are all effective liquid sterilants (20). The item to be sterilized is immersed in the chemical, allowed to remain (with or without mixing) for the required time period. Following the dwell period, the item is either removed from the agent and treated to remove the agent or the agent is chemically neutralized in situ. The steps that follow the sterilization dwell proper must be performed in a aseptic manner that preserves sterility of the object. Removing the item from the chemical agent from the object mimics the removal of a previously sterilized object from its protective wrap. That is it is not a part of the sterilization process, but essential to proper use of liquid sterilization. Depending on the end use of the items and the chemical activity of the materials used, neutralization may have its own adverse material effects to consider. In validation of liquid chemical sterilization, agent removal (whether accomplished by physical or chemical means) is an important part of the overall sterilization process.

Liquid chemicals in aqueous solution capable of sterilizing physical objects as described above include:

- Aldehydes glutaraldehyde, formaldehyde, etc.;
- Acids peracetic, nitric, sulfuric, etc.;
- Bases sodium hydroxide, potassium hydroxide;
- Oxygenating compounds hydrogen peroxide, ozone, chlorine dioxide; and
- Halides sodium hypochlorite, chlorine.

In the simplest of systems, the process is executed in open vessels positioned within an aseptic environment (ISO 5). The process is executed by gowned personnel following a detailed process record providing chemical addition, agitation, and neutralization. The aseptic environment allows for the removal/neutralization of the agent with reduced opportunity for recontamination of the items. In its most evolved form, the process can be carried out in a closed and agitated vessel with considerable automation.

Identical to gas sterilization, liquid sterilant effectiveness varies with concentration and temperature (humidity is provided by the water in the solution); however, because of the mixing it can be considered uniform throughout the vessel and constant over the course of the process. Other factors impacting antimicrobial activity include pH, agitation (if utilized), and presence of soil or other contaminants that might protect the microorganism. Assuring effective liquid chemical sterilization processes is straightforward, due to its simplicity.

As with other forms of sterilization, the effect of the sterilization on the materials must be thoroughly evaluated. The chemical activity of these agents is such that their effect on the items being sterilized can be substantial. Extreme pHs, significant oxidation, and reaction potential, all of which make the agent effective against microorganisms can play havoc on materials (and processing equipment) as well. Chemical activity in the form of materials compatibility is widely available in the literature (21).

VALIDATION OF GAS, VAPOR, AND LIQUID STERILIZATION METHODS

The performance qualification or "validation" activity has been described as documentation that the process or product conforms to expectations as determined through independent parameter measurement and/or intensive sampling or challenge. It is the focus of regulatory attention for any sterilization process. It is common practice in performance qualification to utilize "worst case" challenges in validation, and that is most prevalent with sterilization processes. Typical worst case challenges for gas, vapor, and liquid sterilization include reducing the process (set-point) temperature; reducing cycle dwell time; reduction of both time and temperature; reduction of agent concentration, and the use of resistant biological challenges as bioburden surrogates. More detailed information on the expected practices can be found in the myriad of industry and regulatory publications on this subject (22,23).

Historically, gas sterilization processes have been validated using the half-cycle approach, which uses conservative assumptions about the microbial resistance and number of bioburden microorganisms and was originally developed for use with ETO (24). Prior to the development of parametric lethality calculation for ETO, accurate information on gas concentration, relatively humidity, and temperature was largely unavailable, so the half-cycle method was utilized as a worst case approach. The half-cycle approach mandates a sterilization dwell period that destroys not less than 10^6 spores of a resistant biological indicator. In routine operation, the process dwell period is doubled (thus the term half-cycle) and supports a PNSU of 10^{-6} (5). The half-cycle method as utilized for gas sterilization is graphically depicted in Figure 4. The half-cycle method does not rely substantially on the resistance of biological indicator (as surrogate for the bioburden), because complete destruction of the indicator is required in the "half-cycle". Actual determination of the indicators *D*-value at the chosen parameters requires substantially more effort, and has been ignored by some practitioners. Half-cycle approaches are inherently conservative, and little effort is made to optimize the process dwell period, when it will be arbitrarily doubled in routine use anyway. The half-cycle method evaluates only the effect of time, assuming that the

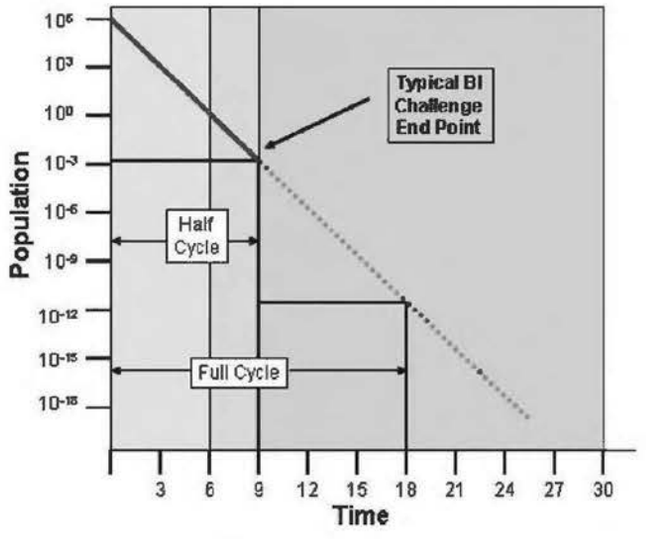

Figure 4 Half cycle sterilization validation.

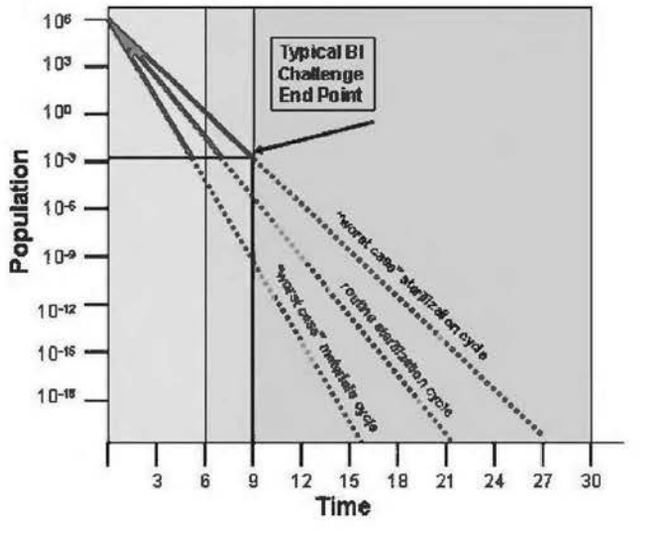

Figure 5 Sterilization validation using bracketing.

effect of lethality of variations in the other essential parameters, gas concentration, relative humidity, and temperature can be ignored. This is a severe limitation of the method.

Another method suited for sterilization validation is a bracketing approach that better supports the extremes of the operating ranges for the critical process parameters (25). In the bracketing approach, a cycle with lower concentration, lower relative humidity, and a shorter dwell period is confirmed by microbial indicator destruction using what are less lethal conditions. Material effects are evaluated in a cycle employing a higher concentration, higher relative humidity, and a longer dwell period where the adverse impact is believed to be greater. Routine operation of the system utilizes conditions that fall between the process extremes that have been evaluated (Fig. 5). This method does not require a precise *D*-value for the biological indicator, because this method supports all of the critical sterilization parameters it is readily defendable without that information.

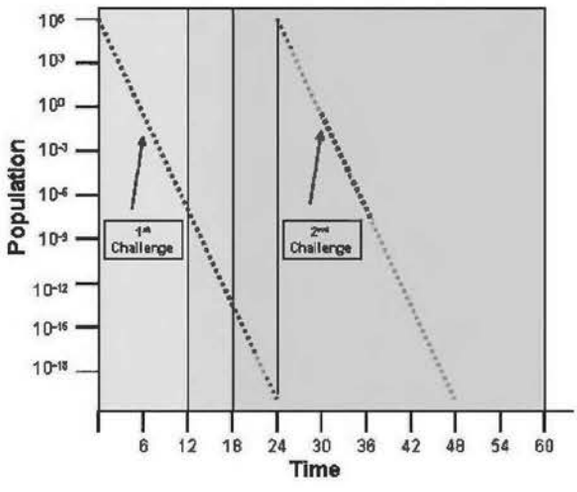

Figure 6 Double spike validation method.

The half-cycle and bracketing approach are fully compatible with sterilization using any of the gas or liquid agents giving the practitioner a choice of methods. As precise *D*-value determination is not required for either of these methods, their use for vapors is also rather simple. The choice between them is between the simple, but less certain efficacy of the half-cycle method versus the more complex, but perhaps more defensible bracketing approach. Where the *D*-value for the agent has been determined at the operating conditions for either gas or liquid sterilization, the process dwell period can be defined more precisely, and a somewhat shorter cycle time established.

Liquid sterilization can also be validated using a double-spike method that has been specifically developed for it (26). It is an adaptation of the half-cycle method in which a second microbial challenge is introduced at the mid-point of the cycle. The microbial challenge is introduced both at the start of the process and again at the same concentration mid-way through the process dwell. Samples are taken and neutralized at intervals after each inoculum to demonstrate microbial kill rates are essentially constant throughout the sterilization process (Fig. 6). The premise in this approach is that the agent might not have sufficient antimicrobial activity over a lengthy process, and thus destruction in the second half of the process might not occur at the same rate. If this is not the case, then the bracketing approach described early can be utilized for liquid sterilization.

Regardless of the validation method utilized, there are common elements in all validation efforts.

- Equipment qualification: The equipment utilized for the sterilization process (pressure vessel or stirred tank) as well as any rooms utilized for pre- or post-cycle processing must be fully documented with respect to installation details and operational characteristics. Equipment qualification serves as the basis for change control for the physical equipment. This effort must of course include calibration of instrumentation and qualification of the control system.
- Empty chamber/vessel parameter distribution: Parameter measurement within the sterilization chamber/vessel is appropriate. Depending on the agent used this may be single or multiple point, with the cost of measurement an important consideration. For vapor systems, the real utility of concentration determination can be questioned as gas phase values will not correspond to liquid phase conditions. The goal in this exercise is to be able to correlate the values obtained during this study to the routine monitoring location(s). Where the vessel is mixed during the process (as is almost universally desirable), this study confirms the effects of that mixing. Overmixing in these processes is not a consideration, as additional mixing can only improve uniformity of

Table 1 Biological Indicators for Common Chemical Agents

Gases	
Ethylene oxide	*Bacillus atrophaeus*
Chlorine dioxide	*Bacillus atrophaeus*
Ozone	*Geobacillus stearothermophilus*
Vapors	
Hydrogen peroxide	*Geobacillus stearothermophilus*
Peracetic acid	*Geobacillus stearothermophilus* or *Bacillus atrophaeus*
Liquids	
All	none established *Bacillus atrophaeus* is perhaps the most appropriate

the process parameters. Biological indicators are not required in the evaluation of the empty chamber/vessel uniformity. The limitations of gas phase measurements in vapor processes must be understood.

- Component and load mapping: These activities are not a part of gas, liquid, or vapor sterilization, because sampling systems placed within the load items would alter agent penetration. This evaluation is best provided by passive biological indicators placed within the load items. The use of physical/chemical indicators placed within the items can be used to support this effort, but as there are no available chemical integrators, this practice is of limited use.
- Biological indicators: The use of a biological indicator for initial validation and routine process control is an integral part of many validation efforts for gases and vapors.[a] The principal exception to that general situation is ETO, where parametric release has been successfully accomplished by numerous practitioners. For all of the other sterilization methods described in this chapter, biological indicators are essential. The biological indicator serves as a worst case surrogate for the bioburden present in routine operations. Biological indicators are conventionally spores of a microorganism (most often a *Bacillus* or *Geobacillus* species) chosen specifically for its greater resistance to the sterilizing process than the expected bioburden. Inactivation of the biological indicator during the validation establishes the lethality of the process across the items being sterilized. The measurement of physical conditions during the validation exercise and routine operation allows for estimations of process lethality. The biological indicators of choice for the various sterilizing agents are listed in Table 1.

  Spore challenges may be either a strip or a coupon positioned within the load or spores inoculated on a load item. Inoculated items should have their population determined by the end user, and where possible their resistance to the sterilization process confirmed. Indicators are placed among the load items at locations believed to be hardest for the agent/humidity. The use of biological challenges for liquid sterilization is limited to the initial validation of the process, as the materials must be in direct contact with the liquid agent making placement and recovery of suitable biological indicators problematic in routine processing. Liquid sterilization processes are customarily established as parametrically released from the onset (a typical situation with many sterilization processes that are utilized in-process).
- Process confirmation/microbiological challenge: The core of the validation activity is the confirmation of acceptable process parameters and inactivation of the microbial challenge. Proof of cycle efficacy is provided in replicate studies in which the biological indicators are killed, and physical measurements are taken as documentation. Differences in resistance are exploited in the validation of these sterilization methods for ease of validation and routine process control.

[a]Where parametric release has been attained, the routine use of biological indicators may not be required.

ROUTINE PROCESS CONTROL

Sterilization processes must be subject to routine controls that support the efficacy of the cycle over time. Validation is not a one-time activity project, but an integral part of a CGMP compliant facility that must be sustained over the useful life of the facility and its products (27). Control over sterilization processes is commonly achieved through practices defined specifically for that purpose including: calibration of instruments, physical measurements of process parameters, use of physical integrators/indicators (and in some cases biological indicators), change control, preventive maintenance and periodic reassessment. In the absence of approvals for parametric release, biological indicators are utilized for routine release of each sterilization load along with documentation from the control system.

ISOLATOR/ROOM DECONTAMINATION

When isolators were first introduced in the health care industry, their internals were decontaminated using a liquid/vapor process using peracetic acid (J. Agalloco and D. Meyer, personal communications, 2002). The corrosive nature of this material and the time required to remove it (via evaporation and air exchange) led to interest in alternative agents for isolator preparation. The first of these to come into widespread use was hydrogen peroxide, as commercialized by Steris Corporation in the late 1980s.[b] The initial VHP-1000 systems that were offered for sale utilized the term "sterilization" in much of the documentation provided. This led directly to the assumption that these systems could readily sterilize the enclosures to which they were connected. Sterilization with these vapor delivery systems is certainly possible; however, considerable care must be taken to establish a process it that will "sterilize" the entire treated volume. Sterilization using H_2O_2 requires careful attention to the details outlined in the preceding text; it is more appropriate to consider the H_2O_2 process as a decontamination that prepares the isolator for use in processing in much the same manner as a manned clean room. Where decontamination is the process objective, the treatment needs be less aggressive, shorter, and thus less harmful to the isolator materials (especially the gloves and gaskets) and is more in line with the real objective of the treatment.[c] In more recent years, the notion that isolators need only be decontaminated rather than sterilized has eased the implementation of this technology. Chlorine dioxide has been applied to isolator treatment as well, and because it is a true gas it offers a simpler methodology; nevertheless while again capable of sterilization, decontamination is the more appropriate target.

The treatment of isolators using gases and vapors has reinvigorated the means by which clean rooms are prepared for use. Hydrogen peroxide, chlorine dioxide, and ozone have all been successfully used in the decontamination of processing environments achieving a degree of consistency and lethality unattainable with manual decontamination (28). The applications have included buildings contaminated with *Bacillus anthraces*, facilities with mold and other microbial infestations, and health care processing environments. The gassing/fogging processes utilized are substantially more effective than the manual practices they replaced, nevertheless the temptation to consider these sterilization processes should be resisted, as that is rarely the goal of these treatments.

CONCLUSION

This chapter provides an overview of the prevalent gas, vapor, and liquid sterilization methods and their validation. This chapter has broadly outlined the primary considerations with respect to each of these sterilization processes. The reader is encouraged to review the substantially larger body of knowledge available on these processes before their implementation. The accompanying bibliography outlines some recommended sources on this topic.

[b] Steris Corporation acquired AMSCO which had introduced Vapor Phase Hydrogen Peroxide as a means for isolator treatment in 1990.

[c] The isolator is not a drug or medical device and will not be injected into a human, thus its sterility is not essential for use as a processing environment.

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11 | Dry heat depyrogenation and sterilization

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INTRODUCTION

Dry heat is one of the oldest methods of sterilization. Dry heat is used in the pharmaceutical industry mainly for depyrogenation of glassware and equipment going into an aseptic processing area. Because of the high heat required for the depyrogenation process, products that have been validated for depyrogenation are considered to be validated for sterilization without additional work (1). More limited use of dry heat processes are for sterilization alone.

This chapter includes information derived from a review of existing literature and publications on dry heat sterilization and depyrogenation, as well as additional information gained from practical experience. A technical information report was published by the Parenteral Drug Association (PDA) in 1981, on validating dry heat sterilization and depyrogenation processes (2). The technical report is currently being updated and is due for publication shortly. Regulatory standards have also been published on the subject; most notably ANSI/AAMI ST63, published in 2002 on validation of dry heat processes used in the healthcare industry (1). This standard is currently undergoing global harmonization and is expected to be published soon as an ISO standard. Although the focuses of the standards are sterilization in the health care industry, the basic premises are applicable to the pharmaceutical industry and to development of depyrogenation processes using dry heat. Because the standards are developed as consensus documents with the input of regulatory authorities, they represent the current best practices in the industry.

DEPYROGENATION BY DRY HEAT

One of the most effective ways to inactivate endotoxin, or depyrogenate, is by a dry heat process. This occurs basically through an incineration process. The materials being treated must be heat stabile due to the high temperatures required for an effective process.

The ability to depyrogenate by dry heat is achieved by inactivating known challenges of purified endotoxin, resulting in the demonstration of a 3 log reduction of endotoxin. Purified endotoxin, consisting only of lipopolysaccharide, is pyrogenic in lower doses than naturally occurring endotoxins, where associated proteins and phospholipids are a factor in mediating pyrogenicity (3). Various studies have been conducted, and publications issued, on the time and temperatures necessary for the inactivation of endotoxin.

The United States Pharmacopeia (USP) specifies to inoculate with 1000 or more USP units of bacterial endotoxin; endotoxic substance should be reduced to not more than 1/1000 of the original amount (3 log reduction) (4). The European Pharmacopeia, the British Pharmacopeia, and the Japanese Pharmacopeia are harmonized on their depyrogenation chapters and state temperatures greater than 220°C without specifying a time, resulting in a 3 log reduction in heat resistant endotoxin (5 7).

In the specific chapters in each of these compendia on performing the pyrogen test or the endotoxin test, the temperature of not less than 250°C for 30 minutes is noted as the commonly used minimum time and temperature settings for depyrogenating glassware and apparatus used in laboratory testing. The USP is also more specific in both the pyrogen and the endotoxin test chapters and says to depyrogenate all glassware and other heat-stable materials in a hot-air oven using a validated process with the commonly used minimum time and temperature settings of 30 minutes at 250°C.

Several studies evaluating temperature and time for endotoxin inactivation are noted in the literature. Tsuji and Lewis looked at the destruction kinetics of lipopolysaccharides from *E. coli*, S. *marcescens*, and *S. typhosa* at temperatures ranging from 170 to 250°C, and postulated that the destruction kinetics were 2nd order, with a z-value of 46.4 (8). In this study, at 250°C, a *D*-value of 4.99 minutes was identified for *E. coli* lipopolysaccharide. The destruction curve for lipopolysaccharide in studies since has been postulated to consist of two distinct linear phases;

the first occurring at a rapid rate and the second phase of the curve flattening out, with the reduction of LPS occurring at a much slower rate (9,10).

Nonlinearity of destruction curves of bacterial spores has been attributed to the lack of homogeneity of the spore population (8); a similar principle may be at work here. Because of the variety of responses in development of inactivation curves, it has been difficult to apply standard F_H calculations to depyrogenation studies, since an accurate z-value is necessary for these calculations (11). It has also been noted that D-value calculations rely on first-order kinetics throughout the entire process (12); the biphasic model for endotoxin inactivation would indicate that estimation of a D-value is not appropriate.

Because of the variability in endotoxin inactivation and recovery, the efficacy of the depyrogenation process is demonstrated by inactivation of the endotoxin indicator, rather than relying on empirical calculations of time and temperature. That being said, the measurement of heat input remains an important factor in monitoring the process to ensure an ongoing state of control.

Developmental studies are conducted to evaluate endotoxin reduction or inactivation. The items to be depyrogenated are inoculated with endotoxin, exposed to the desired temperature for various times, and then tested to measure endotoxin inactivation. The purpose of the developmental studies is to determine the minimum time and temperature necessary to demonstrate the required 3 log reduction of endotoxin using the *Limulus* amebocyte lysate (LAL) assay.

Factors affecting the inactivation of endotoxin are the particular endotoxin formulation, the purity of the concentration, the method of application, and the concentration used (11). Endotoxin has a tendency to bind tenaciously to surfaces, which factors in the removal and recovery of the endotoxin. Most often, surfaces are inoculated at greater than three logs, often at five to six logs to facilitate removal of endotoxin. However, with inoculum levels that high, an issue of concern would be whether aggregated endotoxin is being removed, or whether the endotoxin in contact with the surface of the material being evaluated is being removed (13).

Articles to be processed can be directly inoculated with endotoxin, smaller portions of a similar surface material can be inoculated, or a commercially available endotoxin indicator (EI) can be used. For materials that are directly inoculated with endotoxin, inoculate at the desired concentration, determine the recoverable amount of endotoxin, and perform inhibition/enhancement (I/E) testing to ensure that the surface or carrier does not leach any materials that will interfere with endotoxin recovery. Recovery can be performed by using LAL reagent water, generally in the smallest amount that will cover the endotoxin-spiked area, and shaking, vortexing, or sonicating to extract the spike. In some cases, depending on the process being analyzed, a surfactant or additive can be used to assist endotoxin recovery.

The LAL assay (I/E testing) is then performed to determine recoverable endotoxin in the preliminary phase and inactivated endotoxin following exposure to dry heat. Log reduction is calculated by determining the log of the recoverable endotoxin units (EUs) in the positive control minus the log of the EUs remaining in the processed article, carrier, or commercial EI.

STERILIZATION BY DRY HEAT

Dry heat is not widely used as a mode of sterilization because of the inefficiency of the process. Air is a good insulator, causing slow heat transfer from air to the product/items in dry heat processes. The heat conductivity of the items themselves can be somewhat slow, and stratification of air in the chamber can occur. However, for select, heat stable products, dry heat is the mode of choice for implementing the sterilization process.

Dry heat kills microorganisms primarily by reacting with, and oxidizing, their proteins, although other factors such as the depurination of DNA may play a secondary role (14). The effectiveness of dry heat as a microbicidal agent with the ability to kill a wide range of microorganisms has been well established (15).

The microorganism generally selected as a biological indicator for use in dry heat sterilization validations is *Bacillus atrophaeus* (formerly *B. subtilis*). This microorganism has been chosen for its known resistance to dry heat. Depending on the approach taken to the development of sterilization processes, i.e., if a product specific approach is taken, supplemental studies may be needed to evaluate the resistance of the naturally occurring bioburden on the product/items.

PROCESS DEVELOPMENT—DEPYROGENATION

The initial step in process development is to determine the heat stability of the items to be depyrogenated. The higher the temperature used for processing, the more efficient the process will be. The considerations that apply during process development are variations in load density, initial load temperature, and specific heat of the load components.

Temperature distribution studies are performed to understand the dynamics of temperature in the oven or tunnel in which the dry heat processing will occur. Temperature penetration studies are then (or concurrently) performed to understand the dynamics of the particular load being processed, especially to identify the cool spots in the load. Evaluation of the worst case load in terms of thermal mass should be carried out, and the studies or evaluation should be designed to encompass loads with less mass. Decision must be taken whether to utilize a single process for all materials to be processed or if there is a wide variation in terms of thermal mass, then it may be more efficient to design more than one process.

For glass loads, the smallest vial size with the greatest mass is often the worst case because of the density of the load to be processed, and with the greatest heating lag time. However, in some cases, a small load may be the worst case due to quick heat up and cool down times, thus decreasing total heat input. In dry heat tunnels, with various loads and belt speeds, the different combinations should be challenged to identify the combination that results in the lowest heat input. This combination would then be used during the biological validation study.

Critical operating parameters should be defined during process development studies. These are temperature set point and exposure time for an oven, and temperature set point and belt speed for a tunnel. Perform temperature-mapping studies using qualified and calibrated temperature sensing devices.

Temperature studies may be conducted as separate studies or in combination with biological studies using the endotoxin indicators. Place the inoculated materials or endotoxin challenge vials adjacent to duplicate materials or vials with temperature sensors. Determine the temperature and time necessary to achieve the required level of endotoxin inactivation. This data will then be utilized during the temperature penetration and distribution studies to evaluate the conditions required to achieve the minimum temperature in the coldest portion of the load that is necessary to achieve inactivation of endotoxin in the loaded chamber or tunnel.

PROCESS DEVELOPMENT—STERILIZATION

Following are the pieces of information that need to be understood or identified to develop a sterilization process:

- Dry heat resistance of the biological indicator (BI) organism and/or bioburden on the product
- Heat stability of the product being processed
- Temperature distribution in the chamber or tunnel
- Temperature penetration into the product or load
- Identifying the reference temperature for routine processing
- Equating heat input with delivered lethality, and you can calculate the process (time and temperature) necessary to deliver the desired sterility assurance level (SAL).

The intent of the process development studies for dry heat sterilization is to identify the minimum time and temperature necessary to achieve the desired level of lethality for the items being processed. It is necessary to understand the resistance of the microorganisms on the load to be processed, and the amount of heat delivered to the load being processed, to determine the time and temperature necessary for sterilization. The *D*-value is a measure of resistance of the microorganism and is defined as the time required to achieve inactivation of 90% of a population of the microorganism under stated exposure conditions. As noted previously, the microorganism used as a BI in dry heat processes is *Bacillus atrophaeus*.

Process development follows traditional sterilization concepts, as applied in moist heat sterilization. The F_H replaces the F_0 terminology and is a measure of heat input. Historically 170°C was used as a reference temperature with a z of 20°C (2). More recent publications of international standards and compendial references to BIs for dry heat sterilization have used

160°C as the reference temperature for *D*-value analyses. Whether 160°C or 170°C is used as the reference temperature is somewhat arbitrary when calculating equivalent heat input, since the value is used as a reference, and not an absolute, temperature. The *z*-value of a microorganism is a measure of how heat resistance changes with changes in temperature. The *z*-value is the number of degrees that are required to change the *D*-value by one logarithm, or a factor of 10 (1,2).

Process lethality as measured by BIs is determined by the following equation:

$$F_H = D_{170°C}(\text{Log}a \quad \text{Log}b) \quad (2)$$

where F_H is the minimum lethality required (assume z = 20°C), expressed as the number of minutes equivalent time at 170°C that the slowest to heat item in the load should be heated; $D_{170°C}$, the resistance of the BI (this could also be calculated at 160°C, as appropriate); A, the initial population of the BI prior to exposure; and b, the natural log of the total number of BIs tested divided by the number of negative BIs.

Use of the F_H concept can also be used to determine equivalent times and temperatures; for example, if a process is qualified at a certain time and temperature, then the equivalent time in terms of heat input can be determined on the basis of the following equation:

$$F_t^z = \frac{F_H}{L}$$

where F_t^z is the equivalent time at temperature t delivered to an item for the purpose of sterilization with a specific value of z (e.g., 20°C); F_H, the equivalent time of 170°C delivered to an item for the purpose of sterilization; a z-value of 20°C is used.

The use of the F_H concept helps to integrate the lethality that occurs during the heat-up and cool-down phases of the sterilization process. Physical measurements of heat input can then be correlated to the rate and extent of microbiological kill achieved by a dry heat process.

The time and temperature required to deliver the desired lethality are determined through establishment of a lethality curve or kill time, using subminimal conditions of time and/or temperature to determine the rate of microbial inactivation.

Lethal rate is

$$L = \text{Log}^{-1}\frac{T_o \quad T_b}{z} = 10^{(T_o - T_b)/z}$$

where T_o is the temperature within the commodity; T_b, the reference temperature (i.e., 170°C); Z, the temperature increment required to change the *D*-value by a factor of 10. A *D*-value of 20°C is commonly used as the dry heat *z*-value.

The ultimate purpose of the developmental studies is to determine the rate of kill and the minimum conditions required to achieve the desired SAL.

There are three distinct microbiological approaches that can be used for dry heat sterilization they are the bioburden method, the bioburden-biological indicator combined method, and the overkill method, using the biological indicator itself.

The absolute bioburden method evaluates the resistance of the naturally occurring bioburden on the product or items to be processed, and calibrates the sterilization process based on that information. This approach is appropriate when the bioburden and its resistance to dry heat are well understood, and the manufacturing environment is maintained in a good state of control. It may also be appropriate for materials that are more heat sensitive, but for which dry heat is the best choice as a sterilant.

The bioburden-biological indicator approach evaluates the resistance and amount (quantity) of bioburden and combines that information with use of a resistant BI to demonstrate the inactivation of the BI that gives the theoretical kill of the microorganism.

The BI approach evaluates inactivation of a resistant BI at an appropriate population level to demonstrate the desired SAL without necessarily correlating the population level to the product or commodity bioburden.

Both the PDA Technical Report 3 and ANSI/AAMI ST63 have information and detail on these three approaches.

EQUIPMENT/HARDWARE CONSIDERATIONS

One of the primary considerations for the equipment will be to determine if an oven or a tunnel should be utilized for the application. An oven can be utilized for a wide range of applications, but it is a batch process and the loads must be manually transferred from the oven to the downstream processing equipment. In many applications, this transfer would need to be done aseptically in an ISO class 5 (or equivalent) environment. An oven is more simple from an equipment design standpoint because the load is fixed. Thus, the environment/load is heated to the desired temperature, then held at temperature for the specified time and then cooled to a user-defined temperature.

Tunnels are more appropriate for continuous feeding of glassware directly into a unit operation such as an aseptic filler. When used for continuous operations, tunnels are typically fed continuously with vials from a glassware washer. Tunnels generally have at least three temperature-controlled zones to heat-up and cool-down the glassware. The cooling section must be sized to cool the glassware to a user-defined level, and provisions must be in place to sanitize the cooling section after system maintenance or after other events that may have contaminated the cooling section. Many of the newer tunnels are equipped with heating elements so that the cooling section can be hot air sanitized.

Other equipment/hardware considerations include the following:

- Load sizes and throughput requirements
- Cooling requirements: these requirements should be well defined by the user as the specific cooling requirement can impact the cooling system design and can impact the length of the cooling section for tunnels
- Air flow and differential pressure requirements: user requirements should specify the direction of air flows and differential pressure requirements of the oven/tunnel in relation to the load and unload sides of the tunnel/oven
- HEPA filter testing and other validation provisions
- Particulate control and particulate monitoring provisions
- Filter burn-in provisions (as applicable)
- Filter selection
- Sanitization of tunnel cooling sections
- Energy conservation (especially for tunnels during non-production hours)
- Fire safety provisions (especially during a power outage)

INSTRUMENT AND CONTROL CONSIDERATIONS

A key to effective oven/tunnel operation lies in the automated process control system. By eliminating the dependence on operator intervention and data recording, automatic temperature and sequential control provides assurance that the "validated" sterilization and/or depyrogenation cycle is consistently and repeatedly delivered. A typical control system for a new oven/tunnel includes the following hardware components:

- PLC (programmable logic controller)
- Operator interface panel(s)
- Data recorder/data collection system
- Process variable sensors
- Input/output (I/O) devices

The PLC is most commonly used as the primary component of the automated process control system as it provides sequential control of the process, provides control of all analog devices used for temperature and pressure control, controls all digital devices, receives operator input via the operator interface panels, and provides process information (such as process variable information and alarms) to the operator via displays and/or operator interface panels. The PLC typically contains specific recipe information for the various cycles to be utilized. In some cases, the PLC can be used for data collection, but it is much more common to use a separate data recorder/data collection system.

The operator interface panel can be as simple as switches and displays or as complex as a stand-alone PC running a supervisory control and data acquisition (SCADA) with a human machine interface (HMI) software package. These devices are typically used to select the recipe, start the cycle, and display process information during the cycle. The higher level PC-based SCADA type operator interface panels can provide detailed cycle reports and trending information.

The data recorder/data collection system can range from a simple strip chart recorder to a full-blown Manufacturing Execution System (MES) type data collection system. In many cases, the PLC can also provide batch data logging functionality. The minimum variables to record for dry heat sterilization/depyrogenation processes are typically temperatures, exposure times (including belt speeds for tunnels), and differential pressures.

Typical sensors include temperature measurement devices (or thermocouples), pressure measurement devices, and, where applicable, belt speed measurement devices. It is customary that the temperature sensor used to control the process temperature not be used to provide the batch record process data. An independent/secondary temperature sensor for batch reporting provides a high degree of assurance that the cycle actually ran within its defined limits.

The pressure transmitters need to be appropriately placed to maintain the manufacturer's recommended exposure conditions as the ambient conditions can impact the accuracy of the measured pressures.

Newer tunnels and ovens typically utilize variable frequency drives (VFDs) to control the tunnel/oven pressures (internal and differential) as these drives can adjust to changing room pressures.

For input/output devices, there are analog types and discrete types. The analog inputs are typically from process sensors and the analog outputs are typically for control of proportional valves, heaters, and VFDs. The discrete inputs are typically from switch type (operator and process) devices, and the discrete outputs are typically for activating hardware such as valves, lights, etc.

The design and development of the oven/tunnel control system software should follow the principles of ISPE GAMP (Good Automated Manufacturing Practice) 5, a risk-based approach to compliant GXP computerized systems (16). This guideline details a software lifecycle from conception through decommissioning.

VALIDATION OF DRY HEAT PROCESSES

Validation consists of the documented installation and operational qualification of the equipment used to deliver the dry heat process, followed by the documented performance qualification of the physical and biological aspects of the dry heat process.

Installation Qualification

The purpose of the installation qualification (IQ) is to demonstrate that the equipment is suitable for its intended use as installed and that it has been appropriately built according to the user's requirement specifications. It is the documented verification that the facilities, systems, and equipment, as installed or modified, comply with the approved design and the manufacturer's recommendations (17). The IQ also verifies that the documentation required for the equipment's operation, maintenance, calibration, and cleaning/sanitization is provided, and that programs are in place to maintain the equipment in a continued qualified state for operation.

The equipment should have available the appropriate utilities including air supply, electrical, exhausts, cooling water and HVAC (heating, ventilating and air conditioning) provisions to maintain the desired environmental temperatures and differential pressures. Items to be considered during the qualification testing are as follows:

- Safety and alarm features should be installed correctly and tested
- Operations and maintenance manuals should be available for the equipment and chart recorders
- Software should be validated and compliant for appropriate regulatory authorities

- Program logic control manual should be available
- Wiring and "as built" diagrams for equipment should be available
- HEPA filters should be qualified and tested to meet current standards
- Accuracy of temperature, time, airflow, pressure and belt speed (as applicable) monitoring devices should be established and documented
- Calibration certificates should be available for controlling instruments, such as timers, pressure gauges, anemometers, thermocouples, and recording charts
- Sensors and equipment should be placed on a calibration and preventive maintenance schedules
- Details of cycle programming should be available
- Any necessary inventory of spare parts should be considered at this time

IQ testing of the control systems for computerized equipment and systems will be determined by and specific to the type of computer system.

Drawings of equipment and instrumentation are generally developed during the design phase of the project and are used to build the system. These become an important historical document to track the equipment and subsequent changes to the equipment. Items to be considered at this time for documentation and verification include the following:

- HEPA filters
- Validation ports
- Instruments
- Conveyor systems
  - Nominal size (length and width)
  - Materials of construction
  - Drive motor
- Fans
  - Type
  - Rated capacity
  - Motor horse power/revolutions per minute (RPM)/volts/amps/phase
- Motors
- Gates
  - Numbers and settings of gates
  - MOC of the tunnel and gates
- Heaters
  - Type
  - Rated capacity
- Cooling elements
  - Type
  - Rated capacity

Any inconsistencies between the drawings and specifications and the system and componentry as installed should be resolved at this time to ensure that the documentation on file accurately represents the installed system.

HEPA Filter Integrity Testing

Each HEPA filter installed must pass integrity testing in situ to verify the integrity of the filter frame seals and the proper seating of the filter in the frame or grid. Filter testing includes flow rate and integrity testing, and air testing downstream for particulates to ensure that filters do not leak or shed particles. Items to be considered and documented include the following:

- Procedures for HEPA filter integrity testing and repair
- Serial numbers and locations of filters

- Testing medium used for integrity testing
- Upstream concentration of testing solution
- Integrity and leak test report
- Repair and retesting report
- Surface area of repairs
- Velocity of air
- Grid location of repairs.

Support Utilities
Verify any of the critical utilities necessary to support the dry heat oven or tunnel. These will include electrical power, cooling water, and instrument air. Any discharge connections from the equipment should also be verified.

Critical Instrumentation Installation
Verify the installation of any critical instruments, which are those used to make operational decisions or which are a part of the production or maintenance records. These may include the following:

- Temperature sensors, recorders, or display systems
- Timers, recorders, or display systems
- Differential pressure sensors, recorders or display systems
- Belt speed sensors, recorders, or display systems

All of the IQ documents should be reviewed and approved by the appropriately designated individuals responsible for the quality of the installation process.

Operational Qualification
Operational qualification is the documented verification that the facilities, systems, and equipment, as installed or modified, perform as intended throughout the anticipated operating ranges (17). The operational qualification also demonstrates that all controls function properly and that temperature control and uniformity meet functional specifications.

Items for consideration during the operational qualification are as follows:

- Programmable logic reliability testing each stepped sequence
- Door interlock (ovens)
- Gasket integrity (ovens)
- Blower rotation RPM and direction of rotation
- Heater elements ensure that all are working
- Room balance ensure positive pressure to retain the integrity of clean areas
- Air filtration integrity of the air supply, recirculation and exhaust HEPA filters that supply air for ovens. Verify the integrity of in-feed, hot, and cooling zone HEPA filters for tunnels
- Belt speed and speed recorder for tunnels
- Air velocity profiles across the unit
- Monitoring of nonviable particles required to demonstrate the appropriate clean area classification
- Sanitization of cooling section (for tunnels equipped with heating coils)

An operating procedure for the operation of the dry heat oven or tunnel should be written and available at this stage, with documentation of operator training considered for inclusion as a part of the operational qualification protocol.

Any operating controls on the control panel for the oven or tunnel should be tested to ensure that they function according to manufacturer's or system specification. These include any switches, pushbuttons, indicators, controllers, recorders, etc.

Safety and alarm testing should be conducted and include any features necessary to ensure personnel and equipment safety. Some typical alarms and interlocks may include the following:

- High/low temperature alarms
- High/low pressure alarms
- Airflow alarms
- Belt speed alarms
- Belt/temperature interlocks
- Fan/heater interlock alarms
- Emergency stop button
- Differential pressure alarms (across the HEPA filters)
- Gate interlocks
- Abort alarms

Loss of utility testing should be conducted to verify the response of the equipment to loss of electrical power or air supply. It is important that the critical data not be lost, and also to verify that the response of equipment is appropriate upon resumption of power or air supply.

Airflow Velocity Testing
Testing is conducted in critical zones (class 100, ISO 5) to verify and document sufficient airflow velocity across the face of the HEPA filter. This testing is generally conducted at ambient temperature.

Airflow Pattern Testing
Airflow testing is conducted in tunnels to ensure that integrity of clean areas or zones is maintained, and that turbulence does not cause any clean areas to be compromised. Testing should verify unidirectional flow from higher pressure or clean zones to lower pressure or less clean zones. Consideration should be given to performing airflow testing with gates at both maximum and minimum settings. It is preferable to perform any visual verification of airflow patterns through use of a vapor generated in a manner that leaves no residue on the surface of equipment being tested.

Nonviable Particulate Testing
Nonviable particulate testing is applicable in ovens and tunnels where open containers or items are being processed. Testing is generally conducted at processing temperature with the particle sampling probe placed at representative locations.

Empty Chamber Studies
Empty chamber temperature distribution studies are performed to show temperature uniformity across the chamber or tunnel and to identify any cold and hot spots. Temperature sensors should be placed to give the greatest amount of information about the space to be occupied by the load being processed. For an oven, the temperature sensors are often place in a three-dimensional "X" pattern to ensure that the top, middle, bottom, front, and back of the sterilizer are being evaluated.

In a tunnel, if different temperature zones are used, each zone should be monitored with temperature sensors. Sensors can be mounted on a metal bar (usually stainless steel) above the conveyor belt to map the temperature within the tunnel. Temperature sensors should also be placed next to the fixed sensors that will be used to monitor and control during routine processing. The critical parameters should be recorded, and process variability established at this time.

It should be noted that some people forego the empty chamber studies and use the heat penetration and distribution data established during the loaded chamber studies (see below) to establish temperature uniformity data used in validation.

Process Validation

Process validation is the documented evidence that the process, operated within established parameters, can perform effectively and reproducibly to produce a medicinal product meeting its predetermined specifications and quality attributes (17). The performance qualification involves studies of temperature distribution, heat penetration, and endotoxin or BI (microbial) challenges.

Loaded Chamber Studies

Loaded chamber studies are conducted on the worst case loading pattern, utilizing the information gained from the thermal-mapping studies of the empty chamber or tunnel, if applicable. The intent of the loaded chamber studies is to obtain and document the temperature distribution and penetration data with the actual items to be processed. Temperature sensors are placed across the width of the belt in a tunnel, and are placed at the front, middle, and rear of the load passing through the tunnel. As noted previously, temperature sensors in an oven are placed in a three-dimensional pattern that ensures that the top, middle, bottom, front, and back of the oven are being evaluated.

The purpose of monitoring the loaded chamber or tunnel is to ensure that the coolest location in the load reaches the required temperature for the required length of time as identified during the developmental studies. The spread of temperatures throughout the chamber or tunnel is measured, and ability to achieve the desired biological inactivation is demonstrated in the coolest portion of the load. Endotoxin reduction studies could be conducted at this point.

Temperature distribution thermocouples or sensors are intended to monitor the air temperature within the oven or tunnel and should not be in contact with any surface. Temperature penetration thermocouples or sensors are intended to measure the temperature of the items being processed and should be in contact with the surface of the item itself. The locations of all temperature sensors should be documented, showing the location of the sensor within the chamber or tunnel, within the individual items and within the load itself. Temperature sensors should also be placed next to the recording and controlling temperature sensors in the oven or tunnel where possible.

The biological inactivation portion of validation studies demonstrate that the delivered endotoxin or microbial inactivation has been delivered to the product or items being processed, and that the process is repeatable and reproducible. Using the data obtained from the developmental studies, and the heat penetration and distribution studies, the process is run three times, most often at reduced time or temperature for a batch oven and reduced temperature or increased belt speed for a tunnel. Laboratory testing is conducted to evaluate endotoxin inactivation or microbial lethality, and the studies are documented.

Documentation

Items to be considered in the documentation of the qualification studies include the following:

- A conclusion stating whether the objective of the study has been achieved
- Confirmation that all data collection instruments and equipment were within calibration tolerances during the interval comprising the validation studies
- Testing performed during the studies was properly documented and that test methods were validated where applicable
- Certification for the endotoxin and/or biological indicator used in the studies, i.e., manufacturer, origin of endotoxin/microorganism, inoculum level, etc.
- Sampling and numbers of replicate trials are rational and supported
- Operating parameters, process parameters, and environmental parameters have been met as required
- Any nonconformances, their cause, and resolution have been addressed

- Equipment, processes, and products covered by the qualification studies are identified, whether as a result of inclusion and direct testing in the studies, or through use of a bracketing or matrixing approach, or equivalent determination.

POST-VALIDATION ACTIVITIES

Because of the operational importance of depyrogenation/sterilization processes and the potential for adverse consequences to product quality, continuing evaluation, control, and maintenance of depyrogenation/sterilization cycle performance is critical. Evaluation of depyrogenation/sterilization cycle performance is typically accomplished through data monitoring and periodic requalification. Control is achieved through investigation and resolution of cycle deviations and equipment/process change control. Finally, to ensure maintenance of performance, effective preventative maintenance and calibration programs are essential.

Use of Risk Management Postvalidation

Post-validation activities ensure that the system and processes supporting depyrogenation/sterilization continue to operate as intended and achieve desired levels as required by the production process requirements. These activities encompass requalification and revalidation, which have traditionally been executed on a periodic basis, regardless of historical depyrogenation/sterilization process performance or potential impact to product quality. Many in industry have begun to make use of risk management and statistical process control methodologies to identify those systems that pose the greatest risk based on inherent variability or process capability and concentrate post-validation efforts accordingly. For very capable processes, post-validation activities may be limited to periodic or continual monitoring, depending on the level of automation, with revalidation conducted as an event-driven activity.

Routine Monitoring

Following completion of the cycle development and performance qualification exercises, monitoring of the routine operational cycles should be performed to assure an ongoing state of control. Critical parameters should be documented and data recorded (critical data) for each cycle. Routine monitoring data should be analyzed to ensure the system has remained in a state of control as demonstrated by the qualification data. The routine operational cycle is typically controlled to produce additional lethality over the qualified minimum acceptable cycle to provide increased sterility assurance. Cycles that have not met minimum defined critical cycle parameters should be rejected. Deviations from key parameters should be investigated and their impact assessed to consider whether the cycle is acceptable.

An alarm system for temperature and/or pressure may be used to facilitate the detection of any deviation from the defined process parameters.

OPERATIONAL PARAMETERS

Critical operational parameters may include the following:

- **Temperature**
  Temperature should be monitored using calibrated, redundant, independent monitoring devices with defined accuracy.
  Temperature and pressure profiles for the depyrogenation/sterilization cycles should be recorded and assessed on a periodic basis to confirm that no significant change in the qualified state has occurred.
- **Pressure**
  The system differential pressures should be continuously monitored at appropriate locations.
- **Time**
  Time duration of cycle phases should be monitored to ensure the depyrogenation/sterilization cycle remains within the qualified state.
- **Belt speed (for tunnels)**
  The belt speed should be continuously monitored and recorded.

Strategies for the monitoring of depyrogenation/sterilization parameters and their associated alarms should be designed to provide the appropriate data to demonstrate that the depyrogenation/sterilization process was performed successfully. System monitoring may be automated, manual, or a combination of both, provided that the data obtained is accurate and easily retrieved. The information recorded for each run should be linked to the validation of the cycle. Resumption of a depyrogenation/sterilization cycle following resolution of an alarm condition should ensure that the minimum exposure time is achieved.

Change Control/Revalidation

A robust change control system should be in place to maintain the validated state of the depyrogenation/sterilization process.

Any proposed changes to the depyrogenation/sterilization process (including procedures, hardware, software, cycle configuration, supply utilities, filter types/sizes) should be evaluated to determine the potential effects of those changes on the depyrogenation/sterilization cycle and the extent of requalification/revalidation required to demonstrate that the modified process performs as intended and still meets the applicable acceptance criteria.

Periodic Requalification/Revalidation

A periodic review of the system should be performed to ensure the state of control is maintained and to evaluate the impact of cumulative "minor changes" over the review period.

This review should also include review of performance data from various monitoring sources (e.g., engineering, maintenance, and calibration data) to verify that there have been no adverse trends or drifts away from the baseline performance established during validation. A review of change control documentation should be conducted as part of the requalification/revalidation.

Review frequency should be based on the system's intended use and applicable regulatory expectations. For systems claiming sterilization, requalification may include supplemental thermal and/or biological testing.

Preventative Maintenance Strategy

To ensure consistent system performance, a maintenance strategy should be in place that addresses potential changes in material and component performance because of operation, exposure, and time. In particular, the strategy should take into account how thermal and pressure cycles associated with heat-up, exposure, and cool-down may impact the service life of various components, particularly HEPA filters.

During development of a maintenance strategy, special consideration should be given to polymer replacement practices because of their criticality in maintaining system integrity and their limited lifetime. In general, polymer service life is affected by various operational stresses such as thermal conditions, process frequency, product chemistry, and cleaning frequency.

Within the preventative maintenance program, components that are critical to depyrogenation or sterilization performance should be periodically inspected and/or replaced. The frequency of the preventative maintenance may be determined on the basis of component maintenance history, manufacturer recommendations, or risk evaluation and mitigation.

CALIBRATION STRATEGY

The calibration program should include instruments that are used to control and monitor the cycle. Both the control of the depyrogenation/sterilization cycle and the confirmation of successful cycle completion are dependent on the proper indication and recording of critical operational parameters. Calibration serves as both the means to maintain instrument performance as well as to document proof of performance.

Determination of calibration tolerances and periodicity is determined by instrument capability, history, manufacturer recommendations, and process risk. The impact of instruments found outside calibration tolerances during periodic recalibration evaluations should be investigated. A risk assessment can be used to establish instrument calibration requirements.

CONCLUSION

This chapter has provided a brief review of historical literature and current practices in dry heat depyrogenation and sterilization processes. While not as widely used as other modes of sterilization, dry heat does provide a very effective and reproducible means of sterilization and is a very effective process for inactivating endotoxin.

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12 | Radiation sterilization

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INTRODUCTION

The radiation sterilization industry traces its origin back to over 50 years ago. The first irradiator for commercial sterilization of medical device products came on-line in the United States in the 1950s. Over the intervening 50 years, radiation in the form of high-energy electrons produced by high-power accelerators or gamma rays produced by radioisotopes has been used to terminally sterilize a broad spectrum of medical devices and different types of pharmaceutical products. More recently a third source of radiation; that is, X-ray (bremsstrahlung) radiation has been introduced to the radiation sterilization industry. The proven efficacy of the process and available methodologies to validate a sterility assurance level (SAL) of 10^{-6} has made radiation an attractive alternative for terminal sterilization of many types of products. Because radiation sterilization is classified as a cold process, it also can be used to sterilize heat-labile products. As a final attribute, products that have been radiation sterilized can be released on the basis of certification of the absorbed dose of radiation delivered to the product, that is, dosimetric release. There is no need to conduct post-irradiation sterility testing of the product, thereby expediting time to market for critical products and cost for the sterility tests.

This chapter begins with a discussion of the fundamentals associated with the interaction of radiation with materials. A section is devoted to dosimetry, a critical part of the process, which forms the basis for dosimetric release of product. All three modalities that are presently used for radiation sterilization of products are covered including design and operation of irradiators and control of the irradiation environment. The chapter addresses available methods for setting an acceptable minimum dose to achieve the desired SAL as well as an acceptable maximum dose that ensures the safety and performance of the irradiated product over its lifetime. A final step before routine processing of product, termed "performance qualification," completes the discussion of the radiation sterilization process. The last sections of the chapter address radiation chemistry of liquids and solids, radiation effects, and a final section on irradiation of specific drug products. A more comprehensive discussion of the radiation sterilization process can be found elsewhere (1).

INTERACTION OF RADIATION WITH MATERIAL

Sources of Radiation

Three sources of radiation are used in the radiation sterilization process. The first source of radiation is gamma ray emitting radioactive isotopes. Gamma rays are pure electromagnetic energy in the form of quanta of radiant energy called photons. The energy of the photons is dictated by the radioisotopic source. Two isotopes are used in the radiation sterilization industry with the principal isotope being cobalt-60, which emits two photons per disintegration of the nucleus with energies of 1.17 and 1.33 MeV. The other isotope cesium-137 emits one photon per disintegration at an energy of 0.662 MeV. Cobalt-60 is usually the isotope of choice for commercial applications of radiation processing. It can be manufactured in a metallic form, which is inherently stable and produced in much higher specific activities, that is, approximately 100 curies/g, than cesium-137. Because of its high specific activity; cobalt-60 can be fabricated in compact-energy efficient sources. For example, a single source of about 10,000 curies can be fabricated in a geometry that is about 18 in. in length by less than 0.5 in. in diameter. To achieve the megacurie levels of activity that are used in commercial gamma irradiators, literally hundreds of these sources are used to build the source plane(s) in a commercial irradiator. Cesium-137 has one advantage over cobalt-60 in that its half-life, which is a measure of the rate of decay of the radioactive isotope, is much longer than that of cobalt-60. The half-life of cobalt-60 is 5.27 years and of cesium-137 is 30.17 years. Because of its shorter

half-life, cobalt-60 loses about 12% of its activity in a year, whereas cesium-137 only loses about 2% of its activity in a year. For this reason, isotope replacement in a cesium-137 irradiator can be done on a much less frequent basis than in cobalt-60. This fact favors cesium-137 for use in what are referred to as self-contained irradiators such as blood irradiators that are used to prevent transfusion-induced graft-versus-host disease.

The second source of radiation consists of high-power accelerators that generate high-energy electrons. As we will see these high power accelerators are capable of producing output powers up to several hundred kilowatts. Dependent on the accelerator design, electron energies can range from less than 1 MeV up to about 10 MeV. The third source of radiation occurs when the high-energy electrons from a high-power accelerator impinge on a conversion target. Because the conversion efficiency increases as the square of the atomic number of the target material, conversion targets are fabricated from high atomic number materials, for example, tantalum. The high-energy electrons that impinge on the conversion target are deflected in the field of the nucleus of the atoms in the conversion target and in the process of being accelerated emit electromagnetic radiation in the form of X-rays. This form of electromagnetic radiation is referred to as bremsstrahlung radiation, which translated literally means "braking radiation." The radiation appears as a continuous spectrum of energies with a maximum energy equal to the energy of the incident electrons. The maximum energy for this source of electromagnetic radiation is limited to 7.5 MeV, which is dictated by the need to avoid unwanted radioactivity that could potentially be induced in the irradiated materials via photonuclear reactions at higher photon energies (2).

A common attribute of the three sources of radiation that are employed in the radiation sterilization process resides in the fact that the energy of the incident radiation is sufficient to ionize the atoms that make up the molecules of the materials that are irradiated. In the ionization process, sufficient energy is imparted to the orbital electrons of an atom to remove the electrons from their bound state around the atom. Dependent on the specific element, the energy to remove the outer most electrons, that is, first ionization potential, from the atom ranges from a few eV up to approximately 20 eV. Because the energy of the incident radiation is measured in MeV or millions of eV, sufficient energy is obviously available to initiate the ionization process. For this reason, these radiations are referred to as ionizing radiations and are differentiated from nonionizing radiation such as optical radiation, that is, light, and infrared radiation, that is, heat. The energy of the photons from these two sources of electromagnetic radiation is less than a few eV, which is insufficient to ionize an atom. There is no mystique to the ionization process; it is simply a matter of energetics.

Interaction of High-Energy Photons with Materials

At intermediate photon energies that characterize the gamma ray and X-ray (bremsstrahlung) sources of radiation used in the radiation sterilization process, the dominant channel for interaction of the photons with the orbital electrons occurs via a process called Compton inelastic scattering. This method of energy transfer is named after the person that first described the quantum mechanical relationships governing the scattering process (3). A photon undergoing Compton scattering, transfers part of its energy to the orbital electron. The amount of energy transferred to the electron will depend on the quantum mechanical relationships governing the scattering event, but is usually sufficient to not only ionize the atom but also leave the electron with significant kinetic energy. In fact, the most probable Compton scattering event is a backscatter of the photon, which transfers maximum energy to the electron. For gamma rays emitted by a cobalt-60 source, a backscattered photon will deliver about 1 MeV to the orbital electron. These high-energy electrons are referred to as primary electrons. The scattered photon continues to undergo scattering events and generate additional primary electrons until its energy is dissipated. The primary electrons have sufficient energy to ionize other atoms via an electron-electron inelastic scattering process. A whole cascade of secondary electrons can be produced in this manner. From a numerical standpoint, it is these secondary electrons that are the source of the physical and chemical events that lead to the radiation-induced changes in materials and sterilization of the drug product. The photons function only as an initiator of the process that leads to radiation sterilization throughout the bulk of the drug product.

Interaction of High-Energy Electrons with Materials

For most pharmaceutical products and low atomic number materials that make up the bulk of medical device products, high-energy electrons from an accelerator mainly lose energy in the material via a large number of inelastic scattering events with the orbital electrons. The energy loss per collision is relatively low compared to the energy of the incident electron. For example, the average energy loss per scattering event for a 10 MeV electron is on the order of 100 eV per collision or less (4). It is of interest to note that the energy lost by primary electrons that are produced by Compton scattering of photons occurs in the same manner. Therefore, regardless of the modality of the incident radiation, the energy transfer mechanisms that lead to the sterilization of pharmaceuticals and changes in material properties are the same. The effects of high-energy photons are indistinguishable from those produced by the same amount of energy per unit mass (absorbed dose) imparted by high-energy electrons. This equivalence of effects is the basis for the use of both forms of radiation in radiation processing. However, the rate of energy deposition for the different sources of radiation can be quite different, and this parameter may play an important role in the resultant effect on materials that are irradiated. This topic will be discussed in a subsequent section of the chapter.

RADIATION-ABSORBED DOSE AND MEASUREMENT

Definition of Absorbed Dose

Energy must be absorbed by a material to cause change be it sterilization of a drug product or change in a material property. Energy from the incident radiation is transferred to the material by various pathways that are discussed in the previous section. The energy that is absorbed in a material from radiation exposure is termed absorbed dose. It is defined as the quantity of ionizing radiation energy imparted per unit mass of a specified material (5). The SI unit of absorbed dose is the gray (Gy), where 1 gray is equivalent to the absorption of one joule per kilogram of the specified material (1 Gy = 1 J/kg). The previous unit that was used to measure absorbed dose was the rad, which is no longer in use nor recommended (100 rads = 1 Gy). It is of interest to note that absorbed dose is defined in terms of a specified material. For example, two different materials could be exposed to the same incident radiation field yet receive different absorbed doses. Absorbed dose is measured with well-characterized devices called dosimeters and dose is normally recorded as dose delivered to the dosimeter. The standard material in which absorbed dose is usually expressed is water. Many dosimeters that are commonly used to measure absorbed dose have energy absorption characteristics that are water equivalent so absorbed doses are effectively reported in terms of absorbed dose in water. In radiation sterilization applications that involve the biocidal action of radiation on microorganisms, the difference in absorbed dose between microorganisms and water is relatively small. However, this may be a mute point because the same dosimeters that are used to measure absorbed dose during routine processing of a product are oftentimes used to validate the acceptable minimum and maximum doses for irradiation of the product.

Dosimetry—A Critical Part of The Process

Absorbed dose is a critical parameter that impacts the radiation process from its beginning to its end. Dosimetry, that is, measurement of absorbed dose, enters the radiation process during operational qualification (OQ) of an irradiator, which occurs before the pharmaceutical product is irradiated (6). Studies conducted during OQ demonstrate the capability of the irradiator to deliver the range of doses required for the sterilization process that has been previously specified. OQ demonstrates that the irradiator, as installed, is capable of operating and delivering appropriate doses within defined acceptance criteria. As a first step in the radiation sterilization of a pharmaceutical product you need to determine an acceptable minimum dose that ensures the specified SAL is satisfied and an acceptable maximum dose that ensures the safety and performance of the drug product over its lifetime. Established methodologies that involve a matrix of test irradiations are used to validate an acceptable minimum dose. Accurate measurement of absorbed dose delivered to test samples is a critical part of this validation program. An acceptable maximum dose is determined by irradiation of test samples at specified absorbed doses and post-irradiation analysis of the test samples. Doses need to be delivered to the test samples in a precise manner, which requires accurate

measurement of the absorbed doses. Following validation of acceptable minimum and maximum doses, the pharmaceutical product goes through another step before routine processing, which is called performance qualification (PQ) (6). In PQ, the product is loaded into the irradiation containers in accordance with a specified loading pattern, and absorbed dose is measured at prescribed locations in the product load. The information from this dose map is used to identify the location and magnitude of the minimum and maximum doses. Upon completion of the PQ study, the product is ready for routine processing. During routine processing, absorbed dose is measured at various locations in the run to confirm that all product in the run received the minimum absorbed dose and no product in the run exceeded the established maximum absorbed dose. As a final step, the absorbed dose delivered to product along with its certification is used to release the product. This process is referred to as dosimetric release. With dosimetric release there is no requirement or need to perform post-irradiation sterility testing. This topic is discussed later in the chapter.

Because of the importance of absorbed dose in the overall radiation sterilization process, we obviously need to have a quantitative tool for its measurement. Furthermore, the measurements need to be accurate and we must be confident in the measurement results. The quantitative tool that meets these requirements is called a dosimeter and is defined as a device that, when irradiated, exhibits a quantifiable change that can be related to the absorbed dose in a given material using appropriate measurement instruments and procedures. A key word in the definition of dosimeter is "quantifiable." Dosimeters are highly characterized and calibrated devices. Dosimeters are only one part of the measurement system, which is referred to as the dosimetry system. In addition to the dosimeters, you require a calibrated instrument for measuring the dosimeter response as well as standards and procedures. A dosimetry system is defined as a system used to measure absorbed dose, consisting of dosimeters, measurement instruments with associated reference standards, and procedures for the system's use.

Method of Measurement

The dosimetry systems that are used in the radiation sterilization industry are divided into various classes dependent on where they fit in the metrological hierarchy and field of application. Reference standard dosimetry systems are of high metrological quality and are used to calibrate the dosimetry systems that are used for routine measurements of absorbed dose at an irradiator. This class of dosimetry systems may be held at a given location, that is, irradiator site, or take the form of transfer standard dosimetry systems operated by a national standards laboratory or an accredited dosimetry calibration laboratory. Transfer standard dosimetry systems are sent to an irradiator for irradiation and then returned to the calibration laboratory for measurement. The concept of high metrological quality implies a dosimetry system with low uncertainty and traceability to appropriate national or international standards. A routine dosimetry system, which is used for routine measurements of absorbed dose at an irradiation facility, is calibrated against a reference standard dosimetry system. The dosimeters that are used for calibration purposes have high metrological quality and form a separate class of dosimeters from routine dosimeters that are used to measure absorbed dose at an irradiator. Routine dosimeters are still highly characterized and calibrated devices that provide accurate measurements of absorbed dose. Tables 1 and 2 provide examples of dosimeters that are used to calibrate other dosimeters and for routine measurement of absorbed dose (7).

Table 1 Dosimeters for Calibration Applications

Dosimeter	Description	Radiation induced effect	Method of analysis
Alanine	Pellet or film containing alanine, an amino acid	Generation specific stable free radicals	Analysis of radiation induced free radicals using electron paramagnetic resonance (EPR)
Ceric cerous sulphate	Ceric sulphate and cerous sulphate in sulphuric acid	Change in optical absorbance or electropotential	Spectrophotometry or potentiometry

Note: The cited dosimeters are also used for routine measurement of absorbed dose.

Table 2 Dosimeters for Routine Measurement of Absorbed Dose

Dosimeter	Description	Radiation induced effect	Method of analysis
Calorimeter	Mass of energy absorbing material, thermal insulation and calibrated temperature sensor	Increase in temperature	Temperature measurement
PMMA (Perspex)	Calibrated chip of PMMA in sealed sachet	Color, darkening of dyes	Spectrophotometry
Radiochromic film	Thin film containing special dye precursors	Dyes becoming colored	Spectrophotometry

IRRADIATOR ENVIRONMENTS

Gamma Irradiators

Irradiator Categories

Nuclear regulatory agencies have divided gamma irradiators into four categories according to their design and operation. Because only two of the four categories may find significant application for irradiation of pharmaceuticals, the discussion is limited to these categories. Category I irradiators are self-contained, dry source storage irradiators. This category irradiator was noted in the section on the interaction of radiation with material as a possible source for blood irradiation. The design of category I irradiators typically does not allow a large volume of product to be irradiated over a given period of time. Irradiation of blood as well as some types of drug products fit that product profile. Other possible applications for category I irradiators may include irradiation of test product, clinical studies, research, dose validation, and calibration. The radioactive source in category I irradiators remains shielded inside a biological shield at all times, and it is not possible for an individual to come in contact with the source at any time. For this reason, the regulatory agencies treat this category irradiator differently from the other category irradiators. Category I irradiators are relatively small, that is, typically less than several feet in diameter and several feet in height, and could easily fit in the space that normally serves as a room in a laboratory. In fact, if you were to visit a national calibration laboratory such as the one at the National Institute of Standards and Technology (NIST) in Washington, DC, you could find gamma cells, which are a type of category I irradiator, sitting in a laboratory and being used to calibrate dosimetry systems. Category IV irradiators are panoramic, wet source storage irradiators, which are designed for high-throughput operation. When not in use, the sealed gamma source is stored in a large pool of water within a shielded room that is referred to as the cell. When all personnel have safely exited the cell and a safety system is activated, the sources are automatically removed from the pool of water into the room and irradiate product that is within the cell. Because people can enter the room where the sources are stored and used, regulations for operation of category IV irradiators are more stringent than for category I irradiators.

Dependent on the mission, the design and operation of category IV irradiators can vary significantly. However, there are several common features that will be found in all category IV irradiators. First you need a source of ionizing radiation that comes in the form of a radioisotope, usually cobalt-60 that is doubly encapsulated to form sealed sources. Sources of this type are grouped into racks that are stored in a pool of water inside a shielded room called the cell. Because the radiation levels to kill microorganisms are typically 1000 times greater than the levels to kill individuals, you need a biological shield that surrounds the cell. The shield typically comes in the form of concrete walls and ceiling approximately 6 ft in thickness. However, as a means to reduce the size of the cell, the biological shield is sometimes metallic in nature. Of course, you need a redundant safety system to protect personnel and preclude entry to the cell when the sources are exposed. Category IV irradiators are typically high-throughput systems; that is, some are capable of processing several million cubic feet of product per year, and for this reason you need a conveyance system that is capable of moving large volumes of product into and out the cell on a routine basis. A control system that usually takes the form of a programmable logic controller is required and lastly an air exchange system is required to

remove ozone from the cell that is produced from interaction of the gamma rays with oxygen molecules in the air.

Operation of Category IV Irradiators

A conveyance system moves product through the irradiator in various size containers that depend on the design and mission of the irradiator. For example, these containers may consist of aluminum or stainless steel boxes called totes, carriers, or possibly entire pallets of product. Dependent on the irradiator design, totes may vary in length and height from a few feet up to several feet. The width of the tote, which is the dimension through which the gamma rays must penetrate, is typically 2 ft or less in size. A carrier may have a footprint similar to a tote but be several feet in height. Some carriers have a single shelf with a limited volume for irradiation. In some irradiators, an entire pallet of product is loaded onto the conveyance system as an entity. Regardless of the size and design of the irradiation container, most gamma irradiators move product through the cell in what is referred to as a "shuffle-dwell" principle. In a shuffle-dwell operation, the irradiation containers shuffle to a location in the cell where they accumulate in rows that surround the source plane. They dwell at that location for a preset time called the cycle time after which they shuffle to the next location and repeat the operation until the container has fully traversed the cell.

Category IV irradiators are designed to operate in a batch mode or continuous mode. In a batch mode, the irradiation containers are loaded with product and moved into the cell, where they are positioned around the source location. After this operation is completed, the cell is exited, the safety system set, and the source raised into the cell room. The irradiation containers then proceed to increment around the source in a shuffle-dwell mode until the product has received the required dose. The source is then lowered into the pool of water, and the irradiation containers removed from the cell. In a continuous mode of operation, the irradiation containers continuously move into and out of the cell while the source(s) is in the exposed position. This mode of operation can be accomplished by moving the irradiation containers through a maze before entry to the cell. Figure 1 shows a tote box irradiator that operates in a continuous mode. Up to 5 MCi of cobalt-60 can be loaded into this irradiator, so it is a high-throughput system. The irradiation container is approximately 3.5 ft in length by 6 ft in height by 2 ft in width. The irradiation containers are moved into the cell through a maze via a floor conveyer. Once in the cell, the totes accumulate around the source plane and proceed to increment around the source in a shuffle-dwell mode. A cut-a-way of the biological shield and air exchange system also can be seen in Figure 1.

It is of interest to note that in Figure 1 the totes totally surround the cobalt-60 source. This is due to the fact that the radiation field is isotropic in nature; that is, the gamma rays are emitted in all directions from the cobalt-60 source. For this reason, it is important to surround the cobalt-60 source with product containers thereby capturing as many of the source photons as possible and in the process increase the intrinsic efficiency of the irradiator. Because of the size of the tote and volume of product in the tote, not all product in the tote will receive the

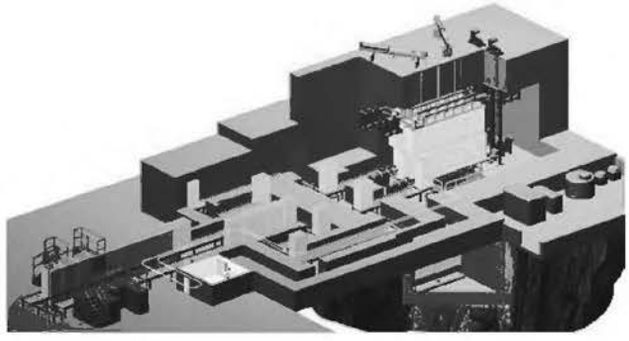

Figure 1 Gamma tote box irradiator.

same absorbed dose. This is due to shielding by the product, that is, absorption of gamma rays by the product, and geometric attenuation, which is due to the fact that the radiation field is isotropic. Even in an empty tote, the absorbed doses are different at various locations within the tote due to the different distances from the source. A similar effect can be noted from the isotropic emission of optical radiation from a light bulb. The ratio of the maximum absorbed dose to minimum absorbed dose delivered to product in the tote is referred to as the dose uniformity ratio (DUR). There are obvious advantages to keeping this ratio as close to one as possible. Techniques for doing so are discussed in the section on methods of control.

Electron Beam Irradiators

Design

A high-power electron beam accelerator is at the heart of an electron beam irradiator. The accelerator serves as the source of radiation analogous to gamma rays in gamma irradiators. The different types of electron beam accelerators that are used in the radiation sterilization share one common attribute, which is high-output power. Power equates to throughput, and electron beam irradiators similar to gamma irradiators are capable of processing millions of cubic feet of product per year. Electron beam irradiators share many of the same design features as gamma irradiators. You need a biological shield to protect individuals from the high levels of radiation that exist in the cell when the accelerator is operational, a conveyance system to transport product in front of the beam of electrons and a safety system that precludes entry to the cell when the accelerator is operational. In addition you need a system for controlling the irradiator and an air recirculation system to remove ozone from the cell. An example of an electron beam irradiator is shown in Figure 2. In this type of accelerator, electrons are accelerated in a resonant cavity that is cylindrical in geometry. This accelerator design is capable of very high-output powers and dependent on the port from which the electrons are extracted can deliver different energy electrons up to 10 MeV. A cut-a-way of the biological shielding is shown in Figure 2 along with the conveyance system that moves product via a floor conveyor under the electron beam. Because electrons are charged particles, they can be steered and directed using magnetic fields. In Figure 2, the electrons exiting the accelerator are defected 90° and steered to the product that is moving on a floor conveyor in a room below the accelerator. Because the electron beam exiting an accelerator is typically only a few centimeters in diameter, the beam needs to be scanned in a transverse direction to the motion of the product on the conveyance system thereby ensuring high-energy electrons uniformly irradiate the entire product surface. Magnetic fields can be used to deflect the beam using a device called a scan horn. Beam scan and conveyor motion need to be synchronized to ensure all parts of the product are irradiated. The irradiator shown in Figure 2 only represents one type of electron beam accelerator and conveyance system that is used to irradiate product. For example, linear accelerators called Linacs may be used as the source of high-energy electrons and product conveyed in carriers horizontally in front of the beam of electrons. Dependent on the application and mission of the irradiator, other configurations are also possible.

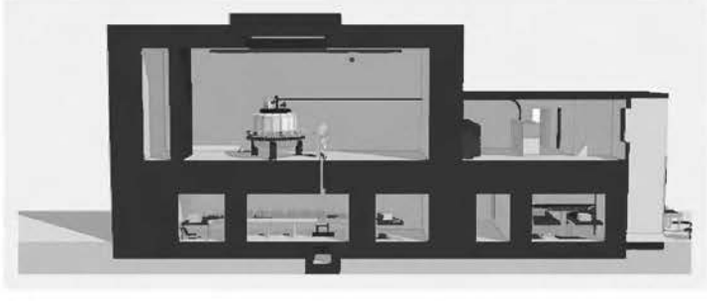

Figure 2 Rhodotron electron beam irradiator.

Operation of Electron Beam Irradiators

Unlike the isotropic radiation environment in a gamma irradiator, the radiation environment in an electron beam irradiator is in the form of a beam that can be steered and directed using magnetic fields. These are nice attributes that can be used to optimize the intrinsic efficiency of the irradiator. Because the radiation emitted by an accelerator is in the form of a beam of radiation, it is only necessary to move the product in a controlled manner in front of the beam; that is, you don't need to surround the accelerator with product as is done in a gamma irradiator. The unit of product that is moved in front of the beam may be in the form of a single box or possibly an entire carrier of product. It has sometimes been noted in the literature that processing time in an electron beam irradiator is much faster than that in a gamma irradiator. This may be true for a single box of product because of the method that is used to convey product through the irradiator. In an electron beam irradiator the box of product is effectively scanned in front of the beam, whereas in a gamma irradiator it needs to be placed in an irradiation container and incrementally moved in a shuffle-dwell method through the entire cell. In one case, the box of product can be literally processed in seconds while in the other case process time can take up to a few hours. However, for large volumes of product, that is, truckload quantities, the output power of the irradiator is the cogent parameter. In any event, it may be somewhat of a mute point given the fact that ship time and queue time often control turn time.

Penetration of the radiation into the target material is more of a concern with high-energy electron than photon irradiation. The mean-free path for the gamma rays from cobalt-60 are more than an order of magnitude longer than the equivalent mean-free path for 10 MeV electrons. The photons produced in an X-ray (bremsstrahlung) irradiator have a mean-free path at least equivalent to cobalt-60 photons. In a gamma or X-ray (bremsstrahlung) irradiator, the high-energy electrons are created internally within the target material via a Compton scattering process whereas in the case of high-energy electrons from an accelerator the electrons effectively need to be driven into the target from the outside. A technique that is frequently used to increase the penetration depth is referred to as two-sided irradiation. In this method, the product is first scanned in front of the beam from one side and scanned from the other side in a subsequent pass. This method has been effectively used for processing a variety of products with bulk densities less than a few g/cc. For higher bulk densities or heterogeneous products that may contain localized high-density regions, special processing techniques may be required.

X-Ray Irradiators

Design

X-ray irradiators contain all the features of a high-power electron beam irradiator and in addition have a target that converts the high-energy electrons into photons (bremsstrahlung radiation). As is the case in gamma irradiators and electron beam irradiators, X-ray irradiators require a biological shield, a conveyance system for transporting the product in front of the beam of X-rays, a control and safety system as well as an air recirculation system. An example of an X-ray irradiator, which is the only X-ray irradiator presently operational in the United States, is shown in Figure 3. Because the conversion efficiency of high-energy electrons to X-ray (bremsstrahlung) radiation is relatively low, that is, 8% for 5 MeV electrons on a Tantalum target and 12% for 7.5 MeV electrons on the same target, very high power electron beam accelerators are required to generate sufficient X-ray output for commercial applications. In Figure 3, the source of electrons is a 190-kW Rhodotron. Dependent on the exit port from which the electrons are extracted from the accelerator, the energy could be 5 MeV or 7.5 MeV, which is the reason two scan horns are shown in Figure 3.

Operation of X Ray Irradiators

As in the case of electron beam irradiators, in X-ray irradiators we are dealing with a beam of radiation rather than an isotropic radiation environment. However, unlike electrons, the forward directed beam of X-rays emanating from the conversion target cannot be steered or directed. In addition, the beam of X-rays has a small angular divergence, which is dependent

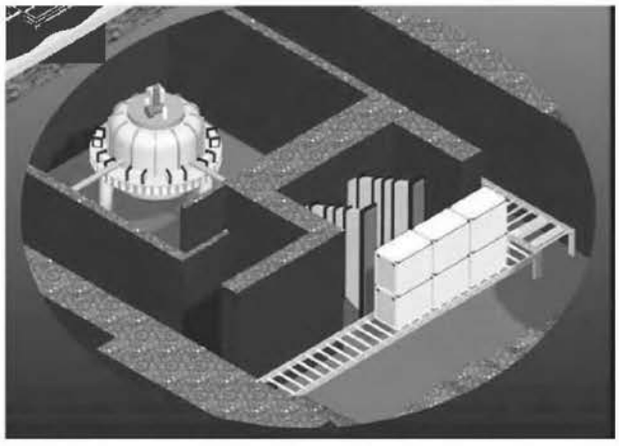

Figure 3 X ray irradiator.

on the energy of the photons and needs to be taken into account in the design of the conveyance system that moves product in front of the beam. Because the radiation environment consists of high-energy photons, penetration of the radiation into the target is not the issue that it is in electron beam irradiators. In fact, the radiation in an X-ray irradiator is effectively more penetrating than the photon radiation in a gamma irradiator. For this reason, as seen in Figure 3, product may be transported in large carriers in front of the beam of radiation. In fact, because of the highly penetrating nature of the X-ray (bremsstrahlung) radiation, X-ray irradiators have been designed to process entire pallet loads of high-density product. Because of the directional nature of the radiation field in an X-ray irradiator, it is not necessary to surround the source with product, as is the case in gamma irradiators, but to increase the intrinsic efficiency of the irradiator; additional rows of carriers may be conveyed in front of the beam.

CONTROL OF THE IRRADIATION ENVIRONMENT

Absorbed Dose and Dose Rate

Absorbed Dose

Absorbed dose is the amount of energy absorbed per unit mass of material. It controls how a material will respond to being irradiated. In gamma irradiators, product is loaded in irradiation containers and moved through the cell in a shuffle-dwell mode of operation. The dwell period is controlled by a preset cycle time. Increasing the cycle time increases the time the irradiation container remains in the cell and is exposed to gamma rays from the source. All other things being equal, a longer resident time in the cell will obviously lead to a higher absorbed in the product. Changing the cycle time is a standard method for changing the absorbed dose delivered to product. Some irradiators offer more than one product path through the irradiator, which allows different absorbed doses to be delivered to the product even at the same cycle time. For example, the tote box irradiator in Figure 1 offers the user an option of using only the outer two passes for incrementing the totes through the cell rather than using all of the four passes that are available. The amount of isotope loaded into the source plane(s) will also dictate the amount of absorbed dose delivered to product at a given cycle time. For electron beam irradiators and X-ray (bremsstrahlung) irradiators, adjustment in the speed of the conveyor system that moves product in front of the beam is a principal method for controlling the amount of absorbed dose delivered to product. The amount of absorbed dose delivered to product also can be adjusted by simply changing the output current of the accelerator. The previous methods allow a wide range of absorbed doses to be delivered to products even within the same irradiator.

Dose Rate

In addition to the amount of absorbed dose delivered to a product, the rate at which energy is delivered to the product may play an important role in its response to the incident radiation.

For this reason, it is important to understand the key parameters affecting dose rate and methods for controlling the dose rate. Dose rate is a function of two parameters. The first of these parameters is the incident power density that is given in units of w/cm^2. In a gamma irradiator, output power is dictated by the amount of isotope that is loaded into the source plane(s) where one megacurie of coblalt-60 equals 14.7 kW of power. Because of the isotropic nature of the radiation field in a gamma irradiator, the power density in w/cm^2 will depend on the distance from the source; that is, the w/cm^2 decreases with distance from the source. In electron beam irradiators and X-ray irradiators, where the radiant energy is delivered in the form of a beam of radiation, the power density will depend on the output power of the accelerator or conversion target and the area over which the power is delivered to the target. The second parameter that controls dose rate is the mass absorption coefficient of the target material. If the mass absorption coefficient is very high, all of the incident radiation will be absorbed in a relatively thin layer of material versus a much thicker layer of material for a low mass absorption coefficient. At a given incident power density, a high mass absorption coefficient will lead to a higher dose rate than a low mass absorption coefficient. The equation for dose rate is given as the product of the preceding two variables.

$$\text{Dose rate} = D(t) = 3.6 \times 10^3 P_A \times \mu_\rho (\text{kGy/hr}) \tag{1}$$

In equation (1), the numerical factor of 3.6×10^3 converts w/g to kGy/hr, P_A is the incident power density in w/cm^2 and μ_ρ is the mass absorption coefficient in cm^2/g.

At equivalent output powers, gamma irradiators have the lowest dose rates, X-ray irradiators higher dose rates, and electron beam irradiators the highest dose rates. By way of comparison, if the dose rate in a gamma irradiator were normalized to 1, dose rate in an X-ray irradiator would be approximately 10 or more, and dose rate in an electron beam irradiator would be greater than 100. For a given modality of irradiation, various methods are available for controlling the dose rates that are delivered to a product. For example, decreasing the output power of the irradiator offers one method for reducing the dose rate. In the case of a gamma irradiator, this would entail loading less isotope in the source plane(s), and for electron beam and X-ray irradiators it could be accomplished by simply dialing down the current of the accelerator. Placement of a shield between the source and target is another method for reducing the dose rate. In a gamma irradiator one can take advantage of the isotropic nature of the radiation field and simply move the product further from the source, which will reduce the power density incident on the target. If dose rate is considered an important parameter in the irradiation of a specific pharmaceutical product, selection of the modality of radiation that best meets the dose rate requirements should be taken into account at an early point in the sterilization project.

Dose Range—DUR

Because of the finite size of a product unit, that is, individual box, tote or carrier, that is transported through an irradiator and shielding by the product itself, all product within a product unit will not receive the same absorbed dose. The product unit will receive different absorbed doses ranging from a minimum absorbed dose up to a maximum absorbed dose. The ratio of maximum to minimum dose is referred to as the dose uniformity ratio or DUR. To satisfy technical criteria for irradiation of the product, no less than the minimum dose must be delivered to the product unit. However, absorbed doses in excess of the minimum dose are not required and in fact if the maximum dose is too high, it may lead to unacceptable degradation of the product. Therefore, it is desirable to keep the DUR as close to one as possible while still allowing product to be processed in an efficient manner. There are various methods for controlling the DUR and range of absorbed doses delivered to product. In this regard, selection of the modality for irradiation, that is, gamma, electron beam, X-ray, should be taken into account during the initial evaluation of the methodology for irradiation of your product. For high-density products and those products that are highly heterogeneous in nature, photon radiation whether gamma or X-ray may be preferred to high-energy electrons. As previously noted, the radiation mean-free path for the photon energies used in the irradiation industry are more than an order of magnitude greater than the radiation mean-free path of 10 MeV

electrons; that is penetration of gamma and X-ray radiation into the product unit is of less concern than for high-energy electrons.

DUR and Gamma Sources

Because of the isotropic nature of the radiation field in gamma irradiators, the DUR depends not only on product shielding but also the geometry of the product unit. Gamma rays are a highly penetrating source of radiation. The penetration of high-energy photons in materials is described by the product of an exponential factor and a semiempirical buildup factor that accounts for scattering of the photons. In lower density materials, gamma rays easily penetrate through a large thickness of material, and shielding is not a dominant factor in the resultant DUR rather it is geometric attenuation. One method of decreasing the effect of geometric attenuation on dose distribution and the resultant DUR is to increase the standoff distance of the product unit from the source. As noted earlier, this option is available for the tote box irradiator shown in Figure 1. Product can be transported around the source plane in the outer two passes only, which significantly increases the standoff distance of the totes from the source plane, thus reducing the impact of geometric attenuation on dose distribution. In irradiators that are designed for precision dosing of product, such as those used in dose validation studies, the irradiation containers are typically offset further from the source than the standoff distance found in production irradiators. Use of lightweight metal framing for the carrier structure also can be used to reduce the effect of shielding by the carrier and resultant effect on the DUR. In category IV irradiators, it is standard practice to equally expose both sides of the irradiation container to the source(s). Figure 4 shows the four-pass product path of a tote though the irradiator shown in Figure 1. As seen from this figure, at the completion of the process cycle both sides of the tote have been exposed to equal amounts of radiation. The symmetric pattern of irradiation shown in Figure 4 also allows the DUR to be decreased by a method called center loading. On the basis of this method, the product is not loaded over the entire width of the irradiation container, rather the width of the product is reduced to a dimension less than the irradiation container width and the product is centered in the irradiation container along its mid-plane. This serves two purposes, first it reduces the amount of shielding due to the reduced target width and second you move the outer surfaces of the target further from the source, which reduces the effect of geometric attenuation on the DUR. Center loading and increasing the standoff distance from the source are but two methods that can be used to reduce the dose spread in the product unit and control the DUR.

DUR Electron Beam and X Ray Sources

Because of the directional nature of the radiation environment in an electron beam irradiator, the effect of geometry on the DUR is less important than in gamma irradiators. However, because of the much higher mass absorption coefficient of high-energy electrons in materials than that of high-energy photons, shielding and its effect on the DUR is a more important

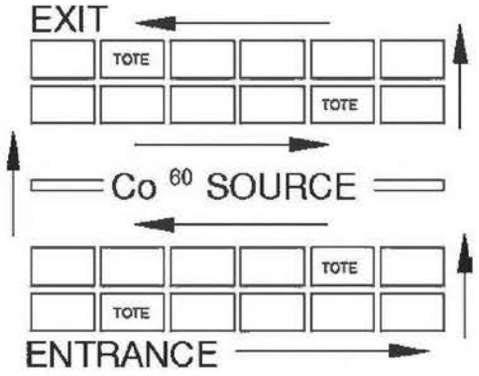

Figure 4 Product flow tote box irradiator.

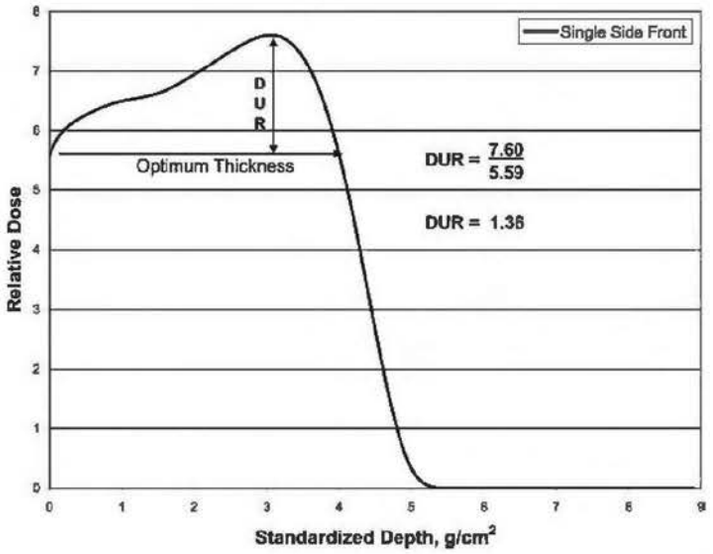

Figure 5 Depth dose profile single sided irradiation.

consideration in electron beam irradiators than in gamma or X-ray irradiators. In homogeneous materials, the depth-dose profile of high-energy electrons in materials is a well-characterized parameter. An example of the depth-dose profile for a beam of 10 MeV electrons incident on a plastic target is shown in Figure 5. The fact that the absorbed dose is greater inside the target than at the surface where the electrons are incident is due to scattering of the electrons as they penetrate deeper into the target. The precipitous falloff in absorbed dose at deeper penetrations into the target occurs after the electrons have given up most of their energy in inelastic scattering collisions. The target thickness is clearly limited by the need to maintain an acceptable DUR. The optimum thickness occurs when the exit dose equals the entrance dose. The horizontal arrow in Figure 5 indicates this thickness and the vertical arrow represents the DUR for single-sided irradiation at the optimum thickness. A standard method for significantly increasing target thickness while maintaining an acceptable DUR is to equally irradiate both exterior surfaces of the target to the beam of high-energy electrons. The result of this two-sided irradiation is shown in Figure 6. Superposition of the dose profiles from irradiation of the two exterior surfaces of the target leads to a DUR that is actually the same as the DUR for the optimum thickness and single-sided irradiation. Other more imaginative methods may be used to reduce the DUR. For example, the product can be displayed in a planar geometry, and the DUR further reduced using metal scatter plates (8).

Of the three modalities for irradiation, an X-ray irradiator offers the potential of delivering the most uniform dosing to product. Dependent on the maximum energy of the X-rays, the mass absorption coefficient can be less than that of cobalt-60 photons. For this reason, shielding is less a concern in X-ray irradiators than in gamma irradiators. In addition, because of the directional nature of the radiation field, geometry is not as important as in gamma irradiators. However, geometrical effects will come into play at boundaries of the product unit due to the beam properties of the X-ray radiation. These so-called edge effects need to be taken into account in controlling the DUR. One method for reducing the effect of edge effects on DUR is to add scatter material at the product unit boundaries. In this manner, photons can also scatter into the material as well as out of the material.

Temperature

The increase in temperature of irradiated products is dependent on three basic parameters. The first of these parameters is the energy absorbed per unit mass of material. Because the energy

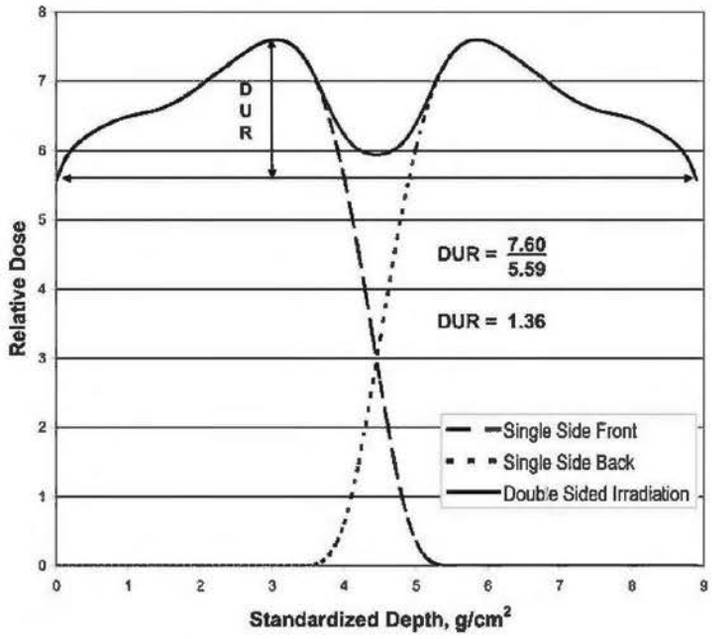

Figure 6 Depth dose profile two sided irradiation.

absorbed per unit mass of material is equal to absorbed dose, it follows that higher absorbed doses should lead to higher excursions in temperature. The second parameter that has an effect on the change in temperature is the rate at which the energy is deposited, that is, dose rate. At high dose rates the material may not have sufficient time to thermally relax, thus leading to higher temperatures in the irradiated product. The last parameter that can significantly affect product temperature is related to the thermal properties of the irradiated material. For equivalent irradiation conditions, materials that are good conductors of heat with similar specific heats should experience a smaller increase in temperature than materials with high thermal resistance. Let's consider each of these parameters and its potential effect on product temperature.

Dose and Dose Rate

The absorbed dose delivered to a product will depend on the minimum dose that is required to achieve the desired SAL and the DUR, which controls the maximum dose delivered to product. As we will see in the section on establishing the sterilization dose, the different methodologies for establishing a minimum dose are bioburden driven, that is, dependent on the initial bioburden. For this reason, a lower initial bioburden on a unit of product translates into a lower minimum dose to achieve the desired SAL. A lower absorbed dose equates to a lower amount of energy absorbed per unit mass and a smaller increase in temperature. At high dose rates the irradiated material does not have an opportunity to thermally relax and essentially behaves in an adiabatic manner. In adiabatic heating the change in temperature is given by the following relationship:

$$\Delta T = \frac{D}{c} \tag{2}$$

In equation (2), D is the absorbed dose and c is the specific heat of the irradiated material.

As an example, consider the case where a minimum dose of 25 kGy is delivered to a product and the DUR is 2, which equates to a maximum dose of 50 kGy or 12 calories/g. If the specific heat of the irradiated material is approximately 0.5 calories/g-°C, which is a

representative number for the types of materials being irradiated, the radiation-induced increase in temperature is 24°C. If the ambient temperature is approximately 30°C, the product temperature in this example could reach 54°C. Therefore, given the right conditions, significant increases in temperature can occur in irradiated products. For some products such as proteins where temperature could have significant impact on the process, an increase in temperature of this magnitude could have an important effect on the response of the protein. The possibility of adiabatic heating is greater in high dose rate environments, for example, electron beam, than low-dose rate environments, for example, gamma.

There are various ways to control the temperature and mitigate its effect on the irradiated product. The first is to irradiate the product at a lower dose while still achieving the desired SAL. The second method of control is to irradiate the product in a low dose rate environment. If it is necessary to irradiate the product in a high dose rate environment, delivering the dose in segments; thus allowing the material to thermally relax between dose deliveries offers one possibility for reducing the increase in product temperature. An additional consideration would involve refrigeration of the product so the initial temperature is below the ambient value.

Thermal Properties

A key thermal property that controls the temperature of irradiated materials is the thermal diffusivity of the material. This parameter, which can be extracted from the heat conduction equation, is

$$\alpha = \frac{k}{\rho c} \tag{3}$$

In equation (3), k is the thermal conductivity of the material, ρ the materials density, and c its specific heat. The unit of thermal diffusivity is cm^2/sec, which is a measure of the rate at which a heat front moves through a material. Because the thermal diffusivities of typical pharmaceutical products and medical devices are relatively low; that is, they are not good conductors of heat such as metals, the rate of diffusion of thermal energy from the region being irradiated is normally quite low. Although little can be done to remedy this condition, it may be possible to enhance heat flow by appropriate selection of packaging materials and other materials that may surround the product unit. In this regard, removal of packing material such as Styrofoam that may encase the product or replacement with a more efficient heat conducting material may be beneficial.

ESTABLISHING THE STERILIZATION DOSE AND MAXIMUM DOSE

Inactivation of Microorganisms

The biocidal effect on microorganisms from exposure to radiation is well documented (9,10). The number of surviving microorganisms decreases with increase in the absorbed dose. The dose survivor curve, which plots survivors versus dose, can take on different shapes, but a common shape obeys first-order kinetics and follows an exponential decrease in surviving microorganisms with dose (11). A key parameter that characterizes the dose-survivor curve is the incremental dose that is required to reduce the number of survivors by one log or a factor of 10. This parameter is referred to as the D_{10} value, and for microorganisms that typically reside on pharmaceutical products and medical devices, D_{10} values range from less than 1 kGy up to several kGy. It is of interest to note that because of the exponential behavior of the dose-survivor curve, absolute sterility is not achievable; that is, no survivors can only be approached in an asymptotic manner. For this reason, sterility is expressed in terms of a sterility assurance level or SAL, which is defined as the probability of a single viable microorganism occurring on a product after sterilization. A commonly accepted SAL that defines a sterile product is 10^{-6} or one chance in a million of finding a viable microorganism on a unit. The total absorbed dose to achieve an SAL of 10^{-6} is a function of the D_{10} value, which defines the slope of the dose-survivor curve, and the initial bioburden that is present on the product unit. Both of these parameters depend on the manufacturing conditions, which define the type and level of microbial contaminants on the manufactured unit. As we will see in the section on establishing

the sterilization dose, the methodologies for setting minimum dose are bioburden driven and for this reason a product that is manufactured under clean conditions with low initial bioburden may be terminally sterilized at a lower minimum dose than products with higher initial bioburdens.

Establishing the Sterilization Dose

During the early years of the radiation sterilization industry, a minimum dose of 25 kGy was generally considered sufficient to achieve an SAL of 10^{-6} (12). The adequacy of this minimum dose was contingent on adherence to good manufacturing procedures. Selection of a 25-kGy minimum dose is still used in some instances (13). In the late1970s, a North American Working Group was convened under the auspices of the Association for Advancement of Medical Instrumentation (AAMI) to develop guidelines for controlling the sterilization of medical devices by radiation. Part of the work of this group included the development of methods for establishing the sterilization dose. Two methodologies, subsequently referred to as Method 1 and Method 2, flowed out of the work of this group. These methods are bioburden driven, that is, dependent on the initial bioburden and, dependent on the level of bioburden present on the unit of product, allow sterilization doses less than 25 kGy, which may be beneficial for terminal sterilization of drug products. A discussion of these methods was first included in the Proceedings of the Second International Kilmer Memorial Conference on the Sterilization of Medical Products (14). These methods are now embodied in ISO standards (15). Over the nearly 30 years since they were first developed, these dose-setting methods have been successfully used to terminally sterilize a broad spectrum of medical device and pharmaceutical products. A more recent method referred to as Method, VD_{max} also has been successfully used over the past several years to terminally sterilize many types of medical device and pharmaceutical products (15,16). Because establishing the minimum dose using Method VD_{max} requires fewer sacrificial samples than Method 1 and Method 2, it offers potential advantages in the case of high-unit value products. Method 1 and Method VD_{max} are both based on testing against a challenge population that is considered to be more resistant to radiation than the natural bioburden that may be present on the product. Experimental verification is required. In Method 2, information is obtained about the resistance to radiation of the natural bioburden present on the product. This information is used in setting the minimum dose for irradiation. The key features of all three of these methods are discussed here. Additional details that pertain to the challenge populations, selection of the SALs for verification dose testing and pass/fail criteria can be found in ANSI/ISO/AAMI 11137-2:2006.

Method 1

Following selection of the desired SAL, the first step in the application of Method 1 is determination of the average bioburden on a product item where a product item is defined in terms of how it is used in clinical practice. A total of 10 product items are selected from each of three independent product batches, and these 30 product items are tested for bioburden. In those cases where manufacturing is limited to a single batch, only 10 product items need to be tested for bioburden. Rather than sampling the entire product item, it is sometimes possible to sample a portion of the item, which is referred to as a sample item portion or SIP. Procedures for using an SIP are defined in the ISO standard on methods for establishing the sterilization dose (6). After the average bioburden has been quantified, the next step is to perform a verification dose experiment at an SAL of 10^{-2}. Selection of the verification dose is based on the average bioburden number, and a standard distribution of resistances (SDR population) that represents a greater challenge than the natural bioburden present on the product. The challenge population used for dose verification in Method 1 was based on testing of several hundred isolate resistances from some 70,000 microbes (17). The SDR population consists of a superposition of different D_{10} values in percentages that were derived from this experimental study. From a comparison of the resultant challenge population with other proposed populations, it was concluded that the SDR population provided a conservative presterilization microbial resistance reference profile. The basis for selection of the SDR as the microbial challenge population is discussed in reference (15). Because the verification dose experiment is

performed at an SAL of 10^{-2}, 100 samples need to be selected from a manufacturing batch for testing. Following irradiation at the verification dose, the 100 samples undergo sterility testing and if the number of positives is less than a preset number, a sterilization dose that is based on the challenge population may be selected. It is important to note that the sterilization dose is dependent on the initial bioburden that is present on a product item. At an average bioburden of 1000 colony forming units (CFU), the 10^{-6} SAL dose is approximately 25 kGy, that is, a sterilization dose commonly used during the early years of the radiation sterilization industry, but for an average bioburden of 0.1 CFU, the 10^{-6} sterilization dose can be as low as 11 kGy. However, the number of sacrificial samples that is needed to complete the test matrix may be problematical for high unit value products. Method 1 may be better suited for products that are manufactured in relatively large product lots, and unit costs are not extremely high.

Method 2

Method 2 actually consists of two methods that are referred to as Method 2A and Method 2B. Method 2A applies to products with average bioburdens per product item greater than about 10 CFU and Method 2B applies to products with consistent and very low bioburdens. In both methods, information is obtained about the resistance to radiation of the natural bioburden present on the product. This is accomplished by exposing product items to a series of incremental doses to estimate the dose at which one in 100 product units are expected to be nonsterile, that is, 10^{-2} SAL dose. The data from the incremental dosing is also used to estimate the D_{10} value of the natural bioburden present on the product. In effect Method 2 provides an estimate of the 10^{-2} SAL dose and slope of the dose-survivor curve, which allows extrapolation to an SAL of 10^{-6}. The number of sacrificial samples that are required for execution of Method 2A or 2B is quite large, that is, at least several hundred, which probably would make application of Method 2 inappropriate for high unit value products or products that are manufactured in small batches. However, for products with low bioburdens and/or products contaminated by microorganisms with low radiation resistance, it may be possible to validate a 10^{-6} SAL dose that is less than 10 kGy.

Method VD_{max}

Method VD_{max} was initially developed for irradiation of product at a sterilization dose of 25 kGy, but subsequently the method was extended to doses down to 15 kGy (15). From an operational standpoint, VD_{max} is similar to Method 1 in that it requires determination of bioburden and performance of a verification dose experiment. However, VD_{max} differs from Method 1 in two basic respects. First, the sterilization dose is fixed to a maximum bioburden number and the sterilization dose does not scale to lower doses with a decrease in bioburden below the maximum value, as is the case for Method 1. The sterilization dose, however, will change with selection of different values for the maximum bioburden. For example, at a maximum bioburden of 1000 CFU, the sterilization dose is 25 kGy and for a maximum bioburden of 1.5 CFU, the sterilization dose is 15 kGy. The second difference between Method VD_{max} and Method 1 involves the number of samples that are required for the verification dose experiment. In Method VD_{max} the verification dose experiment is performed at an SAL of 10^{-1} rather than 10^{-2}, which reduces the number of samples that are needed for testing from 100 to 10. The number of samples that are required for bioburden testing is the same as Method 1, but due to the reduced number of samples that are required for the verification dose experiment, the total number of samples that are sacrificed using Method VD_{max} is only 40 compared to 110 for Method 1. At the maximum bioburden, the VD_{max} sterilization dose converges to the Method 1 dose for the same bioburden. Also, Method VD_{max} rigorously preserves the conservative aspects of the SDRs that represent the challenge population for Method 1.

Establishing the Maximum Acceptable Dose

In addition to establishing a minimum dose that ensures a specified SAL is satisfied, a maximum dose that ensures the safety and performance of the product over its lifetime also needs to be established. The procedure for determining an acceptable maximum dose involves

irradiation of product samples at precise doses followed by post-irradiation testing of the product. In selecting the radiation environment for the tests, it is important to consider the temperature and dose rate in which the product will be irradiated on a routine basis. Dependent on the type of product that will be irradiated, both of these parameters could significantly impact the test results. The test matrix not only includes the drug product and medical device if you are dealing with a combination product, but also any closure system and packaging. Both functionality and biocompatibility are included in the test matrix. The doses that are selected for the tests should take into account a range of doses above the minimum dose that would allow the product to be processed on a routine basis without significant constraints. As we have seen, dependent on the size and density of the product unit as well as the irradiator environment, the product will be exposed to a range of doses that is characterized by a DUR. For example, if the minimum acceptable dose is 15 kGy, initial testing to establish an acceptable maximum dose could begin at a dose of 30 kGy, which would allow for a DUR of 2 during routine processing. If the initial results of post-irradiation testing of the product are acceptable, further testing probably will not be required. However, any negative results would require repeating the tests at a lower dose, for example, 25 kGy. It should be remembered that a tighter allowable dose range could place constraints on how the product can be processed on a routine basis. For example, in the case of gamma irradiation, this may require center loading of the product in a tote, which would reduce the tote load efficiency.

Dosimetric Release

Dosimetric release is an important aspect of the radiation sterilization process. In essence, dosimeteric release allows product to be released following irradiation based on certification that all product in the run received an acceptable minimum dose and no product in the run exceeded an acceptable maximum dose. Post-irradiation sterility testing of product samples is not required and use of biological indicators is no longer a recommended practice. The efficacy of the dosimetric release process rests on the use of an established methodology for selecting an acceptable minimum dose to achieve the desired SAL and certification that no dose exceeded a maximum acceptable value, which was based on a matrix of tests involving functionality and biocompatibility. In addition to establishing acceptable minimum and maximum doses, routine monitoring and control of the irradiation process and maintenance activities that include controls on the manufacturing process and periodic dose audits are important aspects of the radiation sterilization process.

PERFORMANCE QUALIFICATION

Performance qualification (PQ) is the final step in the radiation sterilization process prior to routine irradiation of the product. PQ involves two activities that include loading the product into the irradiation containers in accordance with a specified loading pattern and dose mapping of the product to determine the distribution of dose within the product load and identify the location and magnitude of the minimum and maximum doses. As we have seen, product may be loaded into an irradiation container in different configurations dependent on the carton size, product density, and possible constraints that are dictated by the DUR. Regardless of the load configuration, once established it must be maintained during the irradiation process. The dose distribution in this established load configuration must be measured, and the zones where the dose extremes occur must be identified.

Product Loading Pattern

Dependent on the modality of radiation that is used to sterilize the product, different constraints may be imposed on possible loading patterns. Because of the much longer radiation mean-free path of high-energy photons in materials than energetic electrons, there are typically fewer constraints on loading geometries in gamma and X-ray irradiators than in electron beam irradiators. For many types of health care products that are processed in a gamma or X-ray irradiator, the product within a carton may be treated as effectively homogeneous in nature and only carton size and weight are measured to determine the bulk density. However, some products may contain localized regions of high density that could affect the distribution in dose within the product load and therefore need to be taken into

account. For example, glass vials may be filled with an API powder and surrounded by low-density packing material. In such cases, a measurement of only bulk density may not suffice. For these types of products, it may be necessary to take into account the orientation of a product item within a carton and how the carton may be loaded into the irradiation container. Additional factors other than optimization of the fill efficiency may affect the final load pattern. For example, the final load configuration may take into account dose uniformity requirements, ease of loading, and compatibility with other product runs.

Some pharmaceutical products that are in an aqueous form respond more favorably when frozen and should to be irradiated in a refrigerated state. The loading pattern for refrigerated products requires special consideration. Refrigerants, be it wet ice or dry ice, are high-density materials that can significantly affect the dose delivered to product. For this reason, it is important that the product packaging be properly designed and the refrigerant confined to a specified location within the carton. In selecting the location of the refrigerant within the carton, it is important to consider the geometric relationship between the source of radiation and the product. Whether it is a beam of radiation as is the case for electron beam and X-ray sources or the isotropic radiation environment in a gamma irradiator, placement of the refrigerant between the source and product is generally not a good option. At this location, the refrigerant will significantly attenuate the incident radiation. The situation is further complicated in the case of dry ice, which sublimes, that is, the effect of the refrigerant on dose delivery is time dependent. Of the different possible locations of the refrigerant, perhaps the best place to locate the refrigerant to minimize its effect on dose delivery is in the lead and trail regions of the irradiation container.

Because of the much shorter radiation-free path of energetic electrons in materials compared to high-energy photons, additional considerations should be taken into account in selecting the loading pattern for electron beam irradiation. Loading patterns should be established for each product type. For this modality of irradiation, the loading pattern should take into account the orientation of the product items within the package material as well as any secondary packaging and orientation of the product item with respect to the incident beam of electrons.

Dose Mapping Gamma and X-Ray

For many types of lower bulk density materials and those that are reasonably homogeneous in make-up, the dose map may consist of a standard three-dimensional grid of dosimeters that are placed throughout the product load. For gamma irradiation, geometric attenuation may play an important role in the distribution of dose and for X-ray irradiation, edge effects at product boundaries may be important. These factors need to be taken into account in the placement of dosimeters within the product load. An additional consideration is the region of the product load furthest from the source of radiation. For gamma and X-ray irradiators, this occurs at the mid-plane of the irradiation container. Dependent on how the cartons of product are loaded into the irradiation container, it is frequently possible to place dosimeters only on the outside surfaces of the cartons; that is, it is not necessary to go inside a carton to locate dosimeters.

In those cases, where product may contain localized high-density regions within a carton, it may be necessary to place dosimeters inside the carton and even within a localized high-density region itself. This situation may occur more frequently in the irradiation of pharmaceutical products that are oftentimes formulated in a high-density configuration. Because the maximum dose zone is usually found on an outside surface of the product load, the presence of localized high-density regions usually only affects the location of the minimum dose zone. A customized dose map grid is required whenever it is deemed necessary to place dosimeters inside a carton of product. Whenever, a dosimeter needs to be placed inside a carton or within a product item, it normally is not practical to place a dosimeter at that location during routine processing of the product. In these cases, it is standard practice to measure dose at a reference location, which typically is on an exterior surface in the product load or standard monitoring location and relate the dose measured at the reference location to the dose measured inside the product. The relationship between the dose measured at the reference location and dose at the interior location, is commonly called an adjustment factor (AF) (18).