11

CYCLE DEVELOPMENT

Michael S. Korczynski

MIKKOR™ Enterprises, Inc.
Lake Bluff, IL

Jeanne Moldenhauer

Vectech Pharmaceutical Consultants, Inc.
Farmington Hills, MI

Cycle development is the process of determining the physical properties to be met in a sterilization cycle that will be used to reproducibly and consistently sterilize the product, component, and/or equipment in a defined loading pattern. The goal of cycle development is to provide a proven acceptable range of sterilizing conditions that result in a product/material that is both sterile and functional after the completion of the sterilization cycle. The cycle development goals are often established in a formalized development plan. This frequently is written as a protocol or a standard operating procedure (SOP).

There are many functional group interactions and sterilization-related activities associated with conducting cycle development studies necessary for a regulatory filing. The activities associated with developing sterilization technology data for a new or modified drug product require the interaction of several disciplines. These disciplines may be located within a plant (manufacturing facility) or perhaps at some company location that has support and/or oversight responsibilities for the plant's sterilization technology activities. Frequently, formulation pharmacists, quality assurance, research and development microbiologists, plant and division sterilization process engineers, and regulatory personnel must work closely together to develop the sterilization technology portion of the regulatory

submission. This information and data must be submitted to regulatory agencies for approval prior to the commercialization of a new or modified product and/or sterilization process.

Table 1 includes a summary of many sterilization technology topics that must be submitted in a New Drug Application (NDA). It also contains many of the elements that are described in the Food and Drug Administration's (FDA's) guidance documents. Before discussing the technical areas of cycle development, we must discuss the magnitude of moist heat sterilized drug products. PDA Aseptic Processing Surveys conducted in 1992 (PDA 1992) and 1996 (PDA 1996) indicated that the majority of products that were terminally sterilized were small volume parenteral products (SVPs). These products are 100 mL or less in volume. However, moist heat sterilized products constitute only approximately 20% of the sterile products marketed. The majority of the products manufactured are aseptically filled.

One might ask whether it is acceptable to the FDA to select a sterile filtration aseptic manufacturing process because that is the only equipment that exists in the plant. Another question might be whether a firm can arbitrarily use aseptic processing rather than terminal sterilization. The answer to each of these questions is *"No"!* When submitting an NDA to the FDA, one must show why the product cannot be terminally sterilized by moist heat. The European Agency for the Evaluation of Medicinal Products (EMEA) sterilization decision tree also requires these data for European submissions (CPMP 2000). Data must be supplied to show that the product is not stable or will lose significant quality as a result of moist heat sterilization processing before aseptic processing will be considered as a valid manufacturing method.

Developing an appropriate sterilization cycle is a complex assignment. The types of physical parameters characterized during cycle development activities include exposure time and temperature; pressure requirements; time allowed for heat-up and cooling; load and product heating parameters (e.g., temperature distribution and heat penetration); and loading configuration. Additional considerations may be deemed critical to the efficacy of the sterilization process for some cycles (e.g., time at which overpressure is achieved, and/or stability of heat labile products).

Typical cycle development studies measure physical parameters such as pressure (in the chamber, and, in some cases, in the load components as well) and temperature. Temperature may be measured by temperature sensors and/or resistance temperature detectors (RTDs) placed within the product (heat penetration) and in the sterilizer environment (temperature distribution). Biological indicators (BIs) may be utilized during cycle development, as described in USP <1035>, "Biological Indicators for Sterilization" (USP 2000). It is important to understand the sterilization process utilized and its potential effect on the product. The results of cycle development studies are often

Table 1. Summary of Sterilization Technical Topics Included in an NDA

New Container and/or Closure System:
Complete package engineering descriptions and drawings

The Sterilization Process:
The operation and control of the production sterilizer

The Autoclave Process & Performance Specifications:
Specification of the sterilization cycle

Autoclave Loading Patterns:
Description/Diagram representative of sterilizer loading patterns

Thermal Qualification of the Production Cycle:
Temperature distribution data
Heat penetration data

Container Thermal Mapping Data:
Not generally required

Microbiological Data:

- Biological indicators—Information and data concerning the identification, resistance, and stability of BIs used in the biological validation of the cycle should be included. *ATCC* number, stock number, and D-value test data must be included in each report. Also address the relative resistance of the biological indicator to the typical bioburden.
- Solution D- and z-values
- Data from container/closure microbial inactivation studies for new or modified closures
- Rationale for the selection of the master solution
- Drawings of closure inoculation sites
- Data from subprocess solution and closure production challenge, including associated heat penetration data
- Container/closure maintenance of sterility data, poststerilization following maximum exposure conditions

Bacterial Endotoxin Test Validation Data for Solution and Container Components:
LAL compatibility or kinetic assay compatibility
Bulk drug and final product inhibition/enhancement data

Sterility Testing Methods and Release Criteria:
Bacteriostasis, fungistasis sterility testing data, or parametric release sterility indicating test method

documented in a final summary report that is included with an Investigational New Drug (IND) or NDA submission. The number of studies required may vary based on the desire to standardize cycles, one's knowledge of the sterilization process, and the product and/or load being sterilized.

It may be beneficial to adopt "standard" sterilization cycles to minimize the total number of cycles utilized, and to reduce the on-going costs of qualification/requalification. In this type of strategy, a company analyzes whether us-

ing an existing cycle provides sufficient assurance that the product will achieve the desired sterility assurance level without adversely affecting the stability of the product. When making these assessments, exposure times are usually grouped together in one set of cycle parameters of less than or equal to 5 minutes from the standard cycle. For example, if cycle development studies for three products yield cycle times of 11, 13, and 15 minutes, a cycle of 15 minutes may be used for all three products, providing that they are heat stable.

How Does One Determine Whether the Product Withstands a Thermal Process That Is Encountered in Moist Heat Sterilization?

Generally, pilot plant or bench top laboratory studies are conducted, whereby the formulated product in the intended container/closure system is exposed to maximum anticipated thermal and time conditions. At this point, one would have to speculate on what the maximum conditions of the sterilization process would be. Following thermal exposure per the maximum sterilization cycle conditions, the drug product is tested to ensure that the product remains within the specification limits. More than one set of cycle parameters may be evaluated, such as variable times and temperature conditions, to determine whether a heat sensitive product is compatible with a terminal sterilization process. These evaluations could be classified as initial R&D stability studies. Further, thermally exposed products should be placed on accelerated 40°C stability studies for at least three months. If the product stability data demonstrate that the initial product specification release limits cannot be maintained, a case exists for aseptically processing the product. These data would have to be included in the NDA or European regulatory submission. During the review process, questions may arise regarding whether extractables from the container/closure system influenced the drug or product data. Therefore, the developmental data must show whether the system will meet the USP biological safety testing if the container/closure system is an elastomeric, plastic, or other polymeric material.

Expectations of Regulatory Agencies Concerning Sterilization of the Solution and Cycle Selection on the Basis of Solution Selection—Development Studies That Need to Be Conducted

First of all, the FDA expects that one will provide the D-value of a heat resistant microorganism in or on the subject product, drug, or closure system. Although in Europe there seems to be a fixation concerning the usage of *Bacillus stearothermophilus* (subsequently reclassified by ATCC as *Geobacillus stearothermophilus*) as a BI, within the United States microorganisms such as

Clostridium sporogenes, Bacillus coagulans ATCC 511232, and even *Bacillus subtilis* variety 5230 have been used and accepted as BIs for steam sterilization. However, data must be provided to show that the resistance of the selected BI exceeds that of product bioburden. Many pharmaceutical companies can attest that the D-value of the bioburden in their product is many-fold less resistant than the BI microorganism used to establish sterilization cycles. When solution D-value data are submitted to the FDA, it is generally expected that a minimum of three D- and z-values will have been evaluated. Relative to closure data, the D-value of the spore suspension used to inoculate the closures generally suffices for the regulatory submission.

DEFINING REQUIREMENTS

Prior to initiating cycle development activities, it is important to find out as much information as possible to define the requirements for sterilization. Here are some typical questions:

- *What do you know about the product/component?* Is there literature available to indicate whether the product is steam sterilizable? If so, what are the operating parameters? Does the configuration of the product/component require special sterilization parameters? For example, most prefilled syringes and plastic containers require air overpressure cycles to maintain functionality of the product.
- *What needs to be sterile?* Does the product require sterility on the outside of the container or only a sterile fluid pathway? How is the product used? Does this affect what must be sterile?
- *What level of sterility assurance (SAL) is required?* This is a key concern for determining the sterilization model to be used.
- *What regulatory requirements are applicable to this product?* It is critical to know to which countries product will be exported. Since harmonization of sterilization requirements does not exist (see Chapter 4 of this book for additional details), it is important to clearly understand the applicable regulatory requirements. For most products terminally sterilized by moist heat, a minimum SAL of 1×10^{-6} is required (FDA 1994).

If the product uses a saturated steam cycle and is for export to certain European countries, it may be subject to specific regulations for cycle design and validation, e.g., *HTM 2010 Part 3* (1994) and the EMEA Sterilization Decision Tree (CPMP 2000).

- *What types of sterilization processes are available in the facility?* Obviously, it is less expensive to use existing sterilization processes rather than purchase a new sterilizer. Are the available processes usable for the new sterilization cycle? For example, is the new product plastic, and does it therefore require an air overpressure cycle to maintain its shape? If existing processes do not have this function, either the sterilizer needs to be modified or a new system must be purchased.
- *Do the product development data provide any information that constrains the development of the sterilization cycle?* For example, does the product become unusable above a certain temperature? Does a degradation product exceed limits at a certain temperature? Is the product a suspension or lipid that may require stirring or movement throughout the sterilization cycle to prevent excessive product degradation?
- *How will the load be defined?* After operational qualification and before or during cycle development, the types of loads to be sterilized and the load configurations need to be identified. In determining the load configuration, one should take into account the production efficiency and the sterilization efficiency and effectiveness. How items are placed onto trays, racks, or pallets should be reviewed. For example, how much of the product is in contact with the shelving material? Hard goods may be protected from subsequent contamination after the cycle by wrapping the critical surfaces with commercially available sterilization wrap. Appropriate wraps allow for steam penetration during the cycle and are microbial barriers after the cycle. Wrapped articles should be covered with only enough wrapping material to protect the critical surface while allowing free transfer of saturated steam and air through the material. It is inappropriate to attach the material in such a way that there are several layers of wrap covering areas of steam penetration. Use of sterilization tape should be minimal to avoid obstruction of the steam vapor path. Normally, one to two layers of material are sufficient to protect sterile surfaces and allow appropriate steam penetration. Hard goods sterilization cycles should incorporate vacuum pulsing techniques to remove air from the chamber and loaded equipment, prior to the final charge of the sterilizer with saturated steam. Liquids sterilized in containers may use cycles incorporating air displacement with steam and air-steam mixture or hot water overpressure as appropriate for the type of container.

SELECTION OF A MOIST HEAT STERILIZATION PROCESS

When a new product formulation or a new container/closure system is developed, many moist heat sterilization processes may already exist in the facility. It is very tempting to automatically assign an existing sterilization process to a new product or container/closure system based on past knowledge or judgment. However, an existing sterilization process should not be assigned to the new product or container/closure system until certain key development data are collected.

The product should not be assigned a sterilization process just because the process exists for other products. The product and its resultant capability of being sterilized will dictate the sterilization process. Frequently, new container closure systems are found to provide more sterilization resistance to microorganisms that are inoculated on their surfaces than, for example, the resistance of the same microorganisms that are inoculated in the drug or solution. The process must be designed to ensure that (1) sterilization assurance requirements will be readily met, and (2) the total product attributes will be maintained.

Selection of a sterilization process type is generally based on the type of equipment available, especially if it is already validated at the facility, and the required load considerations. Some material types are only steam sterilizable under certain types of conditions (e.g., plastic requiring overpressure), which may drive the selection of the sterilization process type.

The sterilization cycle should deliver the desired SAL while minimizing the adverse effects on the materials and/or products. The evaluation of worst-case conditions (i.e., subminimal critical parameters) during cycle development should provide confidence in executing routine sterilization procedures. The allowable range of operating parameters (minimum versus maximum) should be developed throughout the development process. It is important to consider the influence of seasonal variation on microbial count and sterilization resistance when conducting such studies. Also, consecutive developmental cycles should be conducted when developing these parameters.

CONTAINER THERMAL MAPPING: DETERMINING THE SLOWEST-TO-HEAT ZONE

Data may be requested by regulatory agencies concerning thermal mapping of the container itself. This is especially true if it is a complex container in an unusual process, with perhaps many unusual container/closure ports on the system. Data may also be required for new technologies (i.e., new to the company), new or modified container/closure systems, or to justify locations that

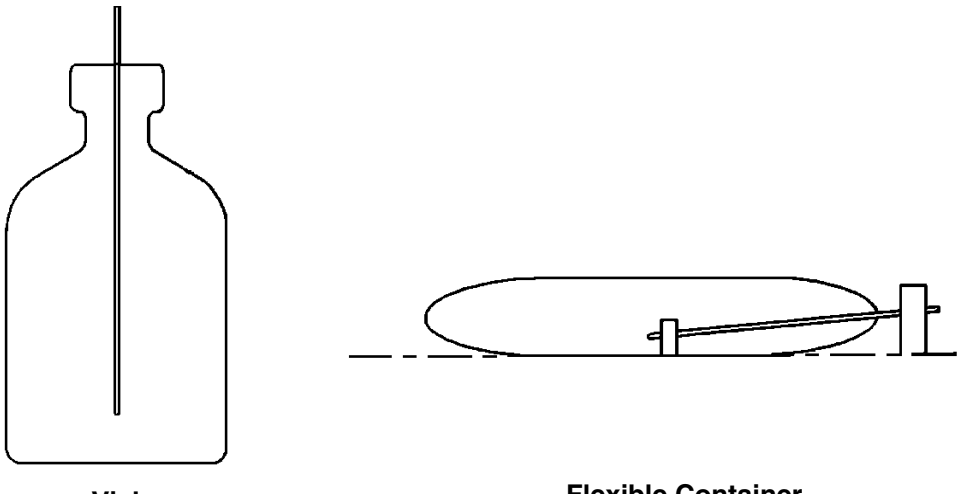
are routinely thermally probed within the container. Information should be generated to show that you understand where the coldest zone is in the container. Therefore, your sterilization process must be developed not only for the coldest zone in the load but also for the coldest zone in the container.

The slowest-to-heat zone (SHZ) of a product is defined as the location within the product that achieves the lowest process lethality (F_o) during the process, i.e., having the lowest temperature delivered throughout the process (Owens 1993). A conservative approach to cycle development assumes that all of the microorganisms in a product exist at the SHZ and are exposed only to the temperatures achieved at that zone. This is considered a conservative approach because using the selected final F_o for the SHZ will result in a greater accumulative microbial lethality (F_o) throughout the rest of the product.

There are two basic ways to determine the SHZ. One method involves placing one temperature sensor at different positions in multiple containers (see Figure 1). Another is to place multiple temperature sensors in different locations within one container (see Figure 2). The choice of which method to use depends on the physical constraints of the item to be tested. For example, it would be difficult to use multiple probes within a 1 mL vial.

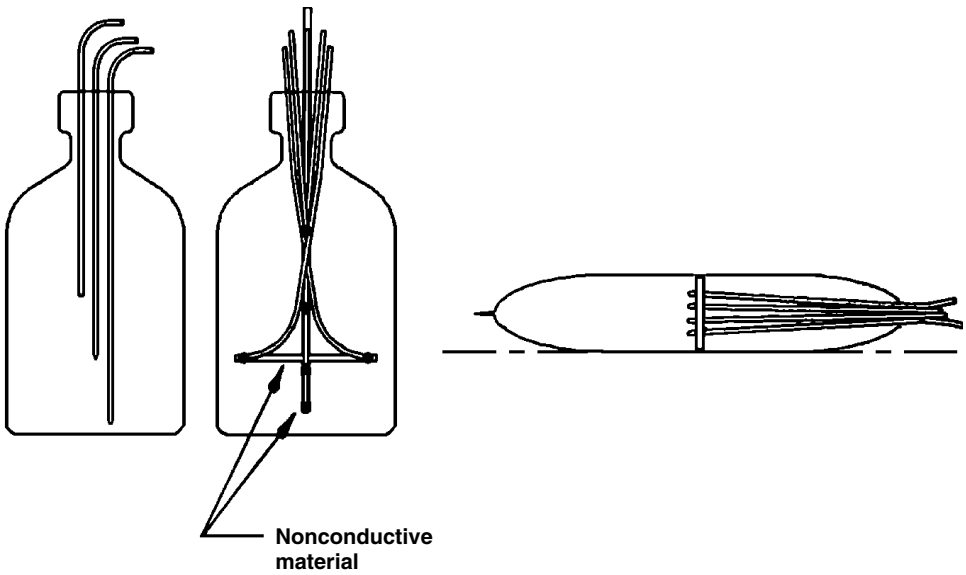
Most sterilization personnel consider the use of multiple probes within one container to be a more reliable method, since it eliminates the variability of using multiple containers. Frequently, a branched probe system or "probe tree" is used in such cases. Statistical tests, such as analysis of variance and Student t-tests, may be used to verify whether alternate probe locations pro-

Figure 1. Placement of a Single Thermocouple in a Container



Vial **Flexible Container**

Figure 2. Placement of Multiple Thermocouples in a Container

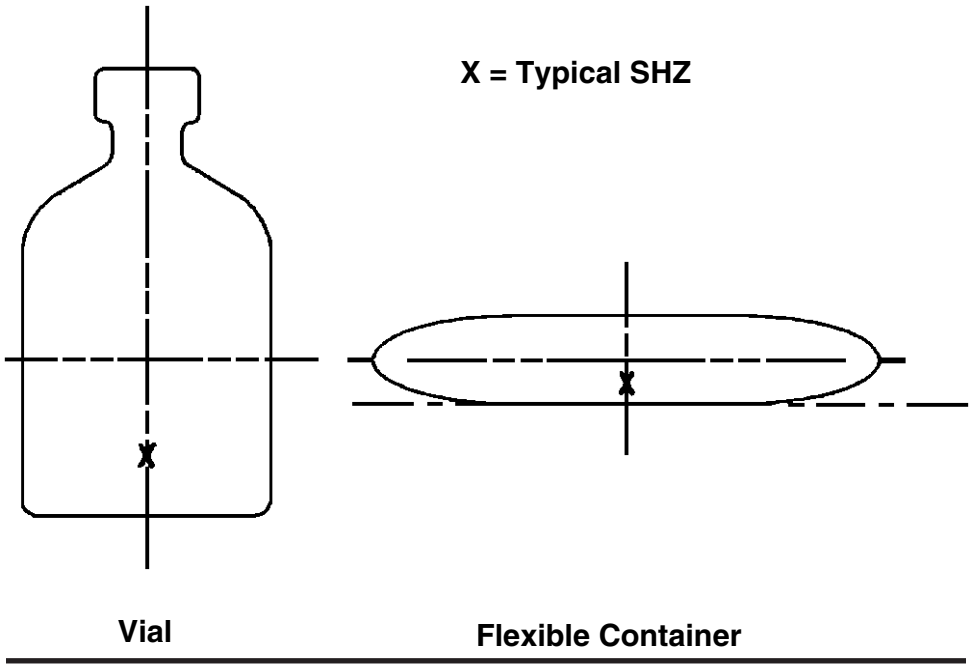


vide heating characteristics that are statistically different from each other. These types of data are necessary to determine where the temperature sensors should be placed for both cycle development and validation activities.

The SHZ for large volume parenterals is typically located between the geometric center of the product and the bottom of the product along the vertical axis (see Figure 3). The very bottom of the container is usually a little hotter than a short distance from the bottom of the container (Owens 1993). This is probably due to the heat transfer from the shelving material. The area where the bottom of the container is in contact with the shelving material is referred to as the footprint of the product. Changing the footprint of the product sterilization can change the quantity of heat delivered to the product. Some newer shelving materials that are available commercially are plastics. The transfer of heat from the shelving material to the product may be different based on the materials of construction. Depending on the time in the cycle, the SHZ may move from near the container sidewall toward the geometric center, due to the change from conduction to convection heating (Owens 1993). An SHZ is not typically determined for SVPs, since the solution heats at almost the same rate as the sterilizer environment.

For elastomeric closures in steam sterilizable bags, the SHZ may vary depending on the number (mass) of closures on the bag, the size and profile of the bag during sterilization, and the amount and type of contact with the

Figure 3. Typical Container Slowest-to-Heat Zone



sterilizer tray. The exact positioning of the bag on the tray becomes important in assessing the actual heat transfer to the closures. A bag arranged in a shingled manner, i.e., overlapping on adjacent bags, will heat differently from a bag lying flat on a tray. Placing a bag over the interface where two trays come together also causes variation in heat transfer.

Figure 4 provides an example of a procedure to determine the SHZ for other filling room equipment. Once the SHZ is determined, it is important to develop a way to verify that the temperature sensor can and will be maintained at the specified locations throughout the process. Probe holders or a mechanical stop may be necessary to keep the temperature sensor at the desired location.

HOW MUCH LETHALITY IS ENOUGH?

Prior to the initiation of the cycle development work, it is imperative to define the minimum-acceptable equivalent lethality at 121°C (i.e., F_o) that will be required to achieve an acceptable SAL or to meet the regulatory standards. Given the D- and z-values of the indicator microorganism in the product, cycle time to achieve a 10^{-6} level of sterility assurance for the product can be projected. To determine this value, there are several things that must be taken

Figure 4. Example of Standard Procedure for Determining Hardest to Sterilize Equipment, Minimum Loads, or Probing Locations

EQUIPMENT BIOLOGICAL CHALLENGE DETERMINATION USING A SUBLETHAL STERILIZATION CYCLE STANDARD PROCEDURE

1. PURPOSE
   This document defines the procedure to biologically determine the most difficult to sterilize area or item or filling room support equipment loads (hard goods) using a sublethal sterilization cycle.
2. APPLICABILITY
   This procedure applies to validation personnel at manufacturing sites performing studies to identify the most difficult to sterilize area of component or filling room support loads (hard goods) in order to determine the minimum load of a particular cycle, or probe and biological indicator (BI) placement for validation studies. This procedure may also be used when the addition or replacement of new equipment is shown not to be equivalent (by Student's t test) or for other load configuration changes, etc.
3. DEFINITIONS:
   Sublethal Study—A sterilization study designed to allow quantifiable BI growth after cycle completion by the reduction of cycle time and/or temperature, generating lead probe or F_0 below nominal operating parameters.
4. MATERIALS/EQUIPMENT
   4.1 Sterilizer and Associated Equipment
   4.2 BIs
5. METHODS
   5.1 Study Preparation
      a. Prepare the componentry/equiupment load per the defined protocol, standard procedure, or investigational study directives.
      b. Place BIs in the desired locations within the load. Ensure that BI placement, componentry/equipment load positioning, and cycle parameters are all well defined and documented. A detailed diagram of each item inoculated with a BI must be included.
      c. Set the sterilizer to deliver reduced exposure time and/or temperature below nominal operational parameters. (For instance, a standard cycle of 121°C for 30 minutes might be reduced to 121°C for 10 minutes.)

   5.2 Perform the cycle, unload the BIs, and deliver them to the Microbiology Department for enumeration. It may be desirable to drop test the BIs when large numbers are needed for a study. Locations showing positive results would be inoculated in a second study. If all the counts are negative (drop test) or equivalent (enumeration), an additional study may be required, with further reduced parameters.

   NOTE: If the exposure temperature was below the standard set-point and the exposure duration was reduced by at least 50% below the standard production time, any equivalent counts will indicate equivalent biological challenge. No second study is required in this case.

   If the subsequent additional study yields all equivalent counts, designate the most difficult to sterilize item in a memo with the following: the situation; rationale for choosing said item; challenge site; and appropriate approvals.

Figure 4 continued on the next page

Figure 4 continued from the previous page

5.3 The most difficult to sterilize item or area will be determined by the highest CFU count achieved from the BI enumeration.
The highest equivalent counts on any two or more items or locations indicate equivalent biological challenge. Therefore, any of these items or locations can be designated as the most difficult to sterilize.

5.4 BI types may be combined, e.g., strips and minidisks, for a sublethal study, but they should have equivalent control count populations and D-values within 0.5 minute of each other. If the D-values vary more than 0.5 minute, F_o values (rather than CFU counts) should be compared to determine greatest biological challenge items or locations.

5.5 If equivalency studies are performed per the appropriate procedure and the items are found to be equivalent, no sublethal studies are required.

5.6 Update the documentation that defines the most difficult to sterilize items and their locations for each set-up and miscellaneous equipment cycle of each component sterilizer, when applicable.

6. REFERENCES

SOP # _____ In-Process Component and Equipment Qualification and Requalification Procedure for Steam Sterilizers

SOP # _____ Component/Equipment Sterilizer Setups and Miscellaneous Equipment Minimum Load Definitions

into consideration: the sterilization model selected, the resistance of the BI chosen for the cycle validation, and the thermal resistance of the bioburden.

What Is the Purpose of the D- and z-values?

D-value is defined as the amount of time (in minutes) required at a specific temperature to reduce the microbial population by 90% or one log. A z-value is defined as the number of degrees temperature, generally stated in degrees Celsius, that will result in a 10-fold (one-logarithm) change of the microbial D-value. To determine the z-value, at least three D-value runs using three different temperatures and three exposure durations are performed for each temperature.

The z-value is important because it will be used to calculate the lethal rate per minute at specific temperature. The lethal rate will be different at each specific temperature of exposure, and then can be summed or accumulated for each minute of exposure at each recorded temperature. This permits the calculation of the accumulative F_{BIO} or the total microbial lethality experienced by the heat resistant spores that were suspended in the given solution.

The z-value can be visualized by plotting the D-value on a log scale against the temperature of exposure on a linear scale. The linear regression line through the points will provide a thermal resistance curve. One can then determine how many degrees C were associated with a one logarithmic change in the D-value to determine the z-value.

The accumulative F_{BIO} can be compared to the F_o value, which is based on a z-value of 10°C and is used as the engineering convention to translate time at temperature into an expression of microbial lethality. Since this convention uses a z of 10°C, it would be expected by this model that 10°C would cause a one-log change in the D-value. If the microorganism had a D-value of one at 121.1°C, then a F_o of 8 minutes, for example, would theoretically be capable of inactivating eight logarithms of the microorganisms.

The F_{BIO}, or lethal rate per minute, in the subject drug product will differ from the thermocoupled measurement-derived F_o because it has used the actual z-value of the BI in the subject drug and not the 10°C convention.

The accumulated F_{BIO} for a sterilization process divided by the determined $D_{121.1°C}$ will yield a predicted spore logarithmic reduction (PSLR) that might occur in the process.

Since several parameters are used to determine the desired minimum lethality required for sterilization, it is important to use these parameters to select cycles that achieve the desired results, do not adversely affect the item being sterilized, and are cost-effective.

For some regulatory bodies, an SAL of at least 1×10^{-6} (i.e., $\leq 1 \times 10^{-6}$) is required. This corresponds to not more than one contaminated unit in a population of one million units. Other regulatory bodies have predefined cycles and acceptance criteria, which must be the first type of cycle to be tried or used. Some regulatory bodies have predefined decision trees that specify the exact steps to be taken to develop an acceptable cycle (CPMP 2000). It is important to be aware of the regulatory requirements for each country that will register and receive product in determining the applicable development requirements.

In determining a sterilization cycle for a specific product, one should take into consideration the BI to be used and the bioburden in the facility. Failure to do so may result in cycles that severely reduce the likelihood of many products to survive terminal sterilization. Sufficient heat must be applied to inactivate bioburden and BIs without destroying the product. An example of the cycle required for two different BIs is given in Figure 5. In this example, the typical bioburden resistance in the facility is ≤ 0.1 minute, so that both BIs provide sufficient heat resistance on stopper XYZ to be a worse case than the environmental isolate, but they would require very different F_o values or time and temperature conditions to achieve an SAL of at least 1×10^{-6}.

When performing process requirement calculations, the value that is used to represent the bioburden is frequently the mean total number of microorganisms per container. One must inactivate at least six logarithms of the biological indicator microorganism(s) beyond or in excess of the mean bioburden count. This is a conservative approach since the entire populations of the bioburden may not be spore formers. When concerned with the potential adverse effects of excessive heat upon the product some may consider

Figure 5. Stopper Sterilization Requirements with Two Different BIs

Stopper XYZ needs to be sterilized:
- $D_{121.1°C}$ for *B. stearothermophilus** is 2.0 minutes.
- $D_{121.1°C}$ for *B. stearothermophilus** on XYZ is 7.9 minutes.
- $D_{121.1°C}$ for *C. sporogenes* is 0.5 minute.
- $D_{121.1°C}$ for *C. sporogenes* on XYZ is 0.5 minute.
- $D_{121.1°C}$ for most heat resistant environmental isolate is 0.1 minute.
- SAL required is 10^{-6}.
- Maximum bioburden allowed is 1 CFU.

For *B. stearothermophilus**,
- D-value increases by 400% when placed on the stopper ($D_{121.1°C}$ rounded to 8 minutes).
- Maximum allowable bioburden is 1 CFU or 10°C.
- A minimum 6-logarithm reduction is required to achieve SAL desired.
- $D_{121.1°C}$ = 8 minutes and 6 log reduction required,
  8 minutes/log × 6 logs = 48 minutes of F_o required.

For *C. sporogenes*,
- D-value shows no increase when placed on the stopper ($D_{121.1°C}$ is 0.5 minute).
- Maximum allowable bioburden in 1 CFU or 10°C.
- A minimum 6-logarithm reduction is required to achieve desired SAL.
- $D_{121.1°C}$ = 0.5 minute and 6 logs reduction required,
  0.5 minute/log × 6 logs = 3 minutes of F_o is required.

This example refers to evaluating which biological indicator to select for a process. This is intended to show the hypothetical differences in achieving the desired SAL.
**Bacillus stearothermophilus* has been reclassified by ATCC as *Geobacillus stearothermophilus.*

it acceptable to use the maximum bioburden number as the maximum allowable number of spore-forming organisms in a product container. The BI population used to challenge the product and process would then be equivalent to the maximum bioburden value. This bioburden type sterilization validation approach is not frequently used. Also, one must be aware that typical bioburden microorganisms may demonstrate long kill times at certain container and closure sites. The kill times for bioburden in certain cases could parallel the inactivation rate for heat resistant spores inoculated at the same sites. This is a result of a nonaqueous environment existing at the inoculated site. Additionally, in cycle developmental studies, one must demonstrate at least a 10^6 inactivation level beyond the bioburden log value action level for the given container/closure system.

Determining the Minimum Microbial Lethality

To determine the lethality required for a new sterilization cycle, one must know the type of indigenous microorganisms present, the moist heat resistance of those microorganisms, the allowable level of contamination in the

environment and in the product, and the comparative heat resistance of the BI(s). Typically, the following formula may be used to establish a minimum acceptable F_o value:

$$F_o = D_{121°C} (\log_{10}N_o - \log_{10}B) \quad (1)$$

where:

F_o = the minimum lethality required.
$D_{121.1°C}$ = the heat resistance of the most resistant organism found in the product or the environment or the heat resistance of the BI.
N_o = the BI population/unit.
B = the maximum acceptable level of probability of survival of microorganisms.

One may also use this formula to estimate the F-value required to inactivate a specific number of BI microorganisms. The above mentioned formula assumes a z-value of 10°C, since the F_o value is used.

The F_o calculation by definition assumes that the z-value is equal to 10°C and that the reference temperature is 121.1°C. Some companies choose to use 121°C. There is no appreciable difference between the two numbers when performing this calculation. The F_o value is expressed as the number of actual minutes of product temperature at exactly 121.1°C. The sterilization and operating specifications should ensure that the coolest container in the load and its coolest zone attain the specified F_o minimum.

Figure 6 provides an example of this calculation. In this example, the numbers selected establish that the coolest container in the sterilizer load should receive an equivalent heat history of at least four minutes at 121.1°C.

Figure 6. Example of the Probability of Survival Calculation

B = probability of survival; A = bioburden level per unit of product

$$\text{Log B} = \text{Log A} - \frac{F_o}{D_{121°C}}$$

Example:

$$\text{Log B} = \log 2000 - \frac{F_o}{D_{121°C}}$$

$$= 3.3 - \frac{6}{0.37} = 3.3 - 16.2$$

$$= -12.9$$

$$B = 1 \times 10^{-12.9}$$

Determination of the Probability of Survival for Bioburden

This is a useful calculation to show the safety factor that is inherent in a developed cycle relative to bioburden. For example, one may be able to calculate a 10^{-60} or 10^{-80} probability of nonsterility for bioburden in a given cycle that was established by the use of a heat resistant BI microorganism.

Once an F_o value is determined for a sterilization cycle, the probability of microbial survival for that cycle is calculated using the following equation:

$$\text{Log}_{10} B = \text{Log}_{10} N_o - F_o/D_{121.1°C} \quad (2)$$

where:

B = probability of survival at the end of the cycle.
N_o = the bioburden in or on the product.
F_o = the equivalent time in minutes that the product would have experienced if the product had been maintained at 121.1°C (assumed z-value = 10°C).
$D_{121.1°C}$ = the time required at 121.1°C to reduce the microbial population by 90% or one log.

DETERMINATION OF THE REQUIRED STERILIZATION PROCESS TIME (IN MINUTES OF F_o) OR CYCLE DEFINITION (LOAD PROBE CONTROLLED CYCLES)

Required Sterilization Process Time

The sterilization process time required for a sterilization cycle to generate the minimum required F_o value can be determined using any of the following procedures:

1. The load cold point or zone is determined using thermocouple data. The sterilization time is adjusted such that the coldest container is at process temperature for the specified amount of time (the number of minutes of F_o required). One does not consider the minor amount of lethality received by the product during the heating and cooling phase of the sterilization cycle at temperatures below 100°C.
2. Alternatively, the load cool point or zone is determined by review of the thermocouple data, and the sterilization time must be adjusted such that the integrated lethality obtained in the coolest container is equal to or greater than the F_o value required for the cycle.

Cycle Definition (Product Penetration Controlled Cycles, i.e., controlled by F_o values in solution filled containers)

Some types of sterilizers are controlled by product penetration thermocouples (i.e., controlled by the F_o values in solution filled units). Defining a cycle for this type of sterilizer involves the sterilization microbiologist, who, in close discussion with the process engineering specialist, generates a printout of a subprocess cycle with all of the temperature and time conditions that might be used in a production cycle. The exception, however, is that the F_o, i.e., the physically measured F_o value, will be slightly less than the minimum of the actual specification for the process.

The microbiologist will calculate the lethal rate or F_{BIO} for each minute or minutes at each temperature provided by the process engineering group. The F_{BIO} would be calculated using a previously obtained z-value for the BI in the solution or master solution. This truly represents a thermal exposure curve that one can expect from the established or new cycle run at slightly subprocess conditions.

Several solutions may be sterilized in the cycle so that a table can be developed that lists the accumulative F_{BIO} for the various solutions that will be sterilized in the process. The accumulative F_{BIO} for each solution will then be divided by the $D_{121.1°C}$ value for the BI in that given solution. This will yield the PSLR for each solution. The solution with the lowest PSLR then becomes the master solution (solution most resistant to sterilization for the BI) and will be the candidate to challenge in validation of the production process, in regulatory submission studies, or in validation studies. If a new formulation drug has been developed, it is tested versus the master solution to determine whether it is more or less resistant than the master solution.

One may set a sterilization specification at $F_o = 8$ minutes. Therefore, one might think that the thermocouple needs to achieve only an $F_o = 8$ minutes to complete the process. Unfortunately, this is not so. One must add the following F_o values to the thermocouple minimum specification to ensure that the absolute coolest zone in a container placed in the coolest zone in the sterilizer will meet the specification minimum:

1. F_o difference between the coolest zone in the container relative to the exact position of the thermocouple in the container.
2. F_o difference between the coolest zone in the sterilizer (previously determined by heat penetration and temperature distribution mapping profiles) to the sterilizer zone where the thermocouple is located.
3. Any F_o differences that may occur as a result of packaging or container configuration. If a multiple pack design is used, perhaps a

package achieves less F_o than the package that would contain the thermocouple.

This may also be stated as a thermal lag time difference between the sterilizing medium and the thermocoupled product. The total difference in this lag time must be established in initial R&D and/or engineering studies and must be added as additional process time required for the coolest container in the load.

All of these F_o differentials or delta $F_o$values, obtained from the probed product thermocouple(s) would need to be added. The sum of those values or differences would be added to the production minimum F_o for the probed product thermocouple(s), and established as part of the process specification and process control. This value could appear in the operating specification for the cycle as additional exposure time required to ensure that the coolest container in the load attains the minimum F_o stated in the sterilization process specification.

If, for example, a cycle is specified as having an F_o = 8 minutes, and the total F_o lag time or delta F_o are found to be F_o = 0.5, the thermocouples that are monitoring the process need to reach an F_o value of 8.5 to ensure that the minimum specification of F_o = 8 minutes has been achieved for the coolest container in the load.

THE CONTAINER/CLOSURE SYSTEM

There are several types of container/closure systems. Closures may be simply rubber stoppers in glass vials and bottles, or rubber ports inserted into plastic tubing, as is frequently found in plastic flexible containers. Also, screw-cap metal or plastic caps with or without a liner may be used. Container/closure systems can provide formidable challenges for the sterilization process. In many cases, it is not the sterilization of the drug or solutions that defines the length of the sterilization process, but the container closure system. The container/closure system can be a rate-limiting factor in the design of the sterilization cycle. It will generally take longer to sterilize a container/closure system to obtain a six logarithmic reduction of spores than in solution.

True F_o Cannot Be Calculated at Closure Sites

Since the microenvironment at the closure may be a dry state, only attaining a moisturized state once mass transfer of moisture arrives at the closure site, it is not subjected to the same moist-heat environment, nor can it be determined in the same manner as the solution sterilization.

During any container/closure challenge run, containers are thermocoupled to record product temperatures and time at temperature with conversion to F_o readings. Such recordings are expressed as heat penetration readings. These readings can indicate the exact time that the process was maintained above 100°C. For purposes of container/closure challenges, the concept of the amount of time above 100°C to achieve kill at the closure sites may be used. However, simultaneously the process F_o that the solution product experienced would also be measured.

Regulatory Expectations for Container/Closure Challenge Data

Rate of bioburden inactivation should be known at the same closure site(s) that were inoculated with BI microorganisms. Inactivation rates in solutions, which can be readily predictable, do not occur at closure sites. The inactivation of bioburden, which has much lower resistance than spore-challenged microorganisms in solution, can be equal to the resistance of spore-challenged microorganisms inoculated at certain container/closure sites.

Some regulatory agencies may want to know the microenvironmental conditions at the closure site(s) and whether there is a different rate of kill for moist heat compared to dry heat resistant microorganisms. This requires that R&D studies be conducted during development of the closure system. Frequently, a developmental sterilizer is used to conduct fractional exposure studies on inoculated closures that are placed on their associated containers. An inactivation curve showing microbial kill for various exposure times is then plotted.

The FDA expects that, in the subprocess production cycle (which is submitted in an NDA), a SAL of at least 10^{-6} will be achieved at the inoculated closure sites. Further, data must be available for multiple sites in or on a closure system if the closure has multiple areas for contact that differ in design. A significant example would be a rubber plug inside a plastic tube, where, on removing the plug, solution in the container flows through the plug or tubing. If the plug contains a recess, the recess will have to be microbiologically challenged as well as the sidewall of the rubber plug adjacent to the inner wall of the plastic tubing.

Figure 7 describes how to design microbial inactivation studies. Appendix A is an example of an SOP for performing these studies at a production facility.

Syringes

Closure kill studies may show that it is extremely difficult to sterilize some of the interfaces, e.g., needle versus needle cover contact. Some companies may choose to presterilize parts of the needle assembly by radiation prior to aseptic assembly to circumvent the difficulties in closure kill.

Figure 7. R&D Microbial Inactivation Kinetic Studies at Container/Closure Sites

When to employ these methods:

1. Only use when developing a new or modified container/closure system.

How to design the study:

1. During the fractional exposure studies, determine the microbial end-point for each closure site that was inoculated and define the time above 100°C that transpired to reach the microbial survival end-point.
2. Repeat the study so that two studies exist and the data may be averaged if the survival curves are very similar.
3. A bioburden action level will need to have been established for closure systems prior to the developmental fractional exposures. While a SAL of 10^{-6} is expected to be demonstrated, one must also add the logarithmic value of the bioburden action level to the 10^{-6} value. Therefore, if one's closure action level is ten, it will be expected that the process is capable of inactivating 10^{-7} indicator microorganisms. The reason for this is that bioburden may be just as resistant as the indicator microorganism at the closure site, based upon the design of the closure and the lack of moisture that may exist at the closure site during the sterilization process.
4. Therefore, it behooves one to also include some bioburden microorganisms in closure sterilization studies that may be found on closure systems prior to sterilization in the R&D fractional exposures of closures. The bioburden should be inoculated using the same inoculum concentration as used for the indicator microorganism.
5. Additionally, since a dry heat condition may be found at the closure site(s), one should employ *B. subtilis* to detect dry heat microenvironments. Cases have been observed where moist heat resistant weak *B. subtilis* have demonstrated more survivors than the moist heat resistant microorganism in specific closure systems.
6. The resulting data will dictate the minimum sterilization process time above 100°C to achieve at least 10^{-6} SAL, for the most difficult closure site to sterilize. If this process time becomes so protracted that a very large solution F_0 value occurs at the detriment of the solution or drug, another process to sterilize the closures may need to be considered.
7. The procedure listed above is used to establish the production cycle in concert with the solution sterilization data and is submitted in the regulatory filing.

Unlike solution sterilization challenge studies, whereby the solution is filtered and the filter sterility tested for microbial growth, closure challenges frequently use the fraction negative method of analysis.

Why use the fraction negative or F/N method?

1. The F/N method is used when the sterilization process conditions are such that the end-point of the microbial kill curve is being approached. If one were to use a direct plate counting method, the level of surviving microbes may be so low that the direct plate count method may not reveal the presence of one to ten surviving microorganisms.
2. This area of the kill curve, where the end-point of survival is being reached or attained, has been referred to as the quantal zone.
3. A number of individuals agree that in their experience, the F/N method at the survival end-point is about one logarithm more sensitive in detection than the direct plate count method.

Figure 7 continued on the next page

Figure 7 continued from the previous page

How does one test samples when using the F/N method?
The samples are directly transferred to culture medium, e.g., the samples are sterility tested, therefore resulting in increased sensitivity for recovery of the surviving microorganisms.

From what is the method derived?
The method is derived from the Most Probable number determinations and has a relation to the Poisson distribution, which is designed to calculate very low numbers at the tail of a population distribution.

When should one use the F/N method?
1. When running the inoculated container/closure R&D fractional exposures to establish kill kinetics for the BI in the container/closure system. The F/N method is used when one believes that the area of the quantal zone of survival is being approached. Note: Where larger populations of survivors are expected in shorter periods of exposure, one may use the direct plate count method.
2. The method will be used when one conducts subprocess production challenges and quantal range zone of recovery is expected.
3. This method is also used to verify the D-values associated with commercial moist heat sterilization biological indicators.

THE MASTER SOLUTION—BIOLOGICAL CHALLENGES

If only two or three solutions are being submitted in a regulatory application, one may as well microbiologically challenge each solution in the subprocess or minimum sterilization run. However, if there are many solutions that will be submitted in the regulatory application, they are all contained in the same container/closure configuration, and the developmental data indicate that they can all be sterilized in the same cycle, a master solution concept may be used. The master solution concept is based on determining which solution, based on D- and z-values, will afford the most resistance to the challenged microorganism using subprocess conditions for the cycle. This means determining which solution has the highest D-value, and a z-value that results in the lowest accumulated lethality per minute in the subprocess cycle thermal profile (see previous section). In other words, when the calculated F_{BIO} or lethality per minute divided by the D-value yields the lowest spore logarithmic reduction, that solution becomes the Master Solution. Another way of describing this is that the master solution is the most difficult to sterilize solution.

How Does One Select the Master Solution?

The table of D- and z-values obtained in the development studies can be used to rank the solutions by D-value with associated z-values. Prior to conducting a subprocess run for regulatory submission, both the

microbiologists and process engineering will have fairly well established the cycle that will be used. A model or an actual run from distribution or penetration thermal runs can be used to prepare a minute-by-minute time at temperature profile. Using the D- and z-value information, one can calculate the accumulated lethality that would occur in each solution that will be sterilized in that particular cycle. This approach can be used for the selection of the master solution, but the same data should be prepared using the actual subprocess (qualification) run data for regulatory submission purposes.

For BIs, except *B. coagulans*, electrolytic solutions such as KCl or 0.9% NaCl yield high resistance, whereas mixtures of carbohydrates and electrolytes afford nominal resistance (Feldsine, Schechtman, and Korczynski 1977; Moldenhauer, Rubio, and Pflug 1995; Berger and Nelson 1995). Carbohydrate solutions permit a fairly rapid inactivation of heat resistant spores. *B. coagulans* has the highest resistance in the presence of divalent ions, e.g., calcium gluconate (Moldenhauer, Rubio, and Pflug 1995).

The master solution concept is understood by regulatory agencies, and they will accept these data providing that the given cycle is provided with a table of accumulated lethality rated per minute for each solution. Again, this necessitates knowing the D- and z-values of the BI in each solution. When submitting the calculated lethality rates, the calculated PSLR for each solution of the subprocess cycle must be given.

Special Considerations Related to the Design of the Subprocess Solution Challenge

1. If only several solutions are being submitted in the filing, it may be less complicated to challenge all of them and not be concerned about a master solution concept.
2. High solution viscosity does not necessarily mean that the solution affords the most resistance to spores. However, if a solution is extremely viscous, questions by reviewers will be avoided if the solution is also challenged. In such cases, the heat penetration thermocouples would also be placed in the viscous solution.
3. Sometimes several solutions have very similar PSLRs. In this case, challenge each solution.
4. If the above do not apply, use the master solution concept.

An example of a testing strategy for FDA submission is included in Figure 8.

Figure 8. Example of Testing Strategy for FDA Submissions

Solution/Drug Challenge

1. Conduct Master Solution Challenge or strategies for solutions provided in the text of this chapter.
2. Use the Master Solution or the most viscous solution product for heat penetration data if the given subprocess cycle of these solutions is also processed in the cycle being used for the subject solution/drug.

Closure Site Challenges

1. Two types of challenges should be considered:
   a. Distributed challenge, whereby containers with inoculated closures are distributed throughout 20 locations in the sterilizer chamber.
   b. Discrete zone-located containers with inoculated closures placed in the three coolest chamber zones, as identified by previous engineering heat penetration and temperature distribution studies.
2. Testing of the samples.
   a. The distributed samples should be accompanied by a thermocouple in the near vicinity. The samples are distributed because in some processes, such as spray heating, one may find some interesting data based upon the spray patterns within the chamber. Test inoculated closure sites by direct plate counting. The regulatory agency may question your choice, so be prepared with appropriate answers.
   b. Locate thermocouples near the three discrete zone samples. Test these units (replicates) in each of the three locations by the F/N method. Compare these data to the data received from the distributed samples. Present both sets of data in the regulatory filing.
   c. All port sites should be inoculated with a dry heat-resistant microorganism and a moist heat-resistant microorganism.

CALCULATION OF THE REQUIRED HEAT HISTORY FOR PROCESSES AT TEMPERATURES OTHER THAN 121°C

There is nothing magical about sterilizing products at 121.1°C. Many individuals believe that this is the only sterilization temperature allowed, but that is not true. Different temperatures can be used to achieve sterilization. However, the amount of time required to achieve the desired SAL also changes. Lower temperatures generally require longer exposure times, whereas higher temperatures require shorter exposure times. This is important to recognize when assessing the sterilizability of some types of containers and/or solutions. For example, many plastic containers, which cannot be sterilized at 121.1°C, can be successfully terminally sterilized at lower temperatures.

When using alternate sterilization temperatures, the process times required to provide a given F-value can be calculated by using a mathematical approach. Many calculations exist, the most common being those by Stumbo (1982) and Pflug (1999).

When a process temperature differs from 121.1°C, the equivalent microbial lethality per minute at 121.1°C can be calculated using the following formula where L is the lethal rate/min. for T_a defined as:

$$L = Log^{-1(T_a - T_b)/z} = 10^{(T_a - T_b)/z} \quad (3)$$

In this equation, the variables are defined in accordance with the following:

T_a = the heat penetration temperature within the container or item.
T_b = the reference temperature used for the process, typically 121.1°C.
Z = the temperature in degrees that it takes to reduce the microbial population by 90% or one log. In F_o calculations, it assumes 10°C or 18°F.

Using the lethal rate calculation, one would find that it requires 15.97 minutes at 115.6°C to achieve the same microbial lethality as 4.5 minutes (i.e., $F_o = 4.5$) at 121.1°C.

$$\begin{aligned} L &= \log^{-1(T_a - T_b)/z} \\ &= \log^{-1(115.6°C - 121.1°C)/10°C} \\ &= \log^{-1(-0.55)} \\ &= \text{antilog}^{(-0.55)} \\ &= 10^{-0.55} \\ L &= 0.2818/\text{minute} \end{aligned}$$

If F_o @ 121.1°C = 4.5 min.

then $\frac{4.5}{0.2818}$ = 15.97 min. at 115.6° is required to attain the same microbial lethality as 4.5 minutes (F_o) at 121.1°C assuming a z-value of 10.

FRACTIONAL OR HALF-CYCLE DEVELOPMENT APPROACHES

Fractional studies involve performing a number of developmental validation studies using exposure times that have been established to provide varying F_o values. This may involve running several cycles with exposure times set to be "x" minutes apart, or running fractions of established cycles, e.g. 1, 1/2, 3/4, and a complete cycle. The data from these runs are used to determine a cycle that will yield the desired SAL (Caputo et al. 1967).

A variation of this type of cycle development is the half-cycle approach. This approach is commonly used in ethylene oxide cycles. Studies are run until all biological challenge units containing approximately 10^6 spores per indicator are inactivated and no survivors occur. The cycle time is then doubled to ensure that a 10^{-6} sterility assurance level can be attained.

CONTAINER CLOSURE INTEGRITY TESTING

In addition to evaluating the container/closure system for microbial inactivation, the system must be tested to ensure that the product and package are integral and will maintain sterility following the sterilization cycle. One type of testing used to perform this analysis is the microbial ingress test (Korczynski 1987). In recent years, FDA has allowed this test to be replaced by a validated analytical method that has sensitivity at least equal to the microbial ingress test. For a complete discussion of the available methods and associated advantages and disadvantages, see PDA Technical Report Number 27, *Pharmaceutical Package Integrity* (1998). Since this test is concerned with maintenance of sterility following sterilization, it is performed following a defined worst-case sterilization, at maximum exposure time and maximum exposure temperature (FDA 1995).

The USP has published a draft chapter on this topic in a recent *Pharmacopeal Forum*. This chapter identifies when testing is warranted. The FDA has clearly indicated that the sterility test alone does not provide adequate assurance of the maintenance of sterility (FDA 1994).

Maintenance of sterility testing does not end with the submission of the NDA. This testing must continue when evaluation lot samples have been placed into long-term stability testing stations as part of a marketed product stability program.

In the United States, one must conduct marketed product stability studies following FDA approval of the new product. Long-term stability studies must be conducted from the first three production lots. Additionally, samples must be placed on annual shelf-life stability studies. The samples must be tested at the beginning and end of the shelf-life period, as well as at the expiration date of the product.

THE MASTER SOLUTION—HEAT PENETRATION

Similarly to the master solution concept for the biological challenge, a master solution can be used for heat penetration probes. This solution for engineering heat penetration studies is the most viscous solution produced, or a placebo that is at least as viscous as the most viscous solution produced.

The Master Equipment Challenge

The use of aseptic processing requires that many equipment or component items be individually wrapped for sterilization prior to introduction into the cleanroom areas. For equipment loads that are steam sterilized, there may be many different types of equipment in the load configuration. It is common to validate these types of cycles as minimum and maximum loading configurations. In determining what should comprise a minimum load, some companies perform studies to determine which piece of the equipment is slowest to heat. This is accomplished by probing all of the equipment in the load and determining which one has the lowest accumulated F value at the end of the cycle. This piece of equipment is designated as the slowest to heat and must be included in the minimum load.

What Thermal Distribution and Penetration Data Are Expected?

Heat penetration and temperature distribution studies must be performed to determine the sterilization cycle to be submitted for regulatory approval. Depending on the type of sterilizer used, 12, 24, or even 48 thermocouples or thermoprobes may be used for the heat penetration and temperature distribution studies. The studies may be run concurrently. These studies should ensure that the load is consistently exposed to sufficient heat input to achieve the desired sterility assurance level. The studies should also show uniform heat delivery to the load. A sufficient number of studies should be run to ensure that the subsequent performance qualification studies are likely to be acceptable, and necessary cycle parameters can be defined.

The studies will need to be repeated for each loading configuration that is used unless, as an individual firm, you can present data to the regulatory agency that permits bracketing of loading configurations. Generally, minute-by-minute values will be recorded. The regulatory reviewer may inspect each line of data for each distribution and penetration thermoprobe location and corresponding measured temperature. It is important to identify locations that are inconsistent in measured thermal values or lower than the other recorded values. Outlier values should also be identified.

It is not a regulatory requirement to use thermocoupled or probed product in a sterilization cycle for approval. If product probes are used in routine processing, one must establish a correlation to the coolest area within the container and the coolest zone within the sterilizer.

Heat Penetration (Thermoprobed Product)

Heat penetration studies are also conducted during OQ studies associated with the development of a new process or sterilization cycle. Also, heat penetration studies need to be conducted during performance qualification (PQ)

studies when container and closure systems, and the subject drug, are microbiologically challenged. It is at this time that thermoprobed containers should be located adjacent to the microbiologically challenged or inoculated containers. Studies are performed using the minimum allowable exposure time and temperature, or a subprocess sterilization cycle for product probe controlled cycles.

Containers, closures, fill volumes, headspace volumes, loading, and viscosity contribute to how the product is heated during the cycle. The heat should be delivered to the product such that variability is reduced and to ensure that the coolest item within a loading pattern receives sufficient heat to meet the required sterility assurance requirements.

Many companies use a bracketing approach of minimum and maximum loads to validate the cycle. Most frequently, the minimum load size is more difficult to sterilize for the biological challenge, due to the reduced time it takes to heat and cool the load. FDA frequently accepts brackets using the minimum and maximum container size for products using the same type of container closure system, e.g., glass vials with rubber stoppers (FDA 1994). When bracketing is performed, each product should be characterized for the container size and type, product viscosity, D-value, and appropriate probing location.

Heat penetration limits should be established for the minimum and maximum F_o that must be achieved in the cycle. Some regulatory agencies impose additional limits that require the product load temperature to attain the sterilizer distribution temperature within a specified time period. Usually, these requirements apply to porous loads (HTM 2010 Part 3 1994). Heat penetration studies using maximum F_o conditions for a cycle may provide data that show heat rate differences among containers during the thermal ramp-up before achieving the exposure peak dwell phase of the cycle. Once all thermocouples or thermoprobes attain the defined temperature of the peak dwell phase, generally only small differences are observed among the thermoprobed containers.

Following heat penetration studies conducted during OQ, the location of the coolest zone in the sterilizer or autoclave will be identified. If a thermoprobed container will be used in the load during production to control the process, a correlation factor must be added to the product control thermoprobe to ensure the coolest container in the load will achieve at least the minimum time at the temperature or F_o value stated in the sterilization specification.

SOPs should also be established for the minimum number of probes that must be present and functional for an acceptable cycle. Probes should also be placed at the appropriate location and should not be touching the tray. Some cycles, e.g., water immersion, may have requirements for minimum and maximum heat penetration probe temperatures during the cycle.

When qualifying rotary sterilizers, there are special concerns regarding the use of probes. Since the entire chamber rotates, it may be difficult to find probes that can survive this process. There are currently several types of wireless sensors that may be placed in products to reduce the risk of failure due to damage to probes during the execution of a study.

It is important to note that the heating characteristics of a sterilizer change to a certain degree when repeated cycles are executed, i.e., it stays hotter for the subsequent cycle. Use of a cold sterilizer frequently gives lower readings than would be found when the sterilizer is operated routinely. For this reason, many companies require that a dummy cycle be run prior to production or qualification studies that are executed following periods when the sterilizer has not been in use. It is common to perform several runs to ensure the reliability and consistency of these runs.

Temperature Distribution Studies

On start-up of a new sterilizer or autoclave, such as during initial commissioning of a vessel, an empty chamber heat distribution engineering study may be conducted. This simply means that a separate protocol may be developed that does not have to be incorporated into OQ or PQ documentation. The study is conducted prior to OQ to prove that wide temperature variances do not exist within the sterilizer. These studies should be conducted while the chamber is fully loaded with empty trays or racks.

Variations of loading within a sterilizer chamber can affect the temperature distribution, depending on the configuration and mass of the items in the load. Temperature distribution studies are performed to ensure that the temperature is consistent and that the chamber items receive uniform heat treatment. Multiple studies are executed to obtain confidence that both maximum and minimum studies will achieve the desired temperature requirements. Heat distribution studies can be influenced by the dimensions and configurations of the load trays or racks, the proximity of the trays or racks to each other, the placement of circulating fans for recirculation of the sterilizing medium, and, possibly, door surfaces that are not jacketed and remain cooler than other surfaces during the sterilization cycle.

Probes should be placed throughout the sterilizer chamber to ensure that a uniform temperature is delivered. Of particular concern to regulatory agencies is whether the sterilizer has a defined cold spot. If so, probes are usually required at that location for all qualification runs and production runs.

Studies may be executed using a random or a geometric (placed throughout the chamber in a three-dimensional X to cover all areas of the chamber) pattern. Some probes are assigned designated locations, e.g., drain, adjacent to the primary temperature recorder (if it is not in the drain), and water loop.

The temperature probe located in the drain may have different temperature requirements than the other probes, since it may be substantially lower in temperature compared to the other probes.

The following types of evaluation parameters are established for distribution probes: minimum and maximum temperature during exposure, maximum disagreement between probes for each minute during the stabilized exposure peak dwell phase, and number of probes that must be present and functional. Temperature uniformity is typically expected to be within 1°C (2°F) of the mean chamber temperature.

TIME WINDOWS

The time duration for each phase of the cycle is determined during initial R&D studies. At this time, the optimum conditions for sterilizing the given product are established. The duration of each event in the sterilization process is defined by both product sterilization requirements and compatibility of the product with the sterilization process. The sterilization process must sterilize the product while maintaining the total quality attributes of the product.

There are typically several events in the cycle that must have established time limitations. The number and type of events are frequently defined by process type. All cycle types include limits for come-up time (steam on, start of peak dwell, or exposure start), exposure dwell time, and cooling time. Table 2 identifies the typical time parameters established for different process types.

Cycle Come-Up Time or Heat-Up Time*

The warm-up or come-up time is the time measured between the end of the prevacuum/steam purge phase and the start of the exposure phase or peak dwell phase. The F_o obtained in the heat-up phase may be calculated once the temperature attains 100°C.

Sometimes, a wide variation in heat penetration values may be observed among containers during the heat-up phase due to the mass or composition of the containers within a load. This variation could continue into the peak dwell phase until equalization of heat penetration values are achieved in all containers. This may be ameliorated by purposely protracting the time of the heat-up phase, thereby allowing additional time for the coolest containers in the load to acquire additional heat penetration values before entering the peak dwell phase.

*"Heat-up" is generally a phrase used in the United States, while "warm-up" is generally used in Europe.

Table 2. Typical Events Monitored for Each Cycle Process Type

Cycle Parameter	Saturated Steam-Vented Process	Saturated Steam-Air Removal Process	Air Steam Mixtures-(With and Without Water)	Water Immersion Cycles
Venting time	Yes		Yes	Yes
Heat-up or warm-up time	Yes	Yes	Yes	Yes
Number of prevacuum exposures		Yes		
Vacuum depth and dwell time		Yes		
Water fill time				Yes
Water fill level				Yes
Exposure dwell time	Yes	Yes	Yes	Yes
Overpressure levels/times			Yes	Yes
Number of postexposure vacuums		Yes		
Depth and dwell time of postvacuums		Yes		
Cooling rate/time/ temperature	Yes	Yes	Yes	Yes
Cycle complete	Yes	Yes	Yes	Yes

Exposure Time

Exposure time may be defined directly from the data generated as the SHZ and converted to F_o. The Ball, and later, Stumbo methods provide mathematical calculations to determine the F_o. In this calculation, the time and temperature data are analyzed to yield f and j values, where f is the time required for the straight line portion of a semi-log heating (f_h) or cooling (f_c) curve to transverse one log; and j is a heating (j_h) or cooling (j_c) lag factor. The data are then used to calculate process lethality. The advantage of a mathematical approach is that a new process time may be predicted from the original heat penetration data when sterilizer temperature, initial product temperature, or product heating rate changes occur.

Depending on the design and composition of the container or device that is to be sterilized, it may be necessary to provide additional overpressure during the peak dwell phase to maintain the shape or functionality of the

product during sterilization. This is accomplished by adding sterile air to the sterilizer. The air that is added increases the pressure within the chamber and aids in the mixing of the air/steam mixture during sterilization.

Calculation of Cooling Times

The cooling time of the cycle depends on the rate of cooling, which is limited by the sterilizer's or autoclave's operational capabilities, maintenance of the container shape or integrity, and personnel safety. Rapid cooling of certain products can cause a deformation of the containers or devices. This may result in the need to apply air pressure during the cycle cooling phase. Further, water spray cooling processes may be used in some sterilization processes. In such cases, the ability of the package to withstand such cooling treatments should be verified. The impact of different cooling rate dynamics on the container is generally determined early in the R&D process and product development stages. The cooling of glass containers and vials is necessary for personnel safety. On opening the sterilizer or autoclave, product should be cooled to at least 60°C to minimize major shattering of glass containers if physical shock were to occur.

LOADING PATTERNS AND CONFIGURATIONS

Loading patterns should be established and documented. Drawings of the configurations are required by many regulatory bodies as part of the submission process.

DRYING CYCLES

Following the cooling process, residual moisture may be retained by the product or packaging. Drying cycle development studies may have to be conducted early in the project. Prior to initiating development studies, one must know what the acceptance criteria for drying are. They can vary from visually dry to volume/weight calculations of the specific concentration of moisture present. Drying can be accomplished in many ways, including vacuum pulses, heating, and air flushes. All of these methods are designed to increase the rate of evaporation.

STERILIZATION AND INTEGRITY OF FILTERS

The sterilization of vent and/or air filters is evaluated in the same way as the sterilizer chamber. Temperature probes and BIs are placed in appropriate within the filter housing. The steam is admitted to the housing, sometimes

directly from the chamber. These probes are not considered part of the routine temperature distribution profile, as the temperatures are routinely higher than in the chamber.

A program should be established to ensure that filter integrity is verified periodically. Additionally, filters should be changed in accordance with the manufacturer's recommendations.

COOLING WATER EVALUATIONS

For those process types utilizing water to aid in temperature distribution, the studies performed should show that the water was sterilized during the exposure cycle. Additionally, microbiological samples should be taken during cooling to verify that the water has been sterilized during the cycle and is sterile or less than 10 CFU/100 mL. If the product is subjected to a cooling spray or immersed in cooling water, data concerning the integrity of the container and closure system following such treatment must exist. These data should be obtained by subjecting media-filled containers to the maximum limits of the cycle peak dwell phase followed by the water cooling phase, and then subjecting the cooled containers to a microbial suspension immersion test (Korczynski 1987).

CONCLUSION

Development of an appropriate sterilization cycle is difficult. There is no one panacea that encompasses everything that can happen in the development process. Sterile products that are terminally sterilized by moist heat sterilization require diligent and thorough engineering processing and microbiological planning. Frequently, firms establish the design of the final product packaging system before personnel have had an opportunity to assess the capabilities and/or difficulties associated with sterilizing the new product. This can impose difficulties on the sterilization functions because they must now "fit a product" into some sterilization process without an opportunity to design the product for the sterilization process. It is highly recommended that sterilization functions within a firm become involved during early product design concept stages, so container and closure design can be modified to ensure ease of sterilization, and the maintenance of container/closure integrity following sterilization. The development of an efficacious and yet economic sterilization process is one of the most critical phases of a product development process.

This chapter is intended to provide some guidance on the topic. However, each site needs to have an established cycle development program that takes into account the facilities and equipment actually used. An example of a

cycle development standard procedure is included in Appendix B. In regulatory inspections, it is becoming more common for field inspectors to also ask for data that have been submitted in support of submissions. The expectation is that the technical personnel at the facility understand why the specific sterilization parameters were selected for the sterilization process. This demands a knowledge of both microbiology and engineering principles.

BIBLIOGRAPHY

Berger, T. J., and P. A. Nelson. 1995. The effect of formulation of parenteral solutions on microbial growth-measurement of D and z values. *PDA J. Pharm Sci. Technol.* 49:32–41.

Berger, T. J., T. B. May, P. A. Nelson, G. B. Rogers, and M. S. Korczynski. 1998. The effect of closure processing on the microbial inactivation of biological indicators at the closure-container interface. *PDA J. Pharm. Sci. Technol.* 52.

British National Health Service. 1994. *HTM 2010-Part 3: Validation and Verification (sterilization).* London: Her Majesty's Stationery Office.

Caputo, R. A., T. E. Odlaug, R. L. Wilkerson, and C. C. Mascoli. 1967. Biological validation of a sterilization process for a parenteral product–fractional exposure method. *Bull. Parenteral Drug Assoc.* 33:214–221.

Code of Federal Regulations. 1976. Title 21, Part 212. Current Good Manufacturing Practices–Large Volume Parenterals (Proposed). Washington, DC: U.S. Government Printing Office.

Committee for Propietary Medicinal Products (CPMP). 2000. Decision Trees for the selection of sterilisation methods (CPMP/QWP/054/98 Corr. Annex to Note for Guidance on Development Pharmaceutics).

CPMP. 1998. Note for Guidance on Development Pharmaceutics, CPMP/QWP/155/96.

European Commission. 2000. "Parametric Release." Proposal for an Annex 17 to the EU *Guide to Good Manufacturing Practice.*

European Pharmacopoeia. 2000. *Microbiological Quality of Pharmaceutical Preparations.*

FDA. 1994. *Guidance for industry for the submission of documentation for sterilization process validation in applications for human and veterinary drugs.* Washington, DC: Government Printing Office.

FDA. 1995. *Guidance for industry for packaging information to be submitted to the Office of Generic Drugs.* Washington, DC: Government Printing Office.

Feldsine, P. T., A. J. Schechtman, and M. S. Korczynski. 1977. Survivor kinetics of bacterial spores in various steam heated parenteral solutions. *Dev. Ind. Microbiol.* 18:401–407.

Korczynski, M. S. 1980. Concepts and issues–Container closure validation. *J. Parenteral Drug Assoc.* 34:277–285.

Korczynski, M. S. 1987. Evaluation of closure integrity. In *Aseptic Pharmaceutical Manufacturing,* edited by W. P. Olson and M. J. Groves. Prarie View, IL: Interpharm Press.

Moldenhauer, J. E., S. Rubio, and I. J. Pflug. 1995. Heat resistance of *Bacillus coagulans* spores suspended in various parenteral solutions. *PDA J. Pharm. Sci. Technol.* 49:225–238.

Odlaug, T. E., R. A. Caputo, and C. C. Mascoli. 1981. Determination of sterilization F-values by microbiological methods. *Dev. Ind. Microbiol.* 22:349–356.

Owens, J. E. 1993. Sterilization of LVPs and SVPs. In *Sterilization Technology: A Practical Guide for the Manufacturers and Users of Health Care Products*, edited by R. F. Morrisey and G. B. Philips. New York: Van Nostrand Reinhold.

PDA/FDA Proceedings of the joint conference on aseptic processing. Transcripts issued by PDA October 1993.

PDA, Technical Report 8, Parametric Release of Parenteral Solutions Sterilized by Moist Heat Sterilization. *PDA J. Pharm. Sci. Technol.*

PDA, Technical Report 17, Current Practices in the Validation of Aseptic Processing, *PDA J. Pharm. Sci. Technol.* 46 (supplement), 1992.

PDA, Technical Report 24, Current Practices in the Validation of Aseptic Processing, *PDA J. Pharm. Sci. Technol.* 50 (supplement), 1996.

PDA, Technical Report 27, Pharmaceutical Package Integrity. *PDA J. Pharm. Sci. Technol.* 52(5) supplement, 1998.

Perkins, J. J. 1973. *Principles and Methods of Sterilization in Health Sciences.* Springfield, IL: Charles Thomas.

Pflug, I. J. 1977. Survivor curves of bacterial spores heated in parenteral solutions. In *Spore Research,* 1976, Vol. II, edited by A. N. Barker, J. Wolf, D. J. Ellar, D. J. Dring, and G. W. Gould. London: Academic Press.

Pflug, I. J. 1999. *Syllabus for an Introductory Course in the Microbiology and Engineering of Sterilization Processes,* 10th ed. Minneapolis, MN: Environmental Sterilization Services.

Stumbo, C. R. 1982. *Thermobacteriology in Food Processing.* New York: Academic Press.

USP. 2000. United States Pharmacopeia XV, <1038> "Biological Indicators for Sterilization." Rockville, MD: U.S. Pharmacopeial Convention.