Technical Monograph No. 1

VALIDATION OF STEAM STERILIZATION CYCLES



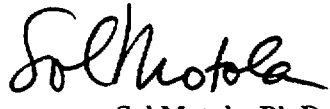
PARENTERAL DRUG ASSOCIATION, Inc.

GENERAL PREFACE

THIS IS THE FIRST of a series of Technical Monographs to be communicated through the efforts of the Parenteral Drug Association Research Committee and Task Groups. These monographs are meant to serve as informative and instructional technical documents designed to provide a basic understanding of certain key areas of parenteral technology.

Due to the dynamic nature of this field of research, we are not posing the monographs as all inclusive, compelling or otherwise absolute in scope. It is our aim to provide current, well founded and practical information of which the reader can make direct use of in the areas of discussion.

Monograph No. 1 titled *Validation of Steam Sterilization Cycles* presents a straightforward approach towards validation procedures for steam sterilization cycles and was the effort of Task Group No. 13 under the chairmanship of Dr. Michael Enzinger.



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PREFACE

Heat sterilization is a probability function dependent on heat exposure, the number of microorganisms, and the heat resistance of those microorganisms. Currently, several health-related trade associations are considering establishing acceptable probabilities for sterility based on the end use of various product classes. In the interim period, it is recommended that steam sterilization processes provide a level of assurance of at least 1×10^{-6} (fewer than one non-sterile unit per one million units) probability of survival (non-sterility) for terminally sterilized parenterals.

This document considers validation studies for steam sterilization. Two basic approaches are currently used by the industry in developing sterilization cycles. The first approach is to establish cycle parameters based on the number of microorganisms in the product (bioburden) and the heat resistance of those microorganisms. The combination of number and resistance will determine the amount of heat required to provide less than 10^{-6} probability of microbial survival in the product. The Probability of Survival approach recognizes that the microbial attributes of a product will determine the F_0 required for adequate sterilization. Generally, this approach would be employed when developing and validating sterilization cycles for "heat labile" products. However, if one chooses, it could be utilized for heat stabile materials.

The second approach is an overkill sterilization process which is normally employed when the material can withstand heat treatment without adverse effects. Employment of the Overkill approach will provide assurance of a 10^{-6} probability of survival regardless of microbial numbers and heat resistance (in most cases the probability will be considerably less than 10^{-6}). When it is possible to use the overkill method, it offers the advantage of eliminating the need for bioburden and resistance studies in developing sterilization cycles. Generally this approach would be employed when developing and validating sterilization cycles for heat stable products.

SCOPE AND ORGANIZATION

It is not the intention of this document to imply that the methods and approaches herein must be followed in validation of a sterilization cycle. It is recognized that technically equivalent methods are currently employed by the industry to validate sterilization cycles. However, it is intended to serve as a step-by-step outline that, if used, will provide an acceptable approach to validation of steam sterilization processes.

This document is subdivided into three sections. The first two sections address the Probability of Survival approach to validation (Laboratory and Plant studies); the last section addresses the Overkill approach to validation of steam sterilization cycles.

Procedures for routine monitoring of sterilization cycles and specifications for sterilizer design and sterilizer performance are not addressed.

OVERVIEW

A. Probability of Survival Approach

Sterilization of heat labile materials requires a thermal process that provides adequate assurance of sterility without resulting in product degradation due to heat exposure. Both overheating and underprocessing will result in an unacceptable product. Therefore, the development and validation of sterilization cycles for heat labile materials by necessity require more comprehensive investigation than heat stable items subjected to "overkill" sterilizing conditions.

The primary objective in sterilizing heat labile materials is to provide the amount of heat that will result in an acceptably low probability of non-sterility yet not adversely affect the product.

In order to establish that materials are consistently exposed to sufficient heat lethality, the following studies should be performed:

1. Laboratory studies to determine the number (bioburden) and resistance of microorganisms associated with the product. These studies will determine the minimum lethality required to obtain a specified probability of a microorganism surviving the sterilization process (Probability of Survival).

2. Calibration of Biological Indicators (Challenge Microorganisms) in order to monitor process lethality.
3. Plant Studies (in production vessels) to determine:
   a. Acceptability of vessel for providing a uniform heating medium (Heat Distribution)
   b. The coolest location within a container and loading pattern (Heat Penetration).
   c. The minimum lethality provided by the sterilization cycle through heat penetration and biological challenge studies.
   d. The reproducibility of the sterilization cycle to assure that the product will consistently receive the specified heat treatment.

B. Overkill Approach

An Overkill approach to sterilization may be employed when sterilizing heat stable materials where the primary concern is assurance of sterilization and not heat degradation. It is implicit in the Overkill approach that sufficient lethality is imparted during the sterilization cycle to provide at least a 10^{-6} probability of microbial survival regardless of the number and heat resistance of naturally occurring microorganisms. When employing the Overkill approach, extremely high F_0 values are generally used. It is desirable that the cycle provide an F_0 that will result in at least a 12-log reduction of microorganisms having a D value of at least 1 minute. A sterilization cycle providing a 10^{12} reduction if $D_{121} = 1$ minute will result in 10^{24} reduction if the $D_{121} = 0.5$ minute, and a 10^{120} reduction if the $D_{121} = 0.1$ minute. Considering typical isolates from product and production environments, only the most resistant will have D_{121} values ranging from 0.5 to 1.0 minutes. When the overkill method is used, there is little need to conduct moist heat resistance studies or probability of survival calculations relative to product bioburden microorganisms.

The primary difference in validation between the Probability of Survival and Overkill approaches is the means by which the minimum required process lethality is obtained. When employing the Probability of Survival approach, the required minimum F_0 value is determined based on the number and resistance of microorganisms in the product. Once this determination is made, validation

studies are performed to assure that the coldest point within the load receives that F_0 value. Bioburden and resistance data are not required to determine F_0 values when employing the Overkill approach. Rather, cycle parameters are adjusted to assure that the coldest point in the loading pattern receives an F_0 that will provide at least a 12-log reduction of microorganisms having a D value of 1 minute. However, individual firms may elect to continue a cursory bioburden screening program, even though excessively high F_0 values are used in sterilization cycles.

It should be noted that the Overkill approach does not imply that less precaution is necessary in preventing microbial contamination. Controlled manufacturing environments and good manufacturing practices which provide barriers to microbial contamination remain of utmost importance.

In order to establish that materials are consistently exposed to sufficient heat lethality when employing the Overkill approach, the following studies should be performed:

1. Calibration of Biological Indicators (Challenge Microorganism) in order to monitor process lethality.
2. Plant studies (in production vessels) to determine:
   a. Acceptability of vessel for providing uniform heating medium
   b. The coldest location within a loading pattern
   c. The minimum lethality provided by the sterilization cycle is sufficient to provide at least a 12-log reduction of microorganisms having a D value of 1 minute.
   d. The reproducibility of the sterilization cycle to assure that the material will consistently receive the specified heat treatment.

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I. LABORATORY STUDIES

A. *Bioburden Studies*

Bioburden studies are necessary to determine the minimum heat sterilization process (F_0) that will provide an acceptable assurance of sterility. The number and frequency of lots tested should be determined by the manufacturer based on lot to lot variation in microbial numbers, the potential for microbial growth prior to sterilization and seasonal variations in bioburden. Depending on the type or form of the product, various microbial recovery methods are available. However, discussion will be limited to parenteral solutions realizing that comparable methods are available for parenterals and other types of products.

1. Conduct microbial counts in an area suitable for microbiological testing.
2. Filter an appropriate volume of parenteral solution, sampled from the final container, through 0.45-μm membrane filters (plate count methods may be used when the number of organisms in the solution is high).
3. Incubate the filters on a suitable non-selective medium (such as Soybean Digest Agar, Tryptone Glucose Extract Agar, Eugon Agar) at 30–35°C for at least 72 hours.
4. Calculate the number of microorganism per container of parenteral solution.

B. *Apparatus for Obtaining Heat Resistance Data*

There are several types of apparatus that allow determination of resistance data in an accurate and reproducible manner. For detailed description of these apparatus consult *Laboratory Manual For Food Canners and Processers,* Volume 1, pp. 171–186 (1) or *Thermobacteriology In Food Processing;* Stumbo pp. 79–92 (2).

The two types of apparatus most commonly used are listed.

1. *Oil or Glycerol Bath*

   Sealed ampoules or capillary tubes containing a liquid suspension are immersed in a bath at a constant temperature for a specified amount of time.

2. *Miniature Retort or Autoclave*

The miniature retort is the most versatile apparatus used in determining heat resistance data. A variety of types of samples (i.e., suspensions in ampoules, paper strips, spores inoculated on solid carriers and inoculated products) can be exposed within the chamber. The miniature retort-autoclave offers the advantage of providing rapid heating and cooling phases during the exposure period.

C. *Laboratory D Value Studies*

When heat labile products will not withstand excessive heat treatment, D value studies of product isolates are necessary to determine the minimum heat sterilization process (F_0) that will provide an acceptable assurance of sterilization. Also D value studies are necessary for calibration of Biological Indicators (such as spores of *Clostridium sporogenes* and *Bacillius stearothermophilus*).

1. *Laboratory* D *value studies should be conducted on:*
   a) Biological indicators whether prepared in house or purchased commercially.
   b) Resistant Product Isolates (those organisms periodically isolated from various products that through screening tests are shown to be heat resistant).
2. *Heat Resistance Screening Tests for Product Bioburden*

The purpose of screening tests is to select those heat resistant microorganisms for which D values should be determined. In order to design a screening test the process lethality (F_0) used in routine sterilization and the number of microorganisms expected in a given product should be considered. Although various screening methods may be employed, an example involving solutions is presented for clarity.

   a) Heat shock the solution for 10–15 minutes at 80°–100°C to eliminate vegetative cells and to stimulate germination of spore forming microorganisms.
   b) Pass the heat shocked solution through a sterile 0.45-μm membrane filter. Incubate approximately one half of the filter in an appropriate aerobic medium and one half in an appropriate anaerobic growth medium (e.g., Soybean Casein Digest Broth, Thioglycollate Broth).

c) Survivors should be characterized with respect to *D* value.

3. *Type of Carrier or Suspending Medium*

The type of carrier or suspending medium used is an important consideration when determining *D* or *z* values since the carrier may significantly affect resistance. When validating sterilization cycles for a given parenteral solution, *D* values are generally obtained by inoculating spores into the solution in question. Also, to allow comparison between different lots of biological indicators, *D* values are generally determined in a standard reference solution (e.g., Sorensen's Buffer, distilled water, etc.). In the case of devices or solid materials, spores can be inoculated onto precut samples of the same composition rather than on the entire device.

D. Laboratory D *Value Methodology*

1. A suspension containing a known number of spores is used to inoculate replicate samples of parenteral solution, paper strips or solid carriers.
2. Positive controls are necessary to determine the number of spores in the suspension (or spore strip etc) to be tested. The number of spores required will be based on individual experimental design but is generally in the order of 10^4–10^7 spores per sample.
3. Expose at least 5 samples at a specified temperature for at least 3 different time intervals.
4. The number of survivors can be determined by colony count, or by quantal methods (fraction negative).

E. Estimation of D *Value by Survival Curves*

1. When survival data is determined by Colony Forming Units a semilogarithmic curve is generated by plotting the logarithm of the number of survivors versus heating time (*U*) as shown in Figure 1. (See Appendix.)
2. A straight line is fitted to the data by linear regression according to the following model. (This equation assumes first order death kinetics.)

$$y = a + bx$$

where:

y = $\log_{10}$ number of survivors at time x
x = heating time u
a = y intercept at time 0
b = slope of the line

The D value is the negative reciprocal of the slope

3. For a more detailed description of D value calculation, including the intercept ratio, consult Pflug (3).

F. Estimation of D *Values by Fraction Negative Methods*

1. At least 10 replicate samples having the same number of organisms are heated at each time interval.
2. After heating, the samples are incubated in a suitable broth (such as Soybean Casein Digest, Fluid Thioglycolate, etc.) at the microorganisms optimum growth temperature for a minimum of 7 days. Record the fraction of samples negative for growth.
3. Two alternative approaches are recommended for evaluating quantal data; (*a*) MPN method used by Stumbo, Murphey & Cochran (2, 4) and (*b*) the Spearman–Karber Estimate (5).

a) MPN

When employing the MPN method, data can only be analyzed when a fraction of the replicates are negative. The D value is estimated according to the formula:

$$D = U/\log_{10} A - \log_{10} B$$

where:

U = heating time at specified temperature
A = initial number of organisms on each replicate

$$B = 2.303 \log_{10} (n/q)$$

where:

n = total number of replicates
q = number of negative replicates

A D value may be determined at each time interval according to the above formula. The average D value (the arithmetic mean

of individual D values at different time intervals) should be calculated.

b) When the Spearman Karber Method is employed, D is calculated according to;

$$D = \frac{2.3026\bar{x}}{(0.57722 + In\ No)}$$

where:

No = initial number of spores on each replicate
where:

$$\bar{x} = \sum_{i=1}^{k} (t_{i+1} - t_i)(P_{i+1} - P_i)$$

where:

t_i = heating time
P_i = proportion of sterile tubes at t_i

When the intervals between heating times are equal the following equation is used

$$\bar{X} = t_k - d/2 - d/n \sum_{i=1}^{k-1} Pi$$

where:

d = interval between heating times
t_k = the first time interval showing all negative replicates
No time interval longer than t_k should have positive replicates. The variance for the above equation is:

$$V(\bar{x}) = \frac{d^2}{N-1} \sum_{i=1}^{k} P_i(1 - P_i)$$

where:

N = the number of replicates
and the 95% confidence interval is

$$\bar{x} \pm 2\sqrt{V(\bar{x})}$$

Consult Pflug (6) for further explanation of the Spearman–Karber Method.

G. Correction for Heating and Cooling Lags When Determining Laboratory D Values

If come-up and come-down time are significant when heating, corrections should be made.

1. Correcting for Cooling and Heating Lags
   a) Temperature is measured by situating a thermocouple within the item being heated. Record temperature and time at frequent intervals until the item reaches the set temperature. Repeat this procedure for the cooling phase by recording time and temperature immediately after the item has been removed from the heating medium or after steam has been shut off.

   b) Calculate the lethality for each time temperature interval above 100°C by the following formula:

$$L = \log^{-1} \frac{(T_0 - T_b)}{z} = 10^{T_0 - T_b/z}$$

It is appropriate to assume $z = 10°C$
T_0 = temperature within item being heated
T_b = temperature at which D value is determined

Lethal Rates may also be obtained from Lethal Rate Tables. An example is provided in Table 1 and 1A (Appendix).

   c) Integrate the lethality accrued during come-up and come-down time. (The Patnashnik method is described in the Appendix, Table 2.)

   d) The lethality during come-up and come-down is added to the time at temperature to determine the value of U.

$$U = \underset{\text{comeup}}{\text{lethality during}} + \underset{\text{at set temperature}}{\text{time of product}} + \underset{\text{comedown}}{\text{lethality during}}$$

If come-up and come-down times are negligible then U = time the product was exposed to heating medium.

   e) Calculate D with the adjusted heating time.

H. Determination of z Values

The z value of a microorganism is a measure of how heat resistance changes with changes in temperature. z is defined as the number of degrees temperature change to change the D value by a factor of ten. It is the z value that allows integration of the lethal effect

of heat as the temperature changes during the heating and cooling phases of a sterilization cycle. The z value is necessary to make calculations that allow comparison of spore lethality at different temperatures. It should be noted that solution characteristics such as pH, electrolyte or carbohydrate concentrations may influence the D and z value of microorganisms. z values can be determined as described below;

1. Determine the D value of an organism at a minimum of three different temperatures.
2. Construct a Thermal Death Curve by plotting the logarithm of D value on the ordinate of graph versus the temperature on the abscissa as illustrated in Fig. 2 (Appendix).
3. Draw a straight line through the data points. The z value is the change in temperature for the D value to change by a factor of ten (or to change by one logarithmic number). The z value is also equal to the negative reciprocal of the slope.

I. z Value Assumptions

For the purpose of process calculations involving the resistance of indigenous microorganisms it is appropriate to assume $z = 10°C$ (18°F). The z value should be verified for biological indicators when the indicator is used to measure integrated lethality during validation of a production autoclave cycle. Descrepancies may occur between F_0 values determined by thermocouple (F_0 assumes $z = 10°C$) and F values determined by biological indicators when the z value of the indicators varies significantly from 10°C.

J. Use of Bioburden Data, z and D Values to Calculate Probability of Survival and Minimum F Value Required for Sterilization

When making process calculations, the value used to represent bioburden is generally the maximum number of microorganisms (total count per container) found in a given product. However, this is a conservative approach because the entire population may not be spore formers. When concerned with the adverse effects of excessive heat it would be acceptable to consider bioburden as the maximum number of spore forming bacteria in a product container. The D values used in process calculations are generally those obtained from the most resistant product isolates. This is also a

conservative approach because it assumes that the entire population consists of the most heat resistant microorganism.

1. The number and resistance of indigenous microorganisms are required to determine the level of sterility assurance provided by a previously established autoclave/retort cycle or to determine the F value required when developing a new sterilization cycle.

   a) Determination of Probability of Survival

   When an F_0 is known for a sterilization cycle, the probability of microbial survival for that cycle is calculated according to:

$$\log_{10} B = \log_{10} A - F_0/D_{121}$$

   where:

   B = probability of survival
   A = bioburden/product
   D_{121} = time at 121°C (250°F) to reduce population of most resistant organism found in product or environment by 90%.
   F_0 = equivalent minutes at 121°C (assume Z = 10°C)

   Example:
   Minimum F_0 provided by a production cycle = 4 minutes (previously determined by thermocouple and/or biological indicator)
   A = 100 spores/filled container (previously determined by bioburden studies)
   D_{121} = 0.4 minutes (most resistant indigenous flora)

$$\log_{10} B = 2 - 4/0.4 = -8$$

$$B = 1 \times 10^{-8} \text{ Probability of Survival}$$

   b) Determination of Minimum Required F_0 Value
   When determining the lethality required for a new sterilization cycle, the bioburden of the product, resistance of indigenous microorganisms and the maximum acceptable level for microbial survival must be known. The following formula is used to establish a minimum F value.

$$F_0 = D_{121}(\log_{10} A - \log_{10} B)$$

where:

F_0 = minimum lethality required; assume $z = 10°C$. Expressed as the number of minutes equivalent to time at 121°C that the coldest container in the load should be heated

B = maximum acceptable level for probability of survival which is usually determined by Quality Control

A = bioburden per container

Example:

$B = 1 \times 10^{-6}$ (1 unit in 1 million will contain a surviving spore)

A = 100 spores/filled container (established from bioburden studies)

D_{121} = 0.5 minutes (established from laboratory resistance studies)

$\therefore F_0 = 0.5\,[2 - (-6)] = 4.0$ minutes

In this example it has been established that the coolest container in the autoclave load should receive the equivalent of 4.0 minutes at 121°C.

c) Determination of Process Time

The process time of a sterilization cycle required to impart the minimum required F value can be determined as follows:

(1) Establish the load cold point by thermocouple and adjust sterilization time such that the coldest container is at process temperature for the specified amount of time (4.0 minutes in the example mentioned above). This does not consider the additional lethality received by the product during the heating and cooling phase of the sterilization cycle.

(2) Establish the load cool point by thermocouple and adjust sterilization time such that the integrated lethality in the coolest container is equal to or greater than F value required. (see Section II, D, E, F)

(3) The process times required to provide a given F value can be calculated by a mathematical approach as described by Stumbo (2) or Pflug (5).

K. Equivalent Times

1. Process Temperatures other than 121°C

When process temperatures differ from 121°C, equivalent times can be determined through use of the following formula:

$$F_t^z = F_{121}^z / L \qquad (1)$$

where:

F_t^z = the equivalent time at temperature T delivered to a container for the purpose of sterilization with a specific value of z.

F_{121}^z = the equivalent time at 121°C delivered to a container for the purpose of sterilization with a specific value of z (when z = 10°C then $F_{121}^z = F_0$)

$$L = \text{Lethal Rate} = \log^{-1} \frac{(T_0 - T_b)}{z} = 10^{T_0 - T_b/z}$$

where:

T_0 = temperature within container or item.

T_b = base temperature (121°C)

(Lethal Rates may also be obtained from Lethal Rate Tables. An example is presented in Table 1 and 1A (Appendix.)

Equation (1) demonstrates that the following time temperature relationships provide equivalent lethality (assume z = 10°C).

$F_{115.6}^{10}$ = 4.5/.288 = 15.6 minutes at 115.6°C (240°F) =

$F_{121.1}^{10}$ = 4.5/1 = 4.5 minutes at 121°C (250°F)* =

$F_{126.7}^{10}$ = 4.5/3.72 = 1.21 minutes at 126.7°C (260°F).

* 121°C is used as the equivalent of 250°F throughout this monograph. It should be noted that 121.1°C is the actual equivalent of 250°F.

II. PLANT STUDIES

A. Calibration of Temperature Monitoring Equipment

1. Thermocouples made of insulated wire suitable for the sterilizing conditions (e.g., iron constantan, copper constantan or chromel constantan) are inserted into the autoclave chamber through pressure tight seals.
2. The thermocouples are then attached to an appropriate multipoint recorder having a temperature scale with gradations of not more than 1°C (2°F).
3. Bundle the sensor ends of the thermocouples as close together as possible and immerse them in a reference bath at the same depth and as close as possible to the bulb of a certified reference thermometer (NBS traceable).
   a) Check calibration before and after a validation study; after thermocouples have been placed in an autoclave or after the electronics in the recorder have been repaired.
   b) If heating bath (oil or glycerol) is used for calibration, it is recommended that a temperature that approximates that of the sterilization cycle also be used.

   Also the accuracy over of the operational temperature range (100°C to sterilization temperature) should be determined to eliminate significant error in integrating lethality during heating and cooling.
4. Turn on the recorder and allow the components to equilibrate before adjustments are made.
5. Compare the recorder readings with those of the reference thermometer. Adjust the recorder such that readings are within 0.5°C (1°F) of the reference readings.
6. Those thermocouples that do not show agreement in readings with the reference thermometer should be checked for shorts, bad connections, reversed polarity, etc. If the problem cannot be corrected, those thermocouples should be replaced.

B. Certification of Sterilizers

Given the various types of steam sterilizers (such as saturated steam, water immersion, water cascade system, air-steam mixtures,

gravity air displacement and vacuum air displacement) it is beyond the scope of this document to precisely define operational characteristics. However, it is recommended that a certification (qualification) program be established prior to the routine use of a new or modified sterilization systems. The purpose of the certification program is to assure that equipment is capable of routinely performing within the limits of established process specifications.

Below is a suggested list of major items to be included.

1. Equipment Specifications; i.e., pressure gauges, timing devices. temperature recording devices, etc.
2. Ancillary Equipment Specifications; i.e., steam generator, air filtration system, etc.
3. Utilities Required; i.e., power source, compressed air, cooling water etc.
4. Operational qualification to establish assurance of consistent operation; i.e., empty chamber heat distribution, heat up and cool down rate, chamber and jacket pressure etc.
5. Calibration procedures, preventive maintenance programs and manuals.

C. Loaded Chamber-Heat Distribution

The loading pattern within a vessel may adversely affect temperature distribution depending on configuration and mass of the items. This study is performed to document that temperature distribution is acceptable such that items within the chamber receive a uniform heat treatment.

1. Perform at least one heat distribution study in each sterilizer with a maximum and minimum load configuration. It should be conducted with different container sizes unless it can be established that one particular size represents the least ideal case.
2. Probes should be distributed evenly throughout the chamber (but not within the product containers) to monitor all representative areas. The probes should not contact solid surfaces.
3. One probe should be located in the steam exhaust line or immediately adjacent to the vessel controller sensor.

4. The sterilization parameters should be those specified for the normal production cycle. (This study may be performed during a routine production sterilization cycle.)
5. At least once during the dwell period (preferably Mid-dwell) the following temperature readings should be compared:
   a) Temperature recording chart on vessel
   b) Reference temperature sensor
   c) Thermocouple adjacent to controller sensor

   The readings should agree within 1°C (2°F).
6. Evaluation of Temperature Uniformity:
   The acceptability of temperature uniformity will be dependent on the type of sterilizer (i.e., saturated steam vs water immersion), the amount of heat received by the product, differences in product heating rates within a given load and the heating rate of the coolest container in the load. Acceptable temperature uniformity should be determined by the manufacturer based on the above criteria. However, significant temperature deviations greater than ±2.5°C (±4.5°F) of the MEAN chamber temperature may indicate equipment malfunction. Generally temperature uniformity can be considered acceptable if the deviation is less than ±1°C (±2°F) of the MEAN chamber temperature.

D. Container Cold Spot Studies for Solutions (Container Mapping)

The container cold spot should be determined on different container sizes and types. (However, the container size may limit the number of probes that can be used. An excessive number of probes per container may introduce experimental error due to disruption of normal convection patterns. It should be noted that small volume containers may not have a discernable cold spot.) Fill volume may affect the location of container cold spot. Container orientation may also affect the location of container cold spots (depending on whether the container is sterilized in a vertical or horizontal position).

A miniature retort or production autoclave may be used to perform this study. It may be assumed that the container cold spot mapping remains constant among different sterilizing chambers using the same heating medium.

1. Sufficient probes are inserted within a container to obtain a representative temperature profile.
2. Repeat studies are required to establish that designated cold points are reproducible. If the cold point is not consistent, additional studies should be performed to determine the point that is coldest the majority of the time.
3. If cold points cannot be determined through visual inspection of the temperature data, calculate F values from the thermocouple data (see Section II, G). The point having the lowest F value is designated as the coldest point.
4. In subsequent heat penetration studies position the probes at the coldest point or a suitable reference point within the containers. If thermocouples are placed at a reference point (i.e., the center of the container) data should be available to allow extrapolation of the reference point data to the cool point.

E. Heat Penetration—Load Cool Point Determinations

Heat penetration studies are conducted to assure that the coolest container within a loading pattern will consistently be exposed to sufficient heat lethality.

It is recognized that many technically equivalent methods are currently employed by the industry to obtain and evaluate heat penetration data. However, two approaches are outlined to provide examples, which if used, will provide acceptable approaches to validation.

1. Load Cool Point Determination
   a) This study should be conducted in each chamber using at least maximum and minimum loading configuration. Perform the study on different container sizes and types. Data may be collected that allows interpolation from one container size to another. The container should have maximum fill volume of a solution with heating characteristics as slow as the slowest to heat solution sterilized by the specified cycle.
   b) When more than one pallet or layer are situated within the chamber, probed containers should be placed equally among each layer (or pallet). This test should be repeated at least once with a different probing configuration and also maintaining equal representation among layers (or pallets).

c) One probe should be located in the steam exhaust line or immediately adjacent to the vessel controller sensor.

d) At least once during the dwell period (preferably Mid-dwell) the following temperature readings should be compared:
1) Temperature recording chart on vessel
2) Reference temperature sensor
3) Thermocouple adjacent to controller sensor
The readings should agree within 1°C (2°F).

e) The sterilization parameters should be those specified for the normal production cycle.

f) The temperatures difference between layers (or pallets) may be compared by determining

$$\Delta F_L = \bar{F}_L - \bar{F}_t$$

where:

$\bar{F}_L$ = average F for each layer
$\bar{F}_t$ = average F for the total load

g) Note the layer with the largest negative ΔF_L. If there is a significant difference in a one-sided T test ($p = 0.05$) between $\bar{F}_L$ and the average F of the load, the majority of probes should be concentrated in this cool layer in subsequent studies. If there is no significant difference, distribute the probes equally throughout the load in subsequent heat penetration studies. (A detailed explanation of the Students t Test can be found in any introductory statistics text, i.e., Introduction to Statistics, 1969, Dixon and Massey, McGraw Hill Co.)

h) When there is a concern with heat degradation of the product, the same approach as described above, may be used to determine the upper limit for F values. This may be important when sterilizing heat labile products which are subject to loss of total product attributes when specific F_0 values are exceeded.

2. Monitoring of Heat Penetration During Routine Production Cycles.
As an alternate approach the following may be employed when F_0 value integrations are used to monitor routine production cycles.

a) F_0 values at the coolest location (and if necessary the hottest location) can be determined and correlated with the F_0 value obtained with a Reference thermocouple that is used to monitor each production load.

b) Conduct multiple heat penetration studies using multiple thermocouples to obtain F_0 values for the coolest and reference locations within the load. During these studies determine:

1) Reference Thermocouple F_0 value = R (The reference thermocouple is positioned within a product container. The location of the container in relation to the rest of the load should remain constant from run to run.)

2) Minimum F_0 Value in load = C

c) Calculate the ratio of R to C for each penetration study and determine the maximum R/C ratio (i.e., ratio of the widest established F_0 range between R and C).

d) Example: If the coolest container in the load must achieve an $F_0 = 8$ (predetermined by bioburden and microbial heat resistance data), then the Reference thermocouple used to monitor production runs should receive an F_0 greater than $8.0 \times$ (maximum R/C ratio) = the lower F_0 limit for the Reference thermocouple used to monitor the cycle.

e) The same approach may be used to calculate the acceptable upper limit for the Reference thermocouple by determining the maximum ratio of R to H (where H = maximum F_0 value in the load). This approach may be necessary when sterilizing heat labile products which are subject to loss of total product attributes when specific F_0 values are exceeded.

3. Reproducibility Studies (Validation of Consistency)

a) Once the cold point of the container and cold area of the loading pattern have been established, reproducibility studies should be conducted by repeating the sterilization cycle with the majority of probes situated in the area designated as the coldest. Probes should also be situated in the area designated as the hottest, if concerned with heat degradation of product. Sufficient repeat studies should be conducted to provide the desired level of confidence that the delivered F_0 value will be consistantly greater than the minimum required F_0 value.

b) The remaining probes should be equally distributed throughout the load. The position of all probes should remain the same during the reproducibility studies.

c) Data collected during heat penetration studies can be evaluated statistically to determine the variation expected in the lethality provided by a given cycle. For example, a mean F for the coldest point and its standard deviation can be calculated. This information can be used to demonstrate that the cycle will provide a given lethality with a given confidence interval. For example;

i. The predicted lower range for the F_0 population mean is

$$\bar{x} - \frac{Sx}{\sqrt{n}} t$$

where:

$\bar{x}$ = mean of minimum F_0 values
Sx = standard deviation
n = number of F_0 values
t = students t value (obtained from one sided t tables)

ii. Assume that bioburden and resistance studies have established that an $F_0 = 5$ minutes is required to provide a 1×10^{-6} probability of survival for bioburden flora.

iii. Assume minimum F_0 values from four heat penetration studies were 8.0, 6.0, 6.5, 6.5.

$$\therefore \bar{x} = 6.8$$

$$Sx = 0.87$$

$t = 2.92$ (from Student's t Table at 95% Confidence level)

iiii. The lower confidence limit for $\bar{x}$ would be;

$$6.8 - (0.87/\sqrt{4} \times 2.92) = 5.5$$

Conclusion: there is greater than 95% level of confidence that the mean of the minimum F_0 values will be greater than the required F_0 value of 5 minutes.

F. *Calculation of Delivered Process Lethality (Alternative Approaches)*

1. Integration of Lethal Rates

   a) The temperature data collected during heat penetration studies is converted to lethal rates at specified time intervals by the following formula:

$$L = \log^{-1} \frac{(T_0 - T_b)}{z} = 10^{T_0 - T_b/z}$$

   where:

   T_0 = temperature within the container
   T_b = process temperature (i.e., 121°C)
   z = temperature increment required to change the D value by a factor of 10. (z = 10°C may be assumed.)

   b) F_0 is then determined by integrating the lethal rates throughout the heating process

$$F_0 = \int_{T_1}^{T_2} L \, dt$$

   c) There are several methods by which lethal rates can be integrated (2, 3), however, the Trapazoidal Rule Computer Program described by Pflug (3) and the Patashnik Method (see Appendix) are the least difficult yet provide reliable estimation of F values.

2. Time at Temperature

The time that the coldest point in the loading pattern is at process temperature can be used to express lethality. (F = time at process temperature) This approach is inherently conservative because it does not consider the lethality during the heating or cooling phases of the cycle (which may be considerable with LVP's or viscous liquids).

3. Sterilization at Temperatures Other Than 121°C (250°F)

When process temperatures other than 121°C are used for sterilization, the amount of time providing equivalent lethality to that at 121°C can be calculated as described in Section I.k. Also, Lethal Rates may be obtained from Lethal Rate Tables (Table 1 and 1A, Appendix).

G. Biological Indicators

The purpose of biological indicators in validating sterilization cycles of heat labile materials is to measure the process lethality provided by the sterilization cycle. In most cases the data will be used to supplement the heat penetration data obtained by physical measurements. The F_0 value can be calculated by use of a calibrated biological indicator as described below (G.b. and I). Also, the biological indicator data can be employed to supplement heat penetration data in assuring that the probability of a microorganism surviving the sterilization process is less than 1×10^{-6} (see G.b.4).

a) Type and Number of Challenge Microorganisms used as Biological Indicators: *Cl. sporogenes* and *B. stearothermophilus* have been widely used as challenge organisms for moist heat by the pharmaceutical and food industries. These species have been chosen because of their relatively high heat resistance. However, other species of spore forming bacteria may be used if properly calibrated. The number and resistance of the spore population are the important criteria when selecting biological indicators for challenging steam sterilization cycles.

b) The number of spores used to monitor a sterilization cycle is dependent on the expected process lethality (F_0) and the resistance of the microorganism. This relationship is described by:

$$F_0/D_{121} = (\log_{10} A - \log_{10} B)$$

where:

$\log_{10} A - \log_{10} B$ = spore log reduction (SLR)

1) In order to obtain an accurate estimation of the F value provided by the autoclave cycle a proportion of the challenge population should survive. Secondly, the method of recovery will influence the number of spores required on a biological indicator. The fraction negative method is capable of enumerating smaller numbers of microorganisms than the plate count method.
2) As a general approximation, the number of spores required for each replicate can be determined by $No = \log^{-1} (F_0/D_{121})$ when employing fraction negative

methods. (No = the number of spores on each replicate.)

3) If plate count methods are used to enumerate survivals; the initial count required can be estimated by $No = \log^{-1}(F_0/D_{121} + \log_{10} B)$, where B equals the number of survivors desired for obtaining a survival count.

4) Biological indicators may be designed to indicate whether the sterilization cycle is providing sufficient lethality to provide a 10^{-6} probability of microbial survival with respect to bioburden. In order to serve this purpose the design criteria for the B.I. can be derived as follows;

$$D_s(\log Ni + 6) = D_{bi}(\log No + 1)$$

where:

Ni = the load of microorganisms on the product to be sterilized
D_s = D value of the most resistant isolate
No = number of organisms on the biological indicator
D_{bi} = D value of Biological Indicator

The number of microorganisms to be used in the biological indicator can be determined by solving for No.

H. Procedure for Use of Biological Indicators

1. Inoculate the carrier of choice with a known volume of calibrated spore suspension. The type carrier will be dependent on the material that will be autoclaved. In the case of solutions spores should be suspended in test containers that are of the same design and fill volume as the actual product container. The suspending medium should be the specific parenteral solutions for which qualification is desired. In the case of devices or solid material, spores can be inoculated onto precut samples of the same composition rather than on the entire device.
2. Positive controls should be conducted to verify that the initial count is correct.
3. The challenge studies should be conducted during the heat penetration studies described in Section II.E. Position a minimum of ten challenge containers containing the Biological In-

dicator throughout the autoclave load, assuring that the coolest locations previously determined by thermocouple measurement are represented. It is suggested that the challenge containers be placed adjacent to containers which are probed with thermocouples.

4. The loading pattern should be the same as that specified for normal production use. If variable size loading patterns are routinely used, the challenge studies should be conducted using the production conditions that yield the minimum F_0 value.
5. Initiate the specified sterilization cycle.
6. After the sterilization cycle, recover the test containers and at least one unchallenged container as a negative control.
7. Each test container should be filtered through a 0.45-μm membrane filter in an appropriate microbiological testing area. Depending on whether survivor counts or quantal information is desired the filters should be placed in the appropriate growth media (agar or broth) and incubated at optimum growth temperature for at least 72 hours. Treat the negative control in the same manner as the test samples.
8. Enumerate the colonies if plated on agar. If fraction negative methods are used to estimate the number of survivors, record the proportion of broth cultures showing positive or negative results.

I. Evaluation of Results

1. The process lethality of the autoclave cycle as measured by biological indicators is determined by the following equation

$$F_t^z = D_t^z(\log_{10} A - \log_{10} B) \qquad (1)$$

D_t^z of the indicator would have been previously determined by laboratory studies (Section I.E. and I.F.).

where:

A = number of challenge spores per container.

B = number of challenge organisms surviving per container. When fraction negative data is collected, $B = 2.303 \log (n/q)$ where n is the total number of replicates and q is the number of negative replicates.

a) Example Employing Fraction Negative Results
Assume:

$$D_t^z = 1 \text{ minute } (Z = 10°C)$$

$$A = 1 \times 10^4 \text{ spores/container}$$

$$B = 20 \text{ replicates/10 negative replicates}$$

Therefore, according to Equation 1

$$B = 2.303 \text{ (log } 20/10) = 0.69 \text{ spores/container}$$

$$F_0 = 1[4 - (-0.159)] = 4.2 \text{ minutes}$$

In the situation where all replicates are sterile, a minimum F value can be estimated by assuming one positive replicate. For example, if ten replicates were used and all were negative, a minimum F could be calculated as follows (assume $D_{121}^{10} = 1.5$ minutes; $A = 10^4$ spores/container and $B = 10$ replicates/9 negative replicates)

$$B = 2.303 \log 10/9 = 0.1054$$

$$\log B = -0.98$$

Therefore:

$$F_0 = 1.5[4 - (-0.98)] = >7.5 \text{ minutes}$$

b) Example Employing Survivor Count Results
Assume:

$$D_t^z = 1 \text{ minute } (Z = 10°C)$$

$$A = 1 \times 10^6 \text{ spores/container}$$

$$B = 1 \times 10^2 \text{ spores/container}$$

Therefore, according to Equation 1,

$$F_0 = 1 (6 - 2) = 4.0 \text{ minutes}$$

III. OVERKILL APPROACH TO STERILIZATION VALIDATION

An Overkill approach to sterilization may be employed when sterilizing heat stable materials where the primary concern is assurance of sterilization and not heat degradation. It is implicit in the overkill approach that sufficient lethality is imparted during the sterilization cycle to provide at least a 10^{-6} probability of microbial survival regardless of the number and heat resistance of naturally occurring microorganisms. In order to assure microbial overkill the cycle lethality should be sufficient to affect at least a 12-log reduction of microorganisms having a D value of 1.0 minute.

A. Laboratory Studies

1. Bioburden and Heat Resistance Studies on Product Isolates. These studies are not routinely necessary when cycle lethality is sufficient to affect a 12-log reduction of microorganisms having a D value of 1.0 minute. Although proper assurance of sterility may be achieved with overkill sterilization cycles, high bioburdens are unacceptable due to the potential presence of endotoxin. Controlled manufacturing environments and good manufacturing practices which provide barriers to microbial contamination remain of utmost importance. If materials or product support microbial growth, periodic bioburdens should be conducted to assess the potential of endotoxin development. If the microbial attributes of the product are unknown, bioburden and thermal resistance studies should be considered during the validation studies to assess the degree of overkill.
2. Calibration of Biological Indicators
   a) Type of Carrier
   The choice of carrier is important when determining D and z values of biological indicators since the carrier may affect resistance. The challenge microorganism may be suspended in solution or inoculated on paper strips or on precut material of the same composition as the item being sterilized. Regardless of the type of carrier used, D and z values of the biological indicator should be determined using the same carrier that is used to monitor the sterilization cycle.

b) Determination of D and z values
D and z values should be determined for biological indicators whether prepared in house or purchased commercially. Survival curve or fraction negative methods may be used as described in Section I.E., I.F., and I.H.

c) Laboratory D value Methodology
Refer to Section I.D.

3. Determination of Process Times
The necessary lethality of a sterilization cycle sufficient to provide a 12-log reduction of microorganisms having a D value of 1.0 is calculated as follows:

$$F_0 = D_{121}(\log_{10} A - \log_{10} B)$$

where:

$D_{121}^{10} = \geq 1.0$
$(\log_{10} A - \log_{10} B)$ = spore log reduction = 12
Therefore:
$F_0 \geq 1(12) \geq 12$ minutes

The process time needed to impart the minimum required F_0 value can be determined as described in Section I.J.1.c.

B. Plant Studies

When the overkill sterilization approach is employed for heat stable parenterals, plant studies may be conducted in the same manner as described for heat labile parenterals (Section II). However, when sterilizing equipment and miscellaneous items used for asceptic filling, the approach may differ slightly due to difficulties in obtaining precise temperature profiles of the loading pattern. Variability in size, mass, and form of equipment may result in numerous permutations with respect to loading pattern configuration. Methodology that is pertinent to validation of cycles for equipment and miscellaneous items are presented in this section:

1. Calibration of Temperature Monitoring Equipment
Refer to Section II.A.
2. Certification of Sterilizers
Refer to Section II.B.
3. Loaded Chamber Heat Distribution
Refer to Section II.C.

4. Cold Spot Determination (Mapping) for Items Other Than Parenterals (in the case of parenterals, refer to II.D).
   a) The determination of cold spots in equipment and miscellaneous items may present unique problems in determining temperature profiles due to variations in size, mass and form. It is important to probe areas of equipment that;
      aa. come into direct contact with a sterile product.
      bb. present a potential barrier to steam penetration.
      cc. are wrapped or packaged in material that may reduce the rate of steam penetration.
   b) Representative items of a specified loading pattern should be probed to determine the cold spot within each item.
   c) It may be assumed that item cool point mapping remains constant among different sterilizing chambers using the same heating medium. If cold spots cannot be determined through visual inspection of the temperature data, calculate F values. (See Section II.F.) The point having the lowest F value is designated as the coldest point. Those items in a specific loading pattern having the lowest F_0 at their respective cold spots are designated as most difficult to heat.
   d) In subsequent heat penetration studies, position the probes at the cold point within the items. The majority of probes should be located at the cold points of those items designated the most difficult to heat.
5. Heat Penetration for Items Other Than Parenterals. (In the case of parenterals, refer to Section II.E.1.)
   a) Load Cool Point Determination
      aa. The load cool point for equipment and miscellaneous items should be determined for maximum and minimum loading configuration. The chamber should be loaded and items wrapped in such a manner that steam has access to all critical surfaces.
      bb. Those items designated as most difficult to heat should be distributed evenly throughout the load. Sufficient trials or probes should be used such that all representative areas in the loading pattern are monitored.
      cc. One probe should be located in the steam exhaust line or immediately adjacent to the vessel controller sensor.

dd. The sterilization parameters should be those designated for the normal production cycle.

ee. At least once during the dwell period (preferably Mid-dwell) the following temperature readings should be compared;

1) Temperature recording chart on vessel
2) Reference temperature sensor
3) Thermocouple adjacent to controller sensor

The readings should agree within 1°C (2°F).

ff. Calculate F_0 (Section II.F) or time at set temperature for the coolest probes in the loading pattern.

b) Reproducibility Studies (Validation of Consistancy)

aa. Once the cold point of equipment and cold area of the loading pattern have been established, reproducibility studies should be conducted by repeating the sterilization cycle with the majority of probes situated in the area designated as the coldest. The position of all probes should remain the same during reproducibility studies. Sufficient repeat studies should be conducted to establish with a given level of confidence that the delivered F_0 value will be consistantly greater than the minimum required F_0 value.

bb. If there is uncertainty whether the delivered F_0 values are significantly greater than the minimum required F_0 value, the heat penetration data can be statistically evaluated as described in Section II.E.3.c.

6. Calculation of Delivered Process Lethality

a) The process lethality can be estimated by integration of lethal rates obtained for the cold point within the load or by determining the time that the coldest point is at process temperature as described in II.F.

b) When process temperatures other than 121°C are used for sterilization, the amount of time providing equivalent lethality to that at 121°C can be calculated as described in Section I.J. When sterilizing heat stable items, cycle times can be significantly reduced by heating at temperatures greater than 121°C.

7. Biological Indicators

Biological indicators provide data that supplements the heat

penetration data obtained by physical methods. The F_0 value can be estimated by use of calibrated indicators.

a) Type and Number of Challenge Microorganisms Used as Biological Indicators.

It should be recognized that biological indicators may not have sufficient resistance and number of spores to precisely monitor overkill sterilization cycles of heat stable materials. Cycles having F values greater than 15 minutes will inactivate the majority of biologically indicators currently in use by the pharmaceutical industry.

When employing overkill sterilization cycles for heat stable items it is recommended that *B. stearothermophilus* be used as the biological indicator because of its relatively high resistance to steam heat. The D value of the biological indicator should exceed 1.0 minutes.

The number of spores used to monitor a sterilization cycle is dependent on the expected process lethality (F_0) and the resistance of the microorganism. This relationship is described by:

$$F_0/D_{121} = \log_{10} A - \log_{10} B$$

where $\log_{10} A - \log_{10} B$ = spore log reduction (SLR)

As a general approximation the number of spores required for each carrier can be determined by $No = \log^{-1} (F_0/D_{121})$ when employing fraction negative methods (No = the number of spores on each replicate).

b) Determination of Survivors

When monitoring cycles having high F values (greater than 15 minutes) microorganisms may not survive the cycle. Enumeration of survivors by fraction negative methods offers the advantage that a smaller number of microorganisms can be detected than with typical plate count methods.

c) Procedure for use of Biological Indicators

(In the case of heat stable parenterals, refer to Section II.H.)

aa. In the case of equipment, inoculate the carrier of choice with a known volume of calibrated spore suspension. The suspension should be dried on the carrier prior to use.

bb. Positive controls should be conducted to verify that the initial count is correct.

cc. The microbiological challenge studies should be performed during the heat penetration studies described in Section III.B.5.b. Position a minimum of ten challenged items throughout the autoclave load assuring that the coldest locations previously determined by thermocouple measurement are represented. It is suggested that thermocouples be placed near the location of the challenge inoculation.

dd. The loading pattern should be the same as that specified for normal production use. If variable sized loading patterns are used, the challenge studies should be conducted using the production conditions that yield the minimum F_0 value.

ee. Initiate the specified sterilization cycle.

ff. After the sterilization cycle, recover the inoculated items and place in an appropriate growth medium (i.e., Trypticase Soy Broth) and incubate at optimum growth temperature for at least 72 hours.

gg. Record the proportion of broth cultures showing positive or negative results.

d) Evaluation of Results

1) The process lethality of the autoclave cycle as measured by biological indicators is determined by the following equation:

$$F_t^z = D_t^z(\log_{10} A - \log_{10} B)$$

D_t^z of the indicator would have been previously determined by laboratory studies (Section I.E. and I.F.).

A = number of challenge spores per container.

B = number of challenge organisms surviving per container. When fraction negative data is collected, $B = 2.303 \log (n/g)$, where n is the total number of replicates and q is the number of negative replicates.

a. Example calculation

With overkill sterilization cycles all biological indicators may be inactivated due to the high F_0 values employed. In these situations, a minimum F value can

be estimated by assuming one positive replicate. For example, if 15 replicates were used and all were negative, a minimum F_0 value could be calculated as follows;

Assume:

$D_{121}^{10} = 2.0$ minutes

$A = 10^5$ spores/carrier

B = 15 replicates/14 negative replicates (one positive replicate assumed)

$B = 2.303 \log (15/14) = 0.069$

$\log B = -1.16$

Therefore:

$F_0 = 2[5 - (-1.16)] = >12.3$ minutes.

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SYMBOLS AND DEFINITIONS

D_t = Time at a given temperature (t) required to reduce the number of microorganisms by 90% (one logarithmic reduction)

F_t^z = The equivalent time at temperature (t) delivered to a container for the purpose of sterilization with a specific value of z

F_0 = The equivalent time at 121°C delivered to a container for the purpose of sterilization. z = 10°C

L = Lethal Rate = $\log^{-1} \frac{T_0 - T_b}{z} = 10^{T_0 - T_b/z}$

where:

T_0 = Temperature within the container (item) being heated

T_b = Process temperature

No = The initial number of microorganisms

SLR = Spore log reduction = $\log_{10} A - \log_{10} B$

where:

A = initial number of spores

B = the number of spores surviving the heat treatment

U = Heating time at process temperature

z = The number of degrees temperature change to change the D value by a factor of ten.

V. APPENDIX

TABLE I

Lethal Rate Table
(Minutes at 250° F Per Minutes at T° F)

Temperature (°F)	z value (°F)								
	12	14	15	16	17	18	19	20	22
200	0.000	0.000	0.000	0.001	0.001	0.002	0.002	0.003	0.005
201	0.000	0.000	0.001	0.001	0.001	0.002	0.003	0.004	0.006
202	0.000	0.000	0.001	0.001	0.002	0.002	0.003	0.004	0.007
203	0.000	0.000	0.001	0.001	0.002	0.003	0.004	0.005	0.007
204	0.000	0.001	0.001	0.001	0.002	0.003	0.003	0.005	0.008
205	0.000	0.001	0.001	0.002	0.002	0.003	0.004	0.006	0.009
206	0.000	0.001	0.001	0.002	0.003	0.004	0.005	0.006	0.010
207	0.000	0.001	0.001	0.002	0.003	0.004	0.005	0.007	0.011
208	0.000	0.001	0.002	0.002	0.003	0.005	0.006	0.008	0.012
209	0.000	0.001	0.002	0.003	0.004	0.005	0.007	0.009	0.014
210	0.001	0.001	0.002	0.003	0.004	0.006	0.008	0.010	0.015
211	0.001	0.002	0.003	0.004	0.005	0.007	0.009	0.011	0.017
212	0.001	0.002	0.003	0.004	0.006	0.008	0.010	0.013	0.019
213	0.001	0.002	0.003	0.005	0.007	0.009	0.011	0.014	0.021
214	0.001	0.003	0.004	0.006	0.008	0.010	0.013	0.016	0.023
215	0.001	0.003	0.005	0.006	0.009	0.011	0.014	0.018	0.026
216	0.001	0.004	0.005	0.007	0.010	0.013	0.016	0.020	0.029
217	0.002	0.004	0.006	0.009	0.011	0.015	0.018	0.022	0.032
218	0.002	0.005	0.007	0.010	0.013	0.017	0.021	0.025	0.035
219	0.002	0.006	0.009	0.011	0.015	0.019	0.023	0.028	0.039
220	0.003	0.007	0.010	0.013	0.017	0.022	0.026	0.032	0.044
221	0.004	0.008	0.012	0.015	0.020	0.024	0.030	0.035	0.048
222	0.004	0.010	0.013	0.017	0.022	0.028	0.034	0.040	0.054
223	0.005	0.011	0.016	0.020	0.026	0.032	0.038	0.042	0.059
224	0.006	0.014	0.018	0.023	0.029	0.036	0.042	0.050	0.066
225	0.008	0.016	0.022	0.027	0.033	0.041	0.048	0.056	0.072
226	0.009	0.019	0.025	0.031	0.038	0.046	0.054	0.062	0.081
227	0.012	0.022	0.029	0.036	0.044	0.052	0.061	0.070	0.090
228	0.014	0.022	0.034	0.041	0.050	0.060	0.069	0.079	0.100
229	0.017	0.031	0.040	0.048	0.058	0.068	0.078	0.089	0.110
230	0.022	0.037	0.046	0.056	0.066	0.077	0.088	0.100	0.123
230.5	0.024	0.040	0.050	0.060	0.071	0.083	0.094	0.106	0.129
231	0.026	0.044	0.054	0.065	0.076	0.088	0.100	0.112	0.136
231.5	0.029	0.048	0.058	0.070	0.081	0.094	0.106	0.119	0.144
232	0.032	0.052	0.063	0.075	0.087	0.100	0.112	0.126	0.152
232.5	0.035	0.056	0.068	0.081	0.093	0.107	0.120	0.133	0.160
233	0.038	0.061	0.074	0.087	0.100	0.114	0.128	0.141	0.169
233.5	0.042	0.066	0.079	0.093	0.107	0.121	0.136	0.150	0.178
234	0.046	0.072	0.086	0.100	0.115	0.129	0.144	0.158	0.187
234.5	0.051	0.078	0.093	0.107	0.123	0.138	0.153	0.168	0.197

Table 1 (continued)

Temperature (°F)	z value (°F)								
	12	14	15	16	17	18	19	20	22
235	0.056	0.085	0.100	0.116	0.131	0.147	0.162	0.178	0.208
235.5	0.062	0.092	0.108	0.124	0.140	0.156	0.172	0.188	0.219
236	0.068	0.100	0.117	0.133	0.150	0.167	0.183	0.200	0.230
236.5	0.074	0.108	0.126	0.143	0.160	0.178	0.195	0.211	0.243
237	0.083	0.118	0.136	0.155	0.172	0.190	0.207	0.224	0.256
237.5	0.091	0.128	0.147	0.165	0.184	0.202	0.220	0.237	0.269
238	0.100	0.139	0.159	0.177	0.197	0.215	0.234	0.251	0.284
238.5	0.111	0.151	0.171	0.191	0.209	0.230	0.249	0.266	0.300
239	0.121	0.164	0.184	0.205	0.225	0.246	0.264	0.282	0.316
239.5	0.133	0.178	0.200	0.220	0.241	0.261	0.280	0.299	0.332
240	0.147	0.193	0.216	0.237	0.258	0.278	0.298	0.316	0.351
240.25	0.154	0.201	0.224	0.245	0.267	0.287	0.307	0.326	0.364
240.5	0.161	0.209	0.233	0.255	0.276	0.297	0.316	0.335	0.369
240.75	0.168	0.219	0.242	0.264	0.286	0.306	0.327	0.345	0.380
241	0.177	0.228	0.251	0.273	0.296	0.316	0.337	0.355	0.390
241.25	0.186	0.237	0.261	0.284	0.306	0.327	0.347	0.365	0.400
241.5	0.195	0.247	0.272	0.294	0.315	0.337	0.357	0.376	0.411
241.75	0.205	0.258	0.282	0.304	0.326	0.348	0.370	0.387	0.422
242	0.216	0.268	0.292	0.316	0.339	0.359	0.380	0.398	0.433
242.25	0.226	0.279	0.305	0.327	0.350	0.371	0.392	0.410	0.445
242.5	0.238	0.292	0.316	0.340	0.362	0.384	0.404	0.421	0.457
242.75	0.249	0.304	0.329	0.352	0.370	0.396	0.415	0.435	0.469
243	0.261	0.316	0.341	0.364	0.388	0.408	0.429	0.448	0.481
243.25	0.273	0.330	0.356	0.378	0.402	0.421	0.440	0.460	0.493
243.5	0.287	0.344	0.369	0.392	0.415	0.435	0.457	0.473	0.508
243.75	0.301	0.358	0.383	0.406	0.429	0.450	0.469	0.487	0.520
244	0.316	0.373	0.398	0.421	0.444	0.464	0.485	0.501	0.533
244.25	0.331	0.379	0.415	0.436	0.459	0.479	0.500	0.516	0.548
244.5	0.348	0.405	0.431	0.452	0.474	0.495	0.515	0.531	0.563
244.75	0.365	0.422	0.448	0.469	0.490	0.511	0.532	0.546	0.577
245	0.384	0.440	0.464	0.487	0.509	0.527	0.545	0.562	0.593
245.25	0.401	0.459	0.483	0.505	0.526	0.545	0.565	0.578	0.603
245.5	0.421	0.477	0.501	0.523	0.544	0.562	0.581	0.596	0.624
245.75	0.442	0.497	0.521	0.543	0.562	0.581	0.599	0.612	0.641
246	0.462	0.519	0.541	0.563	0.580	0.600	0.616	0.631	0.658
246.25	0.486	0.540	0.565	0.584	0.602	0.619	0.637	0.649	0.675
246.5	0.511	0.562	0.584	0.606	0.621	0.639	0.658	0.668	0.695
246.75	0.536	0.585	0.609	0.625	0.645	0.660	0.676	0.688	0.711
247	0.561	0.610	0.631	0.650	0.666	0.681	0.695	0.707	0.730
247.25	0.590	0.637	0.658	0.674	0.690	0.703	0.719	0.728	0.750
247.5	0.618	0.662	0.681	0.699	0.714	00726	0.741	0.750	0.770
247.75	0.652	0.690	0.709	0.724	0.735	0.749	0.763	0.772	0.790
248	0.683	0.720	0.736	0.750	0.763	0.774	0.785	0.794	0.811
248.25	0.712	0.750	0.769	0.776	0.789	0.799	0.813	0.818	0.833
248.5	0.748	0.781	0.794	0.806	0.815	0.825	0.834	0.841	0.855

Table 1 (continued)

Temperature (°F)	z value (°F)								
	12	14	15	16	17	18	19	20	22
248.75	0.788	0.814	0.825	0.834	0.844	0.854	0.859	0.865	0.877
249	0.824	0.849	0.857	0.865	0.872	0.880	0.885	0.891	0.900
249.25	0.865	0.884	0.891	0.897	0.902	0.909	0.914	0.917	0.925
249.5	0.907	0.920	0.925	0.930	0.934	0.938	0.943	0.944	0.950
249.75	0.952	0.960	0.962	0.965	0.967	0.969	0.971	0.972	0.975
250	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
250.5	1.10	1.09	1.08	1.08	1.07	1.07	1.06	1.06	1.05
251	1.21	1.18	1.15	1.16	1.14	1.14	1.13	1.12	1.11
251.5	1.33	1.28	1.26	1.24	1.22	1.21	1.20	1.19	1.17
252	1.47	1.39	1.37	1.33	1.13	1.30	1.28	1.26	1.23
252.5	1.62	1.51	1.47	1.43	1.40	1.38	1.35	1.33	1.30
253	1.78	1.64	1.59	1.54	1.50	1.47	1.44	1.41	1.37
253.5	1.96	1.78	1.71	1.66	1.60	1.56	1.53	1.50	1.44
254	2.16	1.93	1.85	1.78	1.72	1.67	1.63	1.59	1.52
254.5	2.37	2.09	2.00	1.91	1.85	1.78	1.73	1.68	1.60
255	2.61	2.27	2.15	2.06	1.97	1.90	1.84	1.78	1.69
255.5	2.87	2.47	2.33	2.21	2.11	2.02	1.95	1.88	1.78
256	3.16	2.68	2.51	2.37	2.26	2.16	2.08	2.00	1.88
256.5	3.49	2.91	2.55	2.41	2.41	2.30	2.20	2.11	2.08
257	3.83	3.17	2.93	2.74	2.58	2.45	2.34	2.24	2.08
257.5	4.22	3.44	3.17	2.94	2.76	2.61	2.48	2.37	2.19
258	4.65	3.73	3.42	3.16	2.96	2.79	2.64	2.51	2.31
258.5	5.10	4.05	3.69	3.39	3.16	2.97	2.80	2.66	2.43
259	5.62	4.38	3.99	3.65	3.38	3.16	2.98	2.82	2.56
259.5	6.18	4.76	4.30	3.92	3.62	3.37	3.16	2.99	2.70
260	6.80	5.18	4.65	4.22	3.88	3.60	3.37	3.16	2.86

TABLE IA

Lethal Rate Table
(Minutes at 121° C Per Minute at T° C)

Temperature (°C)	Z Value (°C)					
	7	8	9	10	11	12
100	.001	.002	.005	.008	.012	.018
101	.001	.003	.006	.010	.015	.022
102	.002	.004	.008	.013	.019	.026
103	.003	.006	.010	.016	.023	.032
104	.004	.007	.013	.020	.028	.038
105	.005	.010	.017	.025	.035	.046
106	.007	.013	.022	.032	.043	.056
107	.010	.018	.028	.040	.053	.068
108	.014	.024	.036	.050	.066	.083
109	.019	.032	.046	.063	.081	.100
110	.026	.042	.060	.079	.100	.121
111	.037	.056	.077	.100	.123	.147
112	.052	.075	.100	.126	.152	.178
113	.072	.100	.129	.158	.187	.215
114	.100	.133	.167	.200	.231	.261
114.5	.118	.154	.190	.224	.257	.287
115	.139	.178	.215	.251	.285	.316
115.5	.164	.205	.245	.282	.316	.348
116	.193	.237	.278	.316	.351	.383
116.5	.228	.274	.316	.355	.390	.422
117	.268	.316	.359	.398	.433	.464
117.5	.316	.365	.408	.447	.481	.511
118	.373	.422	.464	.501	.534	.562
118.5	.439	.489	.527	.562	.593	.619
119	.518	.562	.599	.631	.658	.681
119.5	.611	.649	.681	.708	.731	.750
120	.720	.750	.774	.794	.811	.825
120.5	.848	.866	.880	.891	.901	.909
121	1.00	1.00	1.00	1.00	1.00	1.00
121.5	1.18	1.16	1.14	1.12	1.11	1.10
122	1.39	1.33	1.29	1.23	1.23	1.21
122.5	1.64	1.54	1.47	1.41	1.37	1.33
123	1.93	1.78	1.67	1.59	1.52	1.47
123.5	2.28	2.05	1.90	1.78	1.69	1.62
124	2.68	2.37	2.15	2.00	1.87	1.78
125	4.39	3.16	2.78	2.82	2.31	2.15
126	5.18	4.22	3.59	3.16	2.85	2.61
127	7.20	5.62	4.64	3.98	3.51	3.16
128	10.0	7,50	6.00	5.01	4.33	3.83
129	13.9	10.0	7.74	6.31	5.34	4.64
130	19.3	13.3	10.0	7.94	6.58	5.62

TABLE II

Calculating Lethality of Heat Sterilization Process Using the Patashnik Method

Time (minutes)	Temperature (°C)	Lethal Rate $L = 10^{T_0 - T_p/z}$
5	100	0.008
6	103	0.016
7	106	0.032
8	109	0.063
9	112	0.126
10	115	0.251
11	118	0.501
12	121	1.000
13	121	1.000
14	121	1.000
15	118	0.501
16	115	0.251
17	112	0.126
18	109	0.063
19	106	0.032
20	103	0.016
21	100	0.008

$F_0 = \Delta t$ (Σ of lethal rates) = 1 × 4.994 = 5.0 minutes at 121°C, Δt is the time interval between successive temperature measurements.

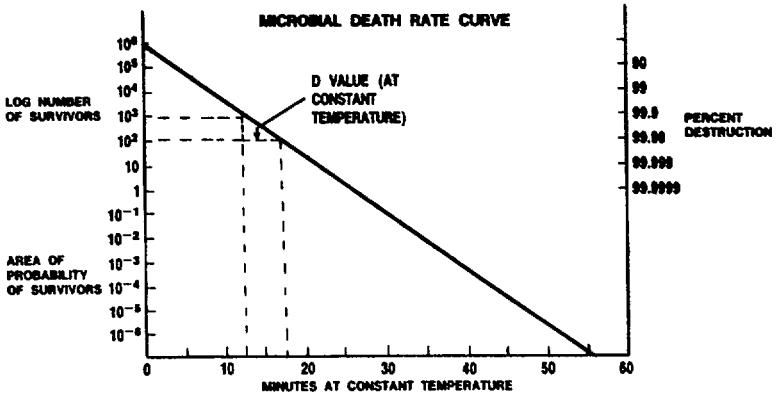


Figure 1—Hypothetical microbial death rate (i.e., survivor curve) at a constant experimental temperature.

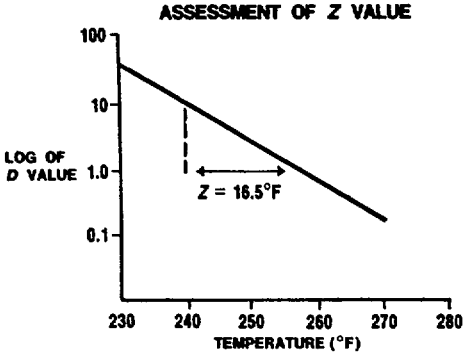


Figure 2—Relationship of *z* value to thermal rate