Technical Report No. 1 Revised 2007 Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control

PDA Journal of Pharmaceutical Science and Technology

PDA Parenteral Drug Association

2007

Supplement

Volume 61

No. S-1

PDA Technical Report No. 1 - Revised 2007

Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control

Contributors

James P. Agalloco, Agalloco & Associates
James E. Akers, Ph.D., Akers Kennedy & Associates
Wilf Allinson, GlaxoSmithKline
Thomas J. Berger, Ph.D., Hospira
Frank Bing, Abbott Laboratories (retired)
Göran Bringert, GE Kaye Instruments
Gary Butler, Steris Corporation
Jean-Luc Clavelin, Eli Lilly & Co.
Peter Cooney, Ph.D., FDA (retired)
Phil DeSantis, Schering-Plough
Peter Dürr, F. Hoffmann-La Roche AG
Kristen D. Evans, FDA
John G. Grazal, AstraZeneca
Nigel Halls, Ph.D., IAGT. Ltd.
Paul Hargreaves, MHRA
Andrew D. Hopkins, MHRA
Martin A. Joyce, Ph.D., GeneraMedix Inc.
David Karle, Steris Corporation
Bernard Kronenberg, Bakrona Basel AG
John W. Levchuk, Ph.D., FDA (retired)
Richard V. Levy, Ph.D., PDA
Steen Loevtrup, Novo Nordisk A/S
Timothy F. Lord, Eli Lilly & Co.
Genevieve Lovitt-Wood, G.I. Lovitt & Associates, Project Manager and Technical Writer
Russell E. Madsen, The Williamsburg Group, LLC
Vittorio Mascherpa, Ph.D., Fedegari Autoclavi Spa (retired)
David W. Maynard, Maynard & Associates
Robert B. Myers, PDA
James E. Owens, Baxter Healthcare (retired)
Irving Pflug, Ph.D., University of Minnesota (retired)
Dario Pistolesi, Ph.D., Fedegari Autoclavi Spa (retired)
Anthony Pochiro, AG Edwards and Sons
Jarmo Saari, Leiras OY
Michael Sadowski, Baxter Healthcare
John T. Shirtz, Baxter Healthcare
Keith Shuttleworth, Keith Shuttleworth & Associates Ltd.
Finlay Skinner, Skinner Pharm-Assist
Ian Symonds, GlaxoSmithKline
Kevin D. Trupp, Hospira
Dieter Witthauer, Ph.D., Novartis
Richard T. Wood, Ph.D. (retired)
William Young, Baxter Healthcare (retired)

Validation of Moist Heat Sterilization Processes: Cycle Design, Development, Qualification and Ongoing Control

Technical Report No. 1 (Revised 2007)
Supplement
Vol. 61, No. S-1

TABLE OF CONTENTS

1.0 INTRODUCTION

PDA's original Technical Monograph No. 1, *Validation of Steam Sterilization Cycles*, published in 1978, introduced the principles of steam sterilization to an entire generation of pharmaceutical scientists and engineers. This revision retains the focus on microbiology and engineering of moist heat sterilization in the original document, and updates it by including contemporary subject matter. References to appropriate and up-to-date scientific publications, international regulatory documents, journal articles, technical papers and books are used where more detail and supportive data can be found.

The primary objective of the task force was to develop a scientific technical report on moist heat sterilization that may be used in all regulatory environments. The report does not always address region-specific regulatory expectations, but provides up-to-date, scientific recommendations for use by industry and regulators. This report should be considered a guide and is not intended to establish standards for sterilization validation. It is intended to be a single-source overview that complements existing documents listed in the reference section.

The task force was composed of European and North American industry and regulatory professionals to ensure the methods, terminology and practices of sterilization science presented reflect sound science and can be used globally. This technical report was disseminated in draft for public review and comment prior to publication to ensure its suitability as a valuable guide to industry in steam sterilization.

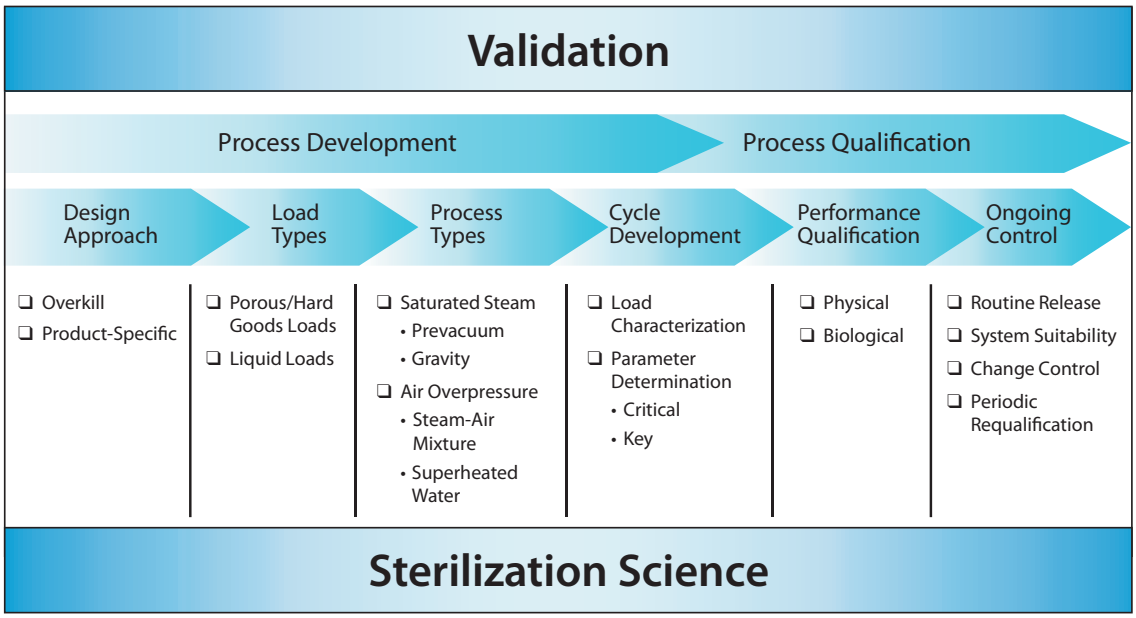
The overarching goal was to provide enough information to an convey understanding of the science of moist heat sterilization with sufficient technical detail to assist in developing a sterilization policy.

1.1 Scope

The new title, *"Validation of Moist Heat Sterilization Processes – Cycle Design, Development, Qualification and Ongoing Control"* reflects the content of this technical report with a focus on manufacturing.

This technical report is organized in a logical progression from the essential elements of sterilization science through sterilization cycle development and qualification, as depicted in **Figure 1.1–1**.

Figure 1.1–1 ■ Application of the Science of Sterilization



In the interest of clarity, the report begins with a glossary of technical terms, including synonyms. With validation as the overarching theme, a discussion of microbiology and sterilization science, including thermal science, (**Section 3.0**) is presented as the foundation, as depicted in **Figure 1.1–1**.

Process development (**Section 4.0**) presents the theory for the design of actual sterilization cycles used in pharmaceutical manufacturing, including overkill and product-specific approaches. A decision tree provides a guide on how to select the most appropriate sterilization process for various load types. Example process parameter tables for liquid and porous/hard goods load types are given to assist in assessing risk associated with different cycle phases. This section takes the user from the theoretical to a practical application of sterilization science for moist heat sterilization of a product or item.

Process performance qualification (**Section 5.0**) is the heart of sterilization process validation. Performance qualification of the sterilization cycle in a manufacturing plant or other facility is addressed using physical and biological qualification approaches. This discussion includes the practice and science necessary to demonstrate delivery of the desired lethality in sterilizer systems.

Ongoing controls (**Section 6.0**) address day-to-day performance considerations to maintain sterilization process state-of-control. The section covers change control, system suitability, and periodic requalification.

Application of the concepts presented in this technical report to laboratories or other non-CGMP applications, including hospitals, is not intended. Other important elements such as facilities, equipment, maintenance, utilities and analytical qualification are not covered in this technical report.

2.0 GLOSSARY OF TERMS

Term usage may differ from company to company, and some terms may be subject to change in the future. However, the terms used in a validation program must be clearly defined and well understood within the company. Regulatory guidelines may offer other definitions that should be considered. For the purposes of this technical report, the following terms are used and are accompanied by their definitions and synonyms, where applicable.

Air Detector: An instrument that may be fitted to a saturated steam sterilizer that detects the presence of air in the chamber.

Air Overpressure Sterilization Process: A moist heat sterilization process that operates at a controlled pressure greater than saturated steam pressure and typically uses compressed air to bring the chamber to the desired pressure.

Air Removal Test: A test used to evaluate air removal and steam penetration in an empty sterilizer that is used for porous/hard goods load sterilization (e.g., Bowie-Dick Test, DART, Lantor Cube, Browns' Test).

Biological Indicator Challenge System (BI): A test system containing viable microorganisms of a pure, specified strain providing a defined resistance to a specified sterilization process. (1) [Synonym: BI Challenge System, Microbial Challenge, Microbiological Challenge System]

Biological Qualification: A component of performance qualification that demonstrates, by use of biological indicators, that the required lethality (F_{BIO}) is achieved consistently throughout the load.

Bracketing Approach: A scientific approach for defining product/load characteristics (e.g., viscosity, container sizes, container fill volumes, item sizes, loading configurations) that are tested (in a qualification study or validation study) at upper and/or lower limits.

Calibration: The demonstration that an instrument or device produces results within specified limits when compared to those produced by a reference standard or a standard that is traceable to national or international standards over an appropriate range of measurements.

Chamber: The primary component of a sterilizer that contains the items to be sterilized. The chamber is a pressure rated vessel.

Chamber Cold Spot: The location(s) within the load zone that achieves the lowest process lethality (F_0) and/or the lowest distribution temperatures during the sterilization process.

Chamber Heat-Up Time: The elapsed time measured from the introduction of steam in the heat-up phase ("steam on") to the point when the temperature of the heating medium within the chamber reaches the exposure temperature set point.

Chamber Leak Test: A test conducted to evaluate possible air infiltration to the chamber under vacuum. [Synonym: Vacuum Leak Test]

Chemical Indicator: Test system that reveals change in one or more predefined process variables based on a chemical or physical change resulting from exposure to a process. (2)

Chemical Integrator: A device that is designed to react in a quantitative manner to multiple sterilization variables, (typically, time and temperature and, in some instances, moisture). (3)

Cool-Down Phase: The phase of a sterilization cycle that occurs after completion of the exposure phase. Parameters of a cool-down phase are typically defined in order to meet applicable user requirements for load cooling and drying.

Container Cold Spot: The location within a sealed liquid container that achieves the lowest process lethality (F_0) during a sterilization process.

Dryness Fraction: An absolute measure of the actual latent heat of a sample of steam relative to the theoretical latent heat of saturated steam.

Dryness Value: A dimensionless test quantity developed to approximate the dryness fraction.

***D_T*-Value:** The time in minutes required for a one-logarithm, or 90%, reduction of the population of microorganisms used as a biological indicator under specified lethal conditions. For steam sterilization, the *D*-value should always be specified with a reference temperature, D_T . For example, a BI system with a $D_{121°C} = 1.4$ minutes requires 1.4 minutes at 121°C to reduce the population by one logarithm.

Equilibration Time: The period that elapses between the attainment of the minimum exposure temperature at

the reference measurement point (typically the drain) and the attainment of the sterilization temperature at all points within the load. This period is an indication of the ability to properly remove air and heat the load items; consequently, it is typically only evaluated by placing heat penetration probes in porous/hard goods loads.

Exposure Phase: The phase of the sterilization cycle in which the appropriate parameters are maintained within defined ranges for the time (exposure time or dwell period) and temperature determined to be necessary to achieve the desired lethality.

***F*-Value (Lethality Factor):** A measurement of sterilization effectiveness. $F_{(Tref,z)}$ is the calculated equivalent lethality (using a specified *z*-value), in terms of minutes at a reference temperature (T_{ref}), delivered by a sterilization cycle to an item.

***F*$_{Physical}$:** A term used to describe the delivered lethality calculated based on the physical parameters of the cycle. The $F_{Physical}$-value is the integration of the lethal rate (L) over time. The lethal rate is calculated for a reference temperature (T_{ref}) and *z*-value using the equation: $L = 10^{(T\text{-}Tref)/z}$.

***F*$_0$:** A term used when the *specific* reference conditions of $T_{ref} = 121.1°C$ and $z = 10°C$ are used to calculate the equivalent lethality. For example, when the *z*-value of the BI is 10°C a cycle with an $F_{(T=121.1°C, z=10°C)}$, or F_0, equal to 8 minutes is equivalent (in terms of delivered lethality) to a square wave cycle of 8 minutes at 121.1°C. A square wave cycle that provided an exposure of 25.9 minutes at 116°C would also yield an F_0 of 8 minutes.

Note: The reference temperature used in calculating F_0 is 121.1°C, which is the approximate mathematical equivalent of 250°F. The reference temperature of 121°C for F_0 will be used throughout this report for brevity.

***F*$_{Biological}$:** A term used to describe the delivered lethality, measured in terms of actual kill of microorganisms on or in a BI challenge system. The $F_{Biological}$-value is calculated as $D_T \times LR$, where D_T is the *D*-value of the BI system at the reference temperature (T) and LR is the actual logarithmic reduction ($\log N_0 - \log N_F$) of the BI population achieved during the cycle.

Fraction-Negative Methods: Fraction-negative methods use the starting population of a biological indicator (N_0) and data in the quantal range to create a two-point line from which the D_T-value can be determined. The quantal range is the exposure period over which a set of replicate test units exhibit a dichotomous response – some are positive for growth and the rest are negative for growth.

Gravity Displacement Process: A sterilization process based on the principle that cold air within the chamber is heavier than the steam entering and will sink to the bottom of the chamber. As steam enters the chamber, air is pushed out the bottom drain and exits, with the condensate, through a steam trap.

Half-Cycle Qualification: A qualification method that uses fifty percent of the exposure time to demonstrate sterilization cycle efficacy. The physical and biological lethality values achieved in the half-cycle exposure time are doubled to project the lethality that will be achieved by the full cycle.

Heat: Energy that is transferred as a result of a temperature difference between an object and its surroundings.

Heat Penetration: Heat penetration testing is a temperature measurement that is used to evaluate the amount of energy that has been transferred to the materials that are to be sterilized within the load. For measurements of heat penetration, the probes should be placed on or in the load items being evaluated.

Heat-Up Phase: The phase of a sterilization cycle that occurs prior to the exposure phase. Process parameters are developed for this phase in order to meet applicable user requirements for load conditioning (e.g., air removal and preheating).

Leak Rate: Leak rate is the quantity of air leakage over time into the sterilizer chamber obtained while performing a chamber leak test. The leak rate should not exceed a level that will inhibit the sterilization process during air removal or vacuum drying stages.

Liquid Load: A load consisting of closed containers of aqueous liquids. The sterilization of the container contents is achieved through transfer of energy through the container into the aqueous liquid.

Load Zone: Area within the sterilization chamber where materials to be sterilized may be placed.

Maximum Load: The maximum quantity or mass of items permitted in a sterilizer load.

Minimum Load: The minimum quantity or mass of items permitted in a sterilizer load.

Minimum Acceptable Cycle (MAC): The minimum cycle (in terms of delivered lethality), as specified in operating procedures, that would be considered acceptable for load release.

Mixed Load: A load that contains multiple item types representing various sterilization challenges. For example, some load items may have air removal challenges, while others pose a challenge due to their mass.

Moist Heat: Steam, steam-air mixtures, and superheated water used for sterilization.

Noncondensable Gases: Air and other gases that will not condense to liquid state, thereby not releasing latent heat under the conditions of sterilization.

Operating Parameters: Values (e.g., time, temperature, pressure) that are controlled and/or measured that collectively define each phase of a sterilization cycle (e.g., heat-up, exposure, cool-down).

Critical Parameters: Values that are controlled and/or measured and are linked to safety and efficacy of a product. Failure to meet a critical parameter should result in rejection of the load.

Key Parameters: Values that are controlled and/or measured and are used to assure the ongoing "state of control" of sterilization runs. Failure to meet a key process parameter should result in an investigation with a documented rationale for the disposition of the load.

Overkill Design Approach: A sterilization design approach where minimal information is required about the product bioburden. A worst-case bioburden assumption is used to determine the delivered lethality needed to achieve a PNSU of 10^{-6} on or in the items being sterilized. When using this approach, the qualification program must demonstrate that both the F_{BIO} and F_{PHY} are greater than 12 minutes.

Parametric Release: A sterility release system based upon effective control, monitoring, documentation, and batch records review of a validated sterilization process cycle in lieu of release procedures based upon end-product sterility testing.

Penetration Probe: A probe placed in contact with the load item or inside a container of liquid to measure the temperature of the load item or liquid.

Physical Qualification: A component of performance qualification that demonstrates that predetermined physical requirements, including temperature distribution and heat penetration, are achieved consistently throughout the load.

Porous/Hard Goods Load (P/HG): A porous/hard goods load consists of items in which the bioburden is inactivated through direct contact with saturated steam. Porous/hard goods load items include: filters, stoppers, tubing (hoses), mops, garments, stoppers, cleaning equipment, or machine change parts.

Prevacuum Process: A sterilization process in which air is removed from the chamber using a vacuum pump or other mechanical system before the exposure phase begins. This method is particularly suited to load items that can trap air such as tubing, filters and filling machine assemblies.

Probability of a Non-Sterile Unit (PNSU): The number that expresses the probability of occurrence of a non-sterile unit after exposure to a sterilization process. Within the pharmaceutical industry, a design end point better than or equal to the probability of one non-sterile unit in a million units is expected, i.e., PNSU ≤ 10^{-6}. [Synonym: Sterility Assurance Level (SAL)]

Process Performance Qualification: Documented verification that a system is capable of consistently performing or controlling the activities of the processes it is required to perform or control, according to written and preapproved specifications, while operating in its specified operating environment.

Product-Specific Design Approach: A sterilization design approach that is based on the characteristics of the bioburden (on or in the load) and the heat sensitivity of the product that delivers the lethality needed to achieve a PNSU of 10^{-6} on or in the items to be sterilized.

Pure Steam: Steam whose condensate complies with the compendial monograph, Water for Injection (WFI). (4)

Resistometer: Test equipment designed to create defined combinations of the physical and/or chemical variables of a sterilization process. Resistometers were formally called biological indicator evaluator resistometers (BIER) vessels. The resistometer is used primarily in the laboratory to determine *D*- and *z*-values. (5)

Routine Operational Cycle: Parameters that are specified for ongoing sterilization operations. The operatio-

nal cycle is typically controlled to produce additional lethality over the qualified minimum acceptable cycle in order to provide increased sterility assurance.

Saturated Steam: Steam that is at a temperature and pressure that corresponds to the vaporization curve of water. It is in a state of equilibrium between being a liquid and a gas, with no entrained liquid water. [Synonym: Dry Saturated Steam]

Saturated Steam Process: A sterilization process, typically used for porous/hard goods loads, where the sterilizing medium is saturated steam.

Steam-Air Mixture (SAM) Process: A sterilization process in which the heating medium used to heat the load is in a mixture of air and steam that is typically used for liquid loads. This addition of air results in an air overpressure condition.

Sterilization: A process used to render a product free of viable organisms with a specified probability.

Sterility Assurance Level (SAL): Probability of a single viable microorganism occurring on an item after sterilization. [Synonym: PNSU]

NOTE: The term SAL takes a quantitative value, generally 10^{-6} or 10^{-3}. When applying this quantitative value to assurance of sterility, an SAL of 10^{-6} has a lower value but provides a greater assurance of sterility than an SAL of 10^{-3}. (6)

Sterilization Cycle: A sequence of defined operating parameters (e.g., time, temperature and pressure) and conditions required to render an item sterile.

Sterilization Run: Execution of a sterilization cycle.

Superheated Steam: Steam whose temperature, at a given pressure, is higher than that indicated by the equilibration curve for the vaporization of water.

Superheated Water: Water in a liquid phase at a temperature above 100°C requiring overpressure to maintain this state.

Superheated Water Process: A sterilization process in which the heating medium is superheated water that is continuously circulated with air overpressure. This process requires air overpressure to keep the water in a liquid state. [Synonyms: water cascade, water spray process, water immersion process, water submersion process, raining water process, steam-air-water process]

Survivor Curve: Graphical representation of the inactivation of a population of microorganisms with increasing exposure to a microbicidal agent under stated conditions. (7)

System Suitability Evaluations: Physical evaluations (e.g., chamber integrity or air removal) conducted on a scheduled frequency to demonstrate ongoing control of the sterilizer system.

Temperature: Temperature is the measure of thermal energy.

Temperature Distribution: Temperature measurement of the heating medium across the chamber load zone.

Terminal Sterilization: A process whereby product is sterilized within its sterile barrier system. (8)

Validation: A documented program that provides a high level of scientific assurance that a manufacturing process will reliably produce acceptable product. The proof of validation is obtained through rational experimental design and the evaluation of data, preferably beginning from the process development phase and continuing through the commercial production phase.

Worst-Case Load: The load configuration that is determined to be most difficult to sterilize. This is a function of the cycle control strategy and load item characteristics (e.g., mass, configuration, or air removal challenges). For porous/hard goods loads, this may not necessarily be the minimum or maximum load.

***z*-value:** The number of degrees of temperature change necessary to change the *D*-value by a factor of 10. The *z*-value allows integration of the lethal effects of heat as the temperature changes during the heating and cooling phases of a sterilization cycle.

3.0 STERILIZATION SCIENCE

This section describes essential scientific tools used for the design, development and qualification of sterilization cycles.

3.1 Sterilization Models

The death of a homogeneous culture of microorganisms exposed to constant lethal stress (also known as the survivor curve) has been shown *empirically* to follow first-order kinetics. (9) The rate of microbial death is a function of the thermal resistance of the microorganism and lethal stress and is independent of the number of microorganisms in the challenge. The survivor curve can be described using the following semilogarithmic, first-order model:

$$\text{Log } N_F = -F_{(T,z)}/D_T + \text{Log } N_0 \qquad \text{[Equation 1]}$$

where,

N_F = Number of microorganisms after exposure of F equivalent minutes

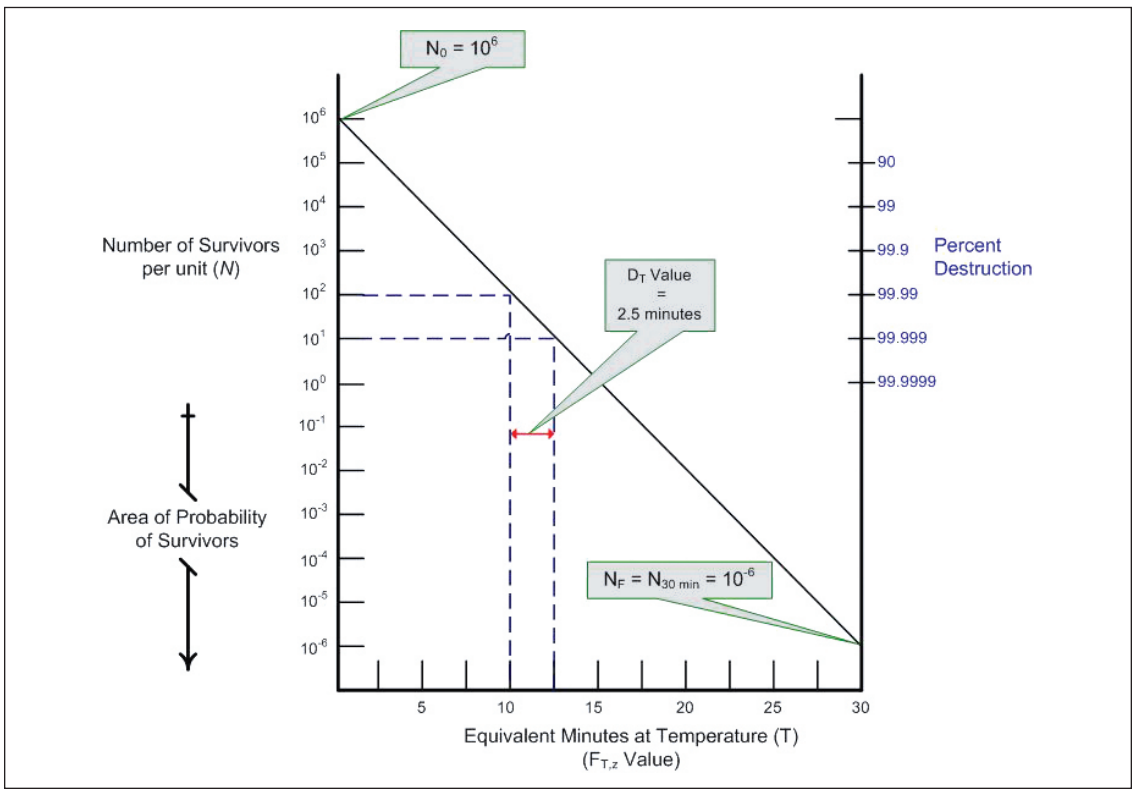
$F_{(T,z)}$ = Equivalent lethality of a cycle calculated as minutes at a reference temperature (T), using a defined temperature coefficient (z)

D_T = Thermal resistance value, in minutes, of the microorganism at a specific temperature (T). Note: This specific temperature must be the same as the reference temperature used for calculating F-value.

N_0 = Number of microorganisms prior to exposure

Figure 3.1–1 illustrates the semilogarithmic survivor curve described above for a biological indicator.

Figure 3.1–1 ■ Microbial Survivor Curve According to the Semilogarithmic Model



In **Figure 3.1–1**, D_T is a measure (the negative reciprocal) of the slope of the semilogarithmic survivor curve; therefore, it describes the relationship between the number of survivors versus equivalent (*F*-value) exposure time. *F*-value is a term used in the model to characterize exposure time to moist heat. By definition, the *F*-value is expressed by a reference temperature so that it truly represents the equivalent exposure time, in terms of lethality, at that reference temperature. Since routine operational cycles are not generally square wave cycles (i.e., the load does not come up to temperature instantaneously, remains at the precise set point throughout the exposure phase, and then cools down instantaneously), the *z*-value, or temperature coefficient, is used in the model to calculate the equivalent lethality at different temperatures. These terms (D_T, *z*, F-value, and *L*) are discussed in greater detail in **Sections 3.1.1–3.1.3**.

In order to use this semilogarithmic model for the survivor curve in the scientific approach to microbial destruction, the challenge must consist of a homogeneous culture, and a constant lethal stress (or ability to calculate equivalent lethal stress – *F*-value) must be applied to the challenge.

The semilogarithmic relationship does not accurately fit all experimental microbial thermal destruction data; (10) but, to date, no other model is known that fits all experimental data. To be useful, a model or mathematical relationship should: (a) represent the system, (b) be predictive of system performance, and (c) be reasonably easy to use and understand.

The Microbial Survivor Curve Example call-out box depicts an example of an application of the survivor curve equation (Equation 1). The semilogarithmic survivor curve model can be used directly and graphically, to both analyze experimental data and design sterilization processes. The model fills the requirement for tools, both in the analysis of experimental data and in the design of moist heat sterilization processes. It allows calculations in both directions – from experimental microbial-destruction data to physical parameters, and from physical parameters to expected microbial survival data.

The use of this model is demonstrated in **Section 4.1** for sterilization process design and in **Section 5.2** for biological qualification of cycles.

3.1.1 Resistance Value (D_T)

D_T-value is the time in minutes required for a one-log, or 90%, reduction of a microbial population under specified lethal conditions, i.e., for a temperature (*T*) in moist heat sterilization. The D_T-value is the negative reciprocal of the slope of survivor curve depicted in **Figure 3.1–1**. One logarithmic cycle on the y-axis (population) represents a ten-fold change in the number of survivors; therefore, the D_T-value is the time, or equivalent time (*F*), on the x-axis for the survivor curve to traverse one logarithmic cycle.

It is important to note that the D_T-value is not strictly a genetic characteristic of any particular microorganism. The D_T-value is determined empirically using the first-order survivor curve model to characterize the resistance of a BI challenge system to a lethal agent. Refer to **Section 3.2.1** for discussion of factors that may affect the resistance of biological indicators.

D-values can be calculated from a survivor curve determined by either 1) direct enumeration or 2) using two data points (N_0 and a point in the quantal range calculated using fraction-negative methods).

3.1.1.1 Direct Enumeration Method

This method involves sub-lethal treatment of the biological challenge followed by enumeration of the survivors (spore counts). The resulting data can be plotted on a semilogarithmic graph (logarithm of surviving population versus equivalent exposure time), and the D_T-value determined from the slope of the line. The D_T-value is the negative reciprocal of the line that best fits the data points. Linear regression analysis can be used to determine the slope of the best-fit line, when appropriate.

Microbial Survivor Curve Example

Using the values in **Figure 3.1–1** the survivor curve equation for this biological indicator (BI) would be:

[Equation 1]

$$\text{Log } N_F = -F_{(T,z)}/D_T + \text{Log } N_0$$
$$\text{Log } N_F = -F/2.5 + \text{Log } 10^6$$

This equation can also be used to calculate the expected population (N_F) after exposure to a lethal stress (F). If F is 30 minutes based on physical parameters, N_F is calculated to be 10^{-6} (i.e., there is a one in a million chance that the BI will show growth after exposure for 30 minutes) as follows:

$$\text{Log } N_F = -F/2.5 + \text{Log } 10^6$$
$$\text{Log } N_F = -30/2.5 + \text{Log } 10^6$$
$$\text{Log } N_F = -12 + 6$$
$$\text{Log } N_F = -6$$
$$N_F = 10^{-6}$$

This equation can also be rearranged to calculate the lethal stress (F) applied to a BI based on the experimental determination of the number of survivors (N_F) on the BI after exposure. If 2×103 survivors were recovered on the BI after an exposure period, the equivalent exposure time (F) would be calculated as follows:

$$F = (\text{Log } N_0 - \text{Log } N_F) \times D_T$$
$$F = (\text{Log } 10^6 - \text{Log } 2 \times 10^3) \times 2.5 \text{ minutes}$$
$$F = (6 - 3.3) \times 2.5$$
$$F = 6.75 \text{ minutes}$$

3.1.1.2 Fraction-Negative Methods

Fraction-negative methods use N_0 and data in the quantal range to create a two-point line from which the D_T-value can be determined. The quantal range is the exposure period over which a set of replicate test BIs exhibit a dichotomous response – some are positive for growth, and the rest are negative for growth. There are two primary methods for analyzing fraction-negative data to determine the N_F for the two-point survivor curve:

- Holcomb-Spearman-Karber Method, where all the data in the quantal range are combined using a weighted average technique to yield the mean time corresponding to an N_F-value of 0.56 average survivors per BI. (11, 12, 13)
- Stumbo-Murphy-Cochran Method (14) where each set of data in the quantal area is individually analyzed using the Halvorson-Ziegler "Most Probable Number (MPN) Method" (15) to determine N_F and, in turn, a D_T-value for each set of data. These D_T-values are then averaged to determine the test D_T-value.

The Holcomb-Spearman-Karber Method allows for calculating a confidence interval for the D_T-value, thereby providing an indication of the quality and reliability of the data.

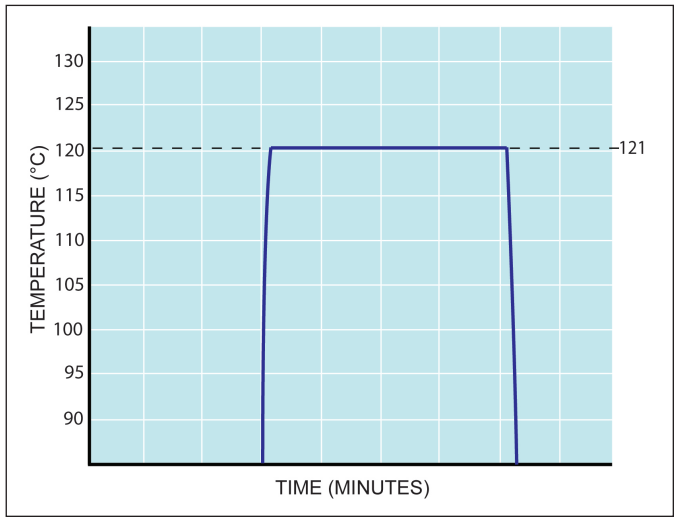
It is important to note that the use of statistical (mathematical) methods for calculating D_T-values requires strict adherence to statistical analysis rules. The BIs exposed throughout the study must be replicates and should be from the same BI lot. If prepared in house, they should all be prepared the

same way from the same stock suspension. Recovery and incubation procedures should be identical for all BIs in the study. The only variable should be the exposure time to the lethal agent.

Furthermore, for each set of replicate test BIs exposed for a certain exposure time, the study should assure that each replicate receives a similar dose of lethal agent. This is generally accomplished in the laboratory by using a resistometer. It is inappropriate to use these methods when true replicates are not part of the study. For example, these methods should not be used to evaluate kill in a production sterilizer where BIs are placed throughout the chamber. In this case, the multiple BIs are not considered replicates, because there is no assurance of uniform lethality throughout the sterilizer.

It is important to provide conditions of consistency from cycle to cycle during D_T-value determination studies. A properly operated resistometer provides an ideal mechanism for this consistency, since it produces a nearly square wave profile with minimum heat-up and cool-down times (**Figure 3.1.1.2–1**).

Figure 3.1.1.2–1 ■ Typical Temperature Curve for a Resistometer



D_T-value determination studies are usually necessary for the following activities:

- To characterize heat-resistant product isolates (obtained from heat shock testing) during design of sterilization cycles when product-specific approaches are used
- To evaluate the effect of the formulation change on resistance
- To characterize heat-resistant microorganisms isolated from the manufacturing environment during routine monitoring
- To establish *D*-values for biological indicators that are prepared by directly inoculating materials or product formulations with heat-resistant spores

3.1.2 Temperature Coefficient (*z*-value)

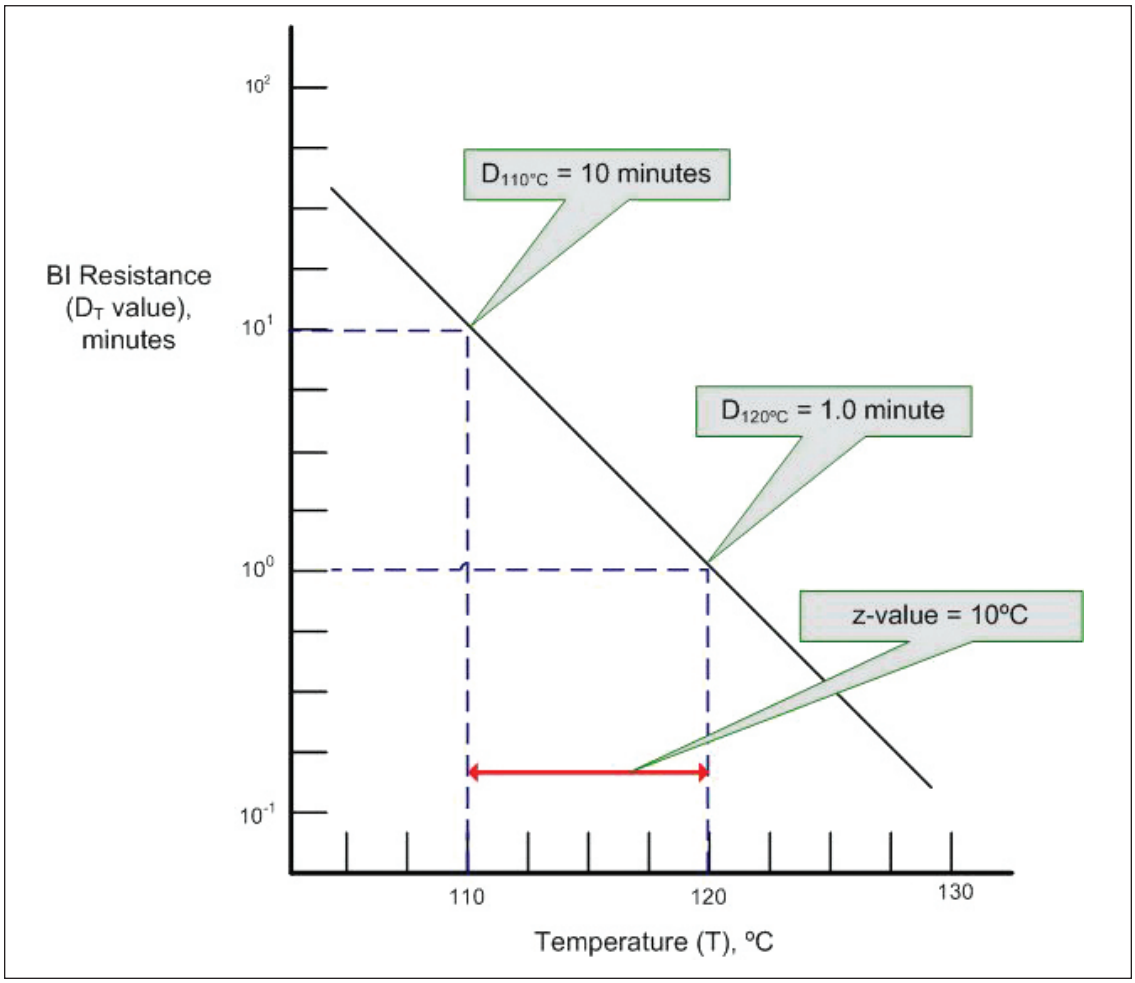
Change in the heat resistance of spores, as a function of the temperature, is represented by the *z*-value. The *z*-value is the number of degrees of temperature change necessary to change the D_T-value by a factor of 10. It is analogous to a temperature coefficient in the semilogarithmic model. The *z*-value is a component of the *F*-value calculation that is used to compare spore lethality at different temperatures.

For example, the D_T of a BI challenge system with a *z*-value of 8°C will change by a factor of 10 for each 8°C change in temperature. If the $D_{121°C}$ of the BI is 1.6 minutes, then the $D_{129°C}$ will be 0.16 minutes and the $D_{113°C}$ will be 16.0 minutes. The correct unit of measurement for temperature differences is the degree Kelvin, which is identical in all respects to a change of 1°C. In the interest of simplicity, degrees Celsius will be used throughout this document.

Moist heat sterilization processes are usually carried out within a small temperature range, e.g., 110–135°C, therefore the experimentally determined *z*-value is usually considered constant for practical purposes. (16) A *z*-value of 10°C is generally used in routine process design and evaluation. In studies where the objective is to reconcile the physical and biological *F*-values delivered to a product, the actual *z*-value of the biological indicator system must be used in the calculation of the physical F_T-values.

The *z*-value can be determined by several methods; however, determining the *z*-value from *D*-values is the most widely-used method. *D*-values for the challenge microorganism are determined at several different temperatures and then plotted with the logarithmic scale on the y-axis and with the temperature on the x-axis. A straight line is drawn through the data points. The *z*-value is the number of degrees of temperature for the *D*-value to change by a factor of 10 (one log cycle), e.g., from 2.0 minutes to 0.2 minute, or from 0.3 minute to 3.0 minutes. Like the *D*-value, the *z*-value is the negative reciprocal of the slope of this line, which is often referred to as the Thermal Resistance Curve (**Figure 3.1.2–1**). (17) It is important to consider the temperature scale when using z-values, and to express the z-value in terms of either Celsius (Kelvin) or Fahrenheit depending on the intended usage.

Figure 3.1.2–1 ■ Thermal Resistance Curve



3.1.3 Lethal Rate (*L*) and Lethality (*F*)

In moist heat sterilization, the ability to relate equivalent minutes at a reference temperature (T_{ref}) with microbial destruction at that same reference temperature is the foundation of all our biological measurements. The development of the measurement and integration of time-temperature data, using the *z*-value to calculate lethal rates, is what has made it possible to model microbial destruction processes.

Sterilizers are designed to operate at a specific designated temperature; however, the actual temperature may fluctuate around the target temperature within a range. Depending on the precision and

responsiveness of the recording device, this oscillation may not be readily apparent on the cycle record. While this fluctuation is usually minimal, it may have a significant effect on *F*-value determination, especially if the process temperature is predominantly on the low or high side of the target temperature. The *F*-value calculation considers all of the fluctuations around the target temperature by reducing the individual temperature observations to a common equivalent value in terms of delivered lethality. It is the integration of the lethal rate over the cycle.

3.1.3.1 Lethal Rate (L)

In order to understand the determination of the lethality (*F*-value) of a process, it is necessary to first understand Lethal Rate (*L*). The lethal rate can be calculated by the following formula: (18, 19, 20)

$$L_{(Tref,z)} = 10^{(T-Tref)/z} \qquad \text{[Equation 2]}$$

where:

T = Temperature of the item being heated

T_{ref} = Reference temperature

z = z-value of the challenge organism (or 10°C if not known)

The lethal rate is an exponential function, thus small temperature differences can have a significant effect on the delivered lethality. For example, for a BI system with a z-value of 10°C, a 1°C degree decrease in temperature reduces the lethality by approximately 20%. This is calculated as follows:

$$L = 10^{(120-121°C)/10} = 10^{-0.1} = 0.79$$

Thus, for a BI with a z-value of 10°C, one minute at 120°C is equivalent to 0.79 minutes at 121°C in terms of lethality.

3.1.3.2 $F_{Physical}$ Value (F_{PHY})

The *F*-value is a measurement of the lethality of a process. $F_{(Tref,z)}$ is the calculated equivalent of time, in terms of lethality, at a reference temperature, T_{ref} , and a temperature coefficient, z, that is delivered to the item being sterilized. *F*-values calculated from physical data (time and temperature) are also referred to as F_{PHY}.

The *F*-value is the integration of the lethal rates throughout the process. In practical terms, this integration is carried out as a numerical summation using the Trapezoid model:

$$F_{Tref} = d(\Sigma L) \qquad \text{[Equation 3]}$$

Where,

d = the time increment between each temperature reading

L = the lethal rate calculated for each temperature reading

The Time-Temperature Example call-out box (next page) provides a step-by-step example of calculating lethal rate and *F*-value. Data acquisition devices are available with programs that perform these calculations automatically and report the incremental and cumulative *F*-values for the process. Due to the inherent inaccuracies of the z-value, which is used to determine the lethal rate, a minimum temperature may be defined (e.g., 100°C) where the integration begins and ends. It is also important to use reference temperatures (T_{ref}) that are close to the operating temperature of the sterilizer during the exposure phase.

Time-Temperature Example

Table 3.3.2–1 ■ Example of Time-Temperature Data with Corresponding Lethal Rates (*L*) and Accumulated Lethality (*F*)

I	II	III	IV
Process Time (*t*)	Item Temperature (*T*)	Lethal Rate (*L*)	Accumulated Lethality ($F_{Physical}$)
d = time interval = 1 minute	Chamber Set Point = 122°C	*z* = 10°C T_{ref} = 121°C	$F = \Sigma L \times d$
Minutes	°C	Minutes at T_{ref} per Minute at *T*	Minutes
0	30.0	0.000	0.000
1	30.0	0.000	0.000
2	30.0	0.000	0.000
3	60.0	0.000	0.000
4	87.0	0.000	0.000
5	102.0	0.012	0.012
6	112.0	0.123	0.135
7	116.0	0.309	0.444
8	118.5	0.550	0.994
9	120.0	0.776	1.770
10	121.0	0.977	2.747
11	121.5	1.096	3.843
12	121.5	1.096	4.939
13	111.0	0.098	5.037
14	91.0	0.001	5.038
15	61.0	0.000	5.038

Column I is the Process Time (*t*). The time interval between temperature readings (*d* = 1 minute) is used in the accumulated lethality calculation (Column IV).

Column II is the Product Temperature (*T*). **Note:** The chamber temperature is set at 122°C.

Column III is the calculated Lethal Rate (*L*) using a reference temperature (T_{ref}) of 121°C and a z-value of 10°C. The lethal rate represents minutes at T_{ref} per minute at *T*. **Figure 3.1.3.2–2** is a plot of the lethal rate versus process time.

Column IV is the accumulated Lethality (F_{PHY}) calculated as the summation of the Lethal Rate times the time interval ($F = \Sigma L \times d$). *F* is also the area under the lethal rate graph (**Figure 3.1.3.2–2**).

The calculated F_{PHY} for this process is 5.038 minutes. Thus, the product temperature profile in **Figure 3.1.3.2–1** is equivalent, in terms of lethality, to a square wave product temperature profile of 5.038 minutes at 121°C.

Figure 3. 1.3.2–1 ■ Example of Item Time-Temperature Graph

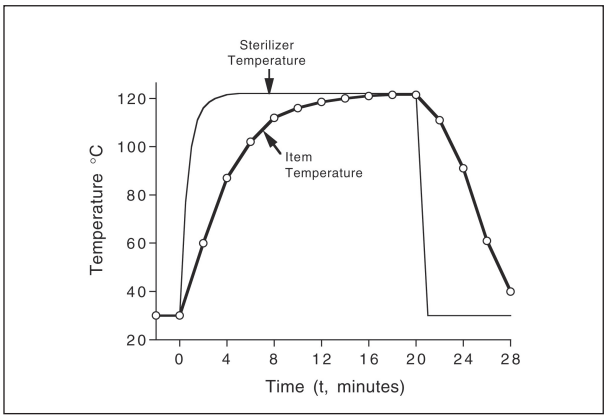
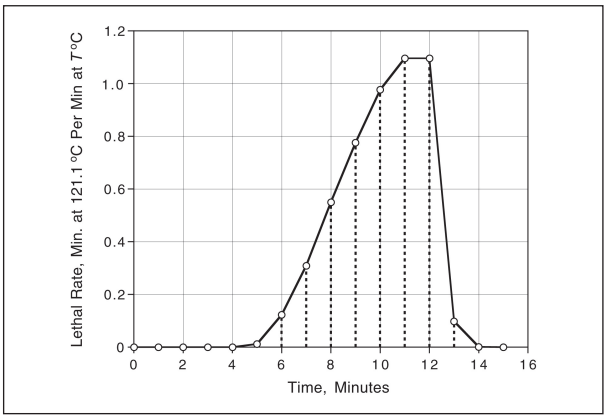


Figure 3. 1.3.2–2 ■ Example of Lethal Rate Graph



3.1.3.3 F_0

The term F_0 is the number of equivalent minutes of steam sterilization at a temperature of 121°C delivered to a container or unit of product. This is calculated using a *z*-value of 10°C. (21)

Note: The reference temperature used in calculating F_0 is 121.1°C, which is the approximate mathematical equivalent of 250°F. The reference temperature of 121°C for F_0 will be used throughout this report for brevity.

Wherever a value is stated in terms of F_0, it is referring to the equivalent time at 121°C. If, for example, a cycle has a stated F_0-value of 8.0 minutes, then the sterilization effectiveness of that cycle is equivalent to 8.0 minutes at 121°C regardless of the load/product temperature and time actually achieved in the cycle.

3.1.3.4 $F_{Biological}$ (F_{BIO})

The term F_{BIO} represents the delivered lethality measured by the actual kill of microorganisms on or in a BI system. The F_{BIO}-value is calculated using the following formula:

$$F_{BIO} = D_T \times LR \qquad \text{[Equation 4]}$$

where:

D_T = *D*-value of the BI at the reference temperature

LR = Log reduction of the BI population achieved during the cycle

3.2 Process Indicators

There are a variety of process indicators that, depending on their design, may be used during the development, qualification, and monitoring of sterilization cycles. Types of indicators and their appropriate use are described below.

3.2.1 Biological Indicators (BIs)

Determination of documented, acceptable probability of survival is based upon the predictable logarithmic death kinetics of microorganisms. This determination is also dependent on the sterilization process used and the sterilization approach selected. Since the biological indicator is a key component in the determination of process lethality, it is imperative that it performs both predictably and reproducibly.

The most common microorganism used as a biological indicator for the qualification of cycles using the overkill design approach is *Geobacillus stearothermophilus* (formerly *Bacillus stearothermophilus*); however, other moist heat resistant microorganisms may be acceptable. Typical microorganisms used for qualification of cycles using the product-specific design approach include: *Clostridium sporogenes*, *Bacillus smithii* (formerly *Bacillus coagulans*) and *Bacillus subtilis 5230*. (22)

The chosen biological indicator typically contains a higher population and resistance than the bioburden on the product or item. Only spores should be used as microbiological challenges for steam sterilization processes. These spores must be clean (essentially free from vegetative microorganisms, microscopic debris and clumping), well-characterized (with respect to their thermal responsiveness), and the "parent" cultures should be obtained from recognized culture collections.

Ideally, the BI's heat resistance is determined in a calibrated physical system capable of square wave heating (e.g., resistometer). (23) Biological indicators challenge systems are available in a variety of configurations, including spore suspensions in solutions, on paper carriers (e.g., spore strips or discs), and on other types of carriers (e.g., metal disks, cotton threads, or ampoules).

If the reliability of a vendor's Certificate of Analysis is established through a supplier qualification program, and the BI is not modified, then the supplied values for resistance of a biological indicator can be used in lieu of confirmatory testing of each lot. (24) Due to potential shipping and handling concerns, confirmatory spore population testing should be conducted upon receipt of biological indicators. It is important to use the same enumeration methodology that the vendor used in order to minimize variables that could lead to differences in spore counts.

A determination of the stability of the population and resistance (D_T-value) of the BI should be performed over its shelf-life. The actual population and resistance to be used should be based on the desired lethality and the qualification approach used (**Section 5**).

The *D*-value of the BI challenge system is a function of not only the species or strain used and prior history, but all facets of the test environment, including, but not limited to, the following:
- The test system (Are the spores suspended in liquid or dried on a solid support? What is the nature or environment surrounding the spores during heating?)
- The medium in which the spores are suspended
- The test temperature
- The primary packaging
- Exposure times that are not corrected for lags in heating and cooling
- Temperature, time and environmental conditions between heating and recovery
- The growth medium used to recover the heated spores
- Incubation conditions

The operation of the test system, including equipment control and technician variability, can make the resulting *D*-value either larger or smaller.

It is important to evaluate the thermal resistance of the biological indicator in the actual configuration to be tested for validation, since the resistance of microorganisms is affected by the solution or substrate onto which they are placed. For example, the heat resistance of *G. stearothermophilus* is increased in the presence of sodium chloride or potassium chloride; the thermal resistance of *B. coagulans* is increased by the presence of divalent calcium and magnesium ions; and the thermal resistance of *C. sporogenes* is increased by the presence of potassium ions. (25) The presence of chelating agents (e.g., citric acid) may also affect the thermal resistance. Strong chelating agents can compete for available ions, yielding changes in spore-coat resistance. (26) Similar resistance differences may be observed based on the substrate that is inoculated (e.g., paper or rubber).

When performing direct inoculation onto substrates, it is important to ensure that the biological indicator remains in the area intended for inoculation and is not reduced during inoculation. The use of multiple smaller volume inoculations typically provides better inoculum placement and control than a single larger volume, which is prone to inadvertent movement.

Compendia (e.g., USP, PhEur) contain monographs defining BIs that may be used in evaluating moist heat sterilization. In the U.S., it is not a requirement to use a compendial BI for industrial sterilization processes. In other regions and countries it is recommended that consideration is given to local regulatory expectations.

3.2.2 Chemical Monitors

A chemical monitor is a device that provides an indication or response to one or more critical sterilization parameters. They can be used as qualitative indicators to verify that a product has been exposed to a sterilization process. Where their reaction can be quantified, they can be used to answer more complex questions. Regardless of their design, chemical monitors cannot be used in lieu of biological indicators and instrumented measurement of temperature/pressure/time during the validation of a steam sterilization process.

3.2.2.1 Chemical Indicators
A chemical indicator is a device that responds to sterilization process parameters in a nonquantitative fashion. Chemical indicators are used to provide immediate results that can serve as a permanent marker (e.g., color change) to indicate that a load or product has been subjected to a sterilization cycle.

Chemical indicators do not indicate that the load is sterile. They only indicate that the threshold temperature was reached, not what the highest temperature achieved was or how long that temperature was maintained. It is important that sufficient indicators be placed throughout the load to prevent inadvertent interchange of sterilized and non-sterilized items. There are several different types (e.g., tape, paper strips or small ampoules) of chemical indicators available.

3.2.2.2 Chemical Integrators
Chemical integrators are designed to react quantitatively to a particular combination of parameters in moist heat sterilization – typically temperature and time and sometimes moisture. These devices react in a quantifiable way to their exposure to certain physical conditions that are critical to the sterilization process. They deliver a measurable record of conditions that may be correlated to microbial inactivation.

When chemical integrators are used, their advantages and limitations should be clearly understood:
- Integrators with simultaneous moisture monitoring may give additional information on the sterilizing conditions achieved in the cycle monitored that are not readily available from temperature and pressure monitoring in commonly used sterilizers.
- Integrators may give an indication of the sterilizing conditions achieved throughout the load that should be correlated to the temperature distribution data achieved during cycle validation.
- Integrators cannot be used exclusively for cycle qualification.

3.3 Thermal Science and Steam Quality

Understanding the basics of thermal science (thermodynamics) is essential to the design and control of moist heat sterilization. There are significant differences in energy content, at a given temperature, in the various heating media used. Superheated water, saturated steam and steam/air mixtures contain different amounts of thermal energy. The strict relationships between temperature and pressure in saturated steam cycles established during cycle design must be achieved in routine production cycles to ensure the efficacy of the sterilization process. The following sections describe the importance of specific parameters in the sterilization process.

3.3.1 Temperature and Heat
Temperature is a measure of thermal energy. Heat is energy that is transferred as a result of a temperature difference between an object and its surroundings.

It is important to understand that thermal energy contained by different heating media at the same temperature (e.g., saturated steam, air/steam mixtures or superheated water) is dramatically different.

The heat of vaporization/condensation is the primary mechanism that imparts energy to the item being sterilized in a saturated steam process. Saturated steam contains 2,675 J/g at 100°C. This is the sum of the energy in the water (at 419 J/g) plus the energy required to create steam (2,256 J/g) or the heat of vaporization/condensation at 100°C. The condensation of one gram of steam imparts 2,256 joules to the object at 100°C.

The heating curve for water shown in **Figure 3.3.1–1** demonstrates this phenomenon. (27) To change the temperature of one gram of liquid water by 1°C, 4.1 joules are required (at 25°C and

Figure 3.3.1–1 ■ Heating Curve of Water at 1.0 Atmosphere

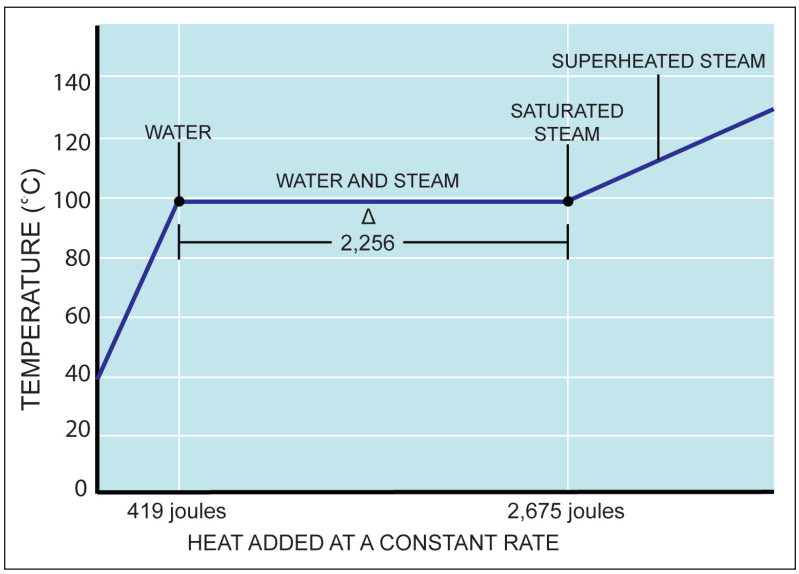
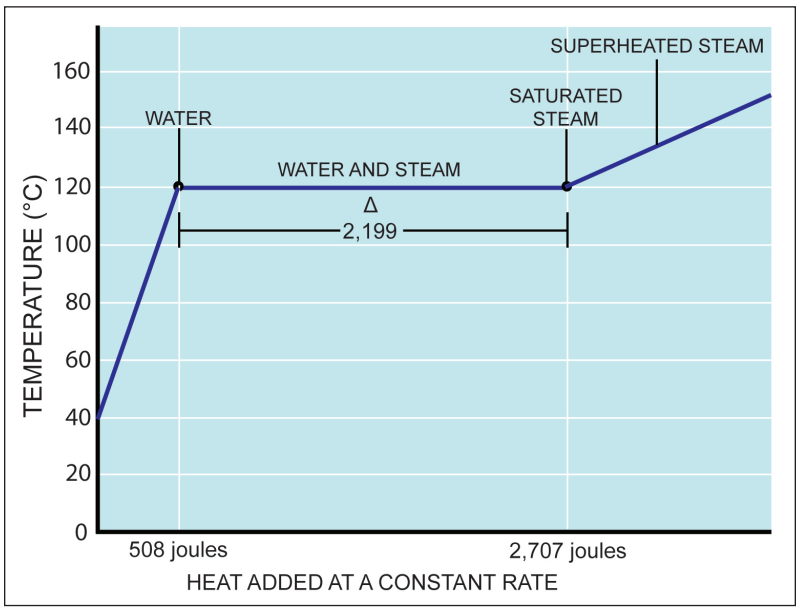


Figure 3.3.1–2 ■ Heating Curve of Water at 2.0 Atmosphere



1.0 atmosphere). The temperature will increase with the input of energy until it reaches 100°C. At 100°C, no further temperature change at 1.0 atmosphere will occur until an additional 2,256 joules have been absorbed by the water to convert all of it to steam. This energy is imparted to an object when the process is reversed and the steam is condensed to water. The following figures are simplified depictions of the thermodynamics involved in the phase change from water to steam and are not drawn to scale.

At 2.0 atmospheres, when the temperature of the water reaches 121°C, it does not change to saturated steam until 2,199 J/g has been added as depicted in **Figure 3.3.1-2**. (27)

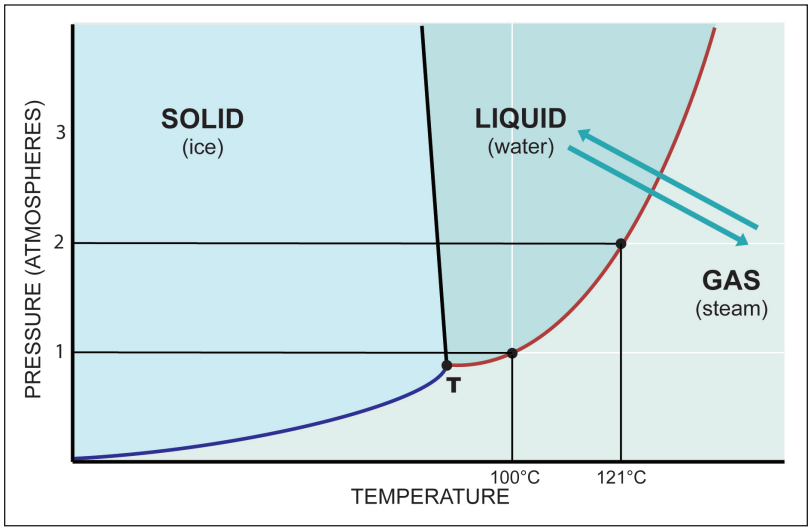
In the 2.0 atmosphere example (**Figure 3.3.1–2**), the transfer of 2,199 joules as a result of the phase change between water and steam occurs only when the system is in "dynamic equilibrium". This occurs when water and steam are in equilibrium at a specific pressure and temperature.

In the simplified pressure and temperature phase diagram (**Figure 3.3.1–3**), (28) the curve shown in red depicts water and steam in equilibrium. Points along this curve can be calculated and used to generate the steam tables, which are then compared to operational results to determine that a system is operating in dynamic equilibrium, and that the steam is "saturated". There is only one pressure that corresponds to a specific temperature on the curve when the steam is saturated. In **Figure 3.3.1–3** two temperature points are given: at 1.0 atmosphere the temperature is approximately 100°C, and at 2.0 atmospheres, the temperature is approximately 121°C.

Steam tables are developed by calculating and listing corresponding temperatures and pressures of a system in dynamic equilibrium. The energy properties contained by water and saturated steam at a specific temperature and pressure may also be listed, since they are well known. Excerpts from the

ASME International Steam Tables are presented in **Table 3.3.1–2**. (29)

Figure 3.3.1–3 ■ Pressure and Temperature Phase Diagram



Pressure, as well as temperature, for any saturated steam cycle should be checked to ensure that the values are in general agreement with the steam tables. If the values are not in general agreement, then this may be an indication that the sterilization cycle is taking place without the full effect of the heat of condensation (saturated steam). In this case, the delivered energy may be less than the calculated energy using **Table 3.3.1–2**.

The mechanisms that transfer energy are conduction, convection and radiation, which may occur separately or in combination.

3.3.1.1 Conduction

Conduction is the transfer of energy through molecular agitation without any required motion of material as a whole. In sterilization science, this is applicable in the following examples:

- Through the container wall to the liquid being sterilized
- Energy transfer to the surface of an item by direct contact with the sterilizing medium (e.g., steam or hot water)

Table 3.3.1–2 ■ Saturated Steam Table

Properties of Saturated Water and Steam (Metric)				
Temperature	Pressure	Enthalpy (Internal Thermal Energy) J/g		
°C	Bar**	Water h_L	Δh*	Steam h_v
100	1.013	419	2256	2675
115	1.692	483	2216	2699
120	1.987	504	2202	2706
121	2.026	508	2199	2707
125	2.322	525	2188	2713
Properties of Saturated Water and Steam (US Customary Units)				
Temperature	Pressure	Enthalpy (Internal Thermal Energy) Btu/lb		
°F	Psia**	Water h_L	Δh*	Steam h_v
212	14.71	180	970	1150
240	24.99	208	952	1160
250	29.84	218	945	1164
260	36.45	228	938	1167
270	41.87	238	931	1170

*Latent heat of condensation or vaporization ($\Delta h = h_v - h_L$)

**1.0 Atmosphere = 1.013 bar = 14.71 psia

3.3.1.2 Convection

Convection is the transfer of energy resulting from contact with a moving fluid.

When this process occurs naturally, as in the movement of a fluid in a container being sterilized, it is referred to as natural convection. Forced convection occurs when the fluid is moved by a fan or pump.

3.3.1.3 Radiation

Radiation is the transfer of energy through electromagnetic waves.

Radiant heat is the energy transfer mechanism that occurs primarily during a cycle drying phase under vacuum, but it can contribute to the heat added to the load during the sterilization phase of the cycle, especially if the jacket temperature is appreciably higher than that of the chamber.

3.3.1.4 Heat Transfer Rate and a Comparison of Heat Capacities of Heating Mediums

The flow of heat from the heating media to a sealed container is dependent on a number of factors that include the temperature difference between the container and the heating media, the geometry and characteristics of the container, and the overall heat transfer coefficient. The heat transfer coefficient is a complex function that includes the thermodynamic characteristics of the heating media. The energy content differences of media and the importance of attaining saturated steam conditions have been covered in earlier sections, but here we briefly cover the heat transfer from the media to the item being sterilized and compare the heat capacities of each alternative (**Table 3.3.1.4–1**).

Table 3.3.1.4–1 ■ Overview of Capabilities and Requirements for Sterilization Processes

Sterilization Processes		Heat Transfer Rate	Circulation Required	Temperature Distribution Challenges	Load Considerations
Saturated Steam		High	No	Low	P/HG & liquid loads that do not require a total pressure greater than the saturated steam pressure
Steam-Air Mixtures		Function of steam-to-air ratio and flow velocity	Yes	High	Liquid and potentially some P/HG loads that require a total pressure greater than the saturated steam pressure
Superheated Water	Water spray with air over pressure	Moderately high, function of flow velocity	Yes	Moderate	Liquid loads that require a total pressure greater than the saturated steam pressure
	Water submersion with air over pressure	High, but function of flow velocity	Yes	Moderate	Liquid loads that require a total pressure greater than the saturated steam pressure

In general, there is an optimal heating system for each application. Items typically heat most rapidly in saturated steam. In some cases, such as large flexible packages, a superheated water submersion/immersion sterilizer is highly efficient.

Tables 3.3.1.4–2 through **3.3.1.4–4** show the heat capacity of steam, water and steam-air mixtures. (30) Heat capacity is the heat given up when the temperature of steam, superheated water and three concentrations of steam-air mixtures are reduced 1.0°F. The values are expressed per pound, BTU/lb.°F and per cubic foot, BTU/ft^3°F.

An evaluation of the data in the charts indicates that on a volume basis (the more meaningful comparison) the heating capacity of superheated water and steam is similar. Both media have high energy content – the steam because of its latent heat and the superheated water due to its mass. In the case of the superheated water sterilizer, however, the transfer of heat is highly dependent on the

Table 3.3.1.4–2 ■ Saturated Steam

Temperature	Latent Heat Capacity (Δ*h*)	
	BTU/lb.°F	BTU/ft³°F
212°F	970.3	36.1
250°F	945.3	68.3

Table 3.3.1.4–3 ■ Water

Temperature	Heat Capacity	
	BTU/lb.°F	BTU/ft³°F
212°F	1.001	59.87
250°F	1.003	58.98

Table 3.3.1.4–4 ■ Steam-Air Mixture

Temperature	Heat Capacity					
	60% Steam		75% Steam		90% Steam	
°F	BTU/lb.°F	BTU/ft³°F	BTU/lb.°F	BTU/ft³°F	BTU/lb.°F	BTU/ft³°F
212°F	13.06	0.61	19.94	0.86	27.6	1.10
250°F	11.56	0.72	17.21	1.12	24.29	1.65

forced movement of the media past the container. Steam does not require forced circulation, since it condenses to liquid at a near-constant temperature and is replaced by more steam.

The heat-delivery rate of steam-air mixtures to containers is a function of the ratio of air to steam and the forced circulation of heating medium throughout the sterilizer. Steam-air mixtures have a much lower heat capacity per unit volume than either pressurized water or steam. However, steam-air mixtures can be an effective sterilant with a properly developed cycle.

3.3.2 Steam

Several types of steam can be used for moist heat sterilization: these include, but are not limited to, plant steam, process steam and pure steam. Since they are all application specific, it should be demonstrated which type of steam is appropriate.

3.3.2.1 Plant Steam

Plant steam is a general purpose, industrial steam generated, distributed and used for a variety of energy transfer purposes such as facility, water and process heating, or to drive engines or turbines. For moist heat sterilization, plant steam is generally considered adequate for supplying the sterilizer jacket (provided the chamber steam is not sourced from the jacket). It is also used to heat water for superheated water processes on the non-sanitary side of heat exchangers.

3.3.2.2 Process Steam

Process steam is similar to plant steam, except the steam is generated using a controlled feed water source to which no volatile additives (amines or hydrazines) have been introduced. Process steam may be appropriate for moist heat sterilization of liquid loads when the containers are filled and sealed prior to sterilization.

3.3.2.3 Pure Steam

Pure steam (sometimes called clean steam or high quality steam) is steam whose condensate complies with the compendial Water for Injection (WFI) monograph. (31) Pure steam is typically produced by purpose-designed steam generators or from the first effect of a multiple-effect still using a feed water of known chemical quality.

Softened, deionized, and purified water as defined in current compendia have all been successfully used as feedwater to pure steam generators. Also important to the proper operation of the system is the appropriate design, placement, and maintenance of steam traps and air vents in the steam distribution system. Condensate pooling should be avoided. Pure steam should always be used for porous/hard goods load sterilization.

3.3.3 Steam Quality Testing for Pure Steam

The semilogarithmic model of inactivation of microorganisms for saturated steam processes assumes that saturated steam is free from noncondensable gases and free from superheat. Wet steam, superheated steam, and steam containing noncondensable gases have the potential to adversely affect the lethality achievable in porous/hard goods load cycles. The extent to which saturated steam sterilization processes may be affected by steam quality depends on the extent of the deviation from ideal steam conditions and the type(s) of items in the load being sterilized.

For sterilization of porous/hard goods, steam quality characteristics should be evaluated as part of the qualification of the steam supplied to the sterilizer and should be repeated at regular intervals and documented in internal company policy or in accordance with applicable regulatory requirements. (32)

3.3.3.1 Noncondensable Gases

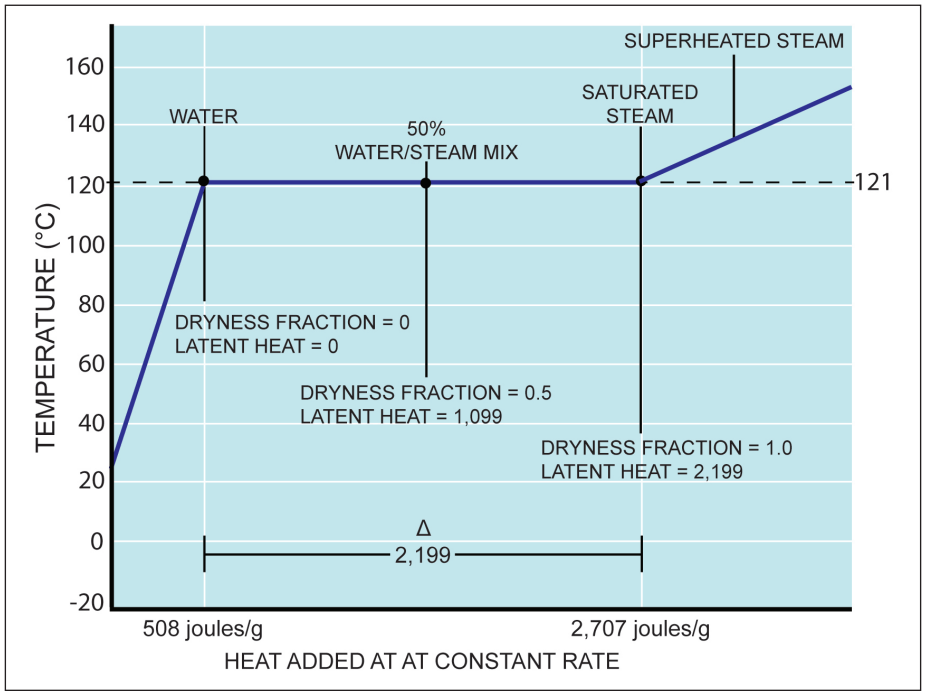
Noncondensable gas is gas (e.g., air, nitrogen and CO_2) that may be carried over in steam from the steam generator. These noncondensable gases change the steam from being pure, vapor-phase water to a mixture of steam and gas.

3.3.3.2 Dryness Fraction and Dryness Value

The dryness value (an empirical determination of dryness fraction) of steam is a measure of the amount of liquid-phase water carried by the steam and applies to a saturated steam process. A value of 0 denotes 100% water and a value of 1.0 represents dry steam with no liquid-phase water. In addition to the problem of causing wet loads for certain items, the energy contained in steam with less than approximately 1.0 can be significantly less than pure saturated steam. Dryness value is determined by testing, and values are generally considered approximate.

The dryness fraction of steam is inextricably linked with the latent heat that it possesses. Steam having an energy level equal to 50% of the latent heat for its saturation pressure will have a dryness

Figure 3.3.3.2–1 ■ Dryness Fraction at 2.0 Atmospheres (Absolute)



fraction of 0.5 indicating a 50:50 water/steam mixture. Therefore, only when steam has its full quotient of latent heat will it be dry saturated and have a dryness fraction of 1.0. (*33*)

Figure *3.3.3.2*–1 illustrates the corresponding loss in energy in the heat transfer process if the dryness fraction is less than 1.0. When 100% water is present at 121°C, there is no latent heat, and the dryness fraction is 0. When 50:50 steam/water mixture is present, the dryness fraction is 0.5, and the latent heat is 1,099 joules/g. When 100% steam is present, the dryness fraction is 1.0 with 2,199 joules/g of latent heat.

3.3.3.3 Superheat

Superheated steam is steam whose temperature, at any given pressure, is higher than that indicated by the equilibration curve for the vaporization of water. For inactivation of microorganisms to conform to the semilogarithmic model, the steam should be saturated, i.e., it should be at its equilibration temperature for the particular pressure achieved (**Section 2.0**). The lethal effect of steam with superheat on microorganisms will be less than that predicted for the particular temperature registered.

The principal causes of superheat are:

- Excessive pressure reduction near the point of use
- The sterilizer chamber jacket is at a higher temperature than the chamber

4.0 STERILIZATION PROCESS DEVELOPMENT

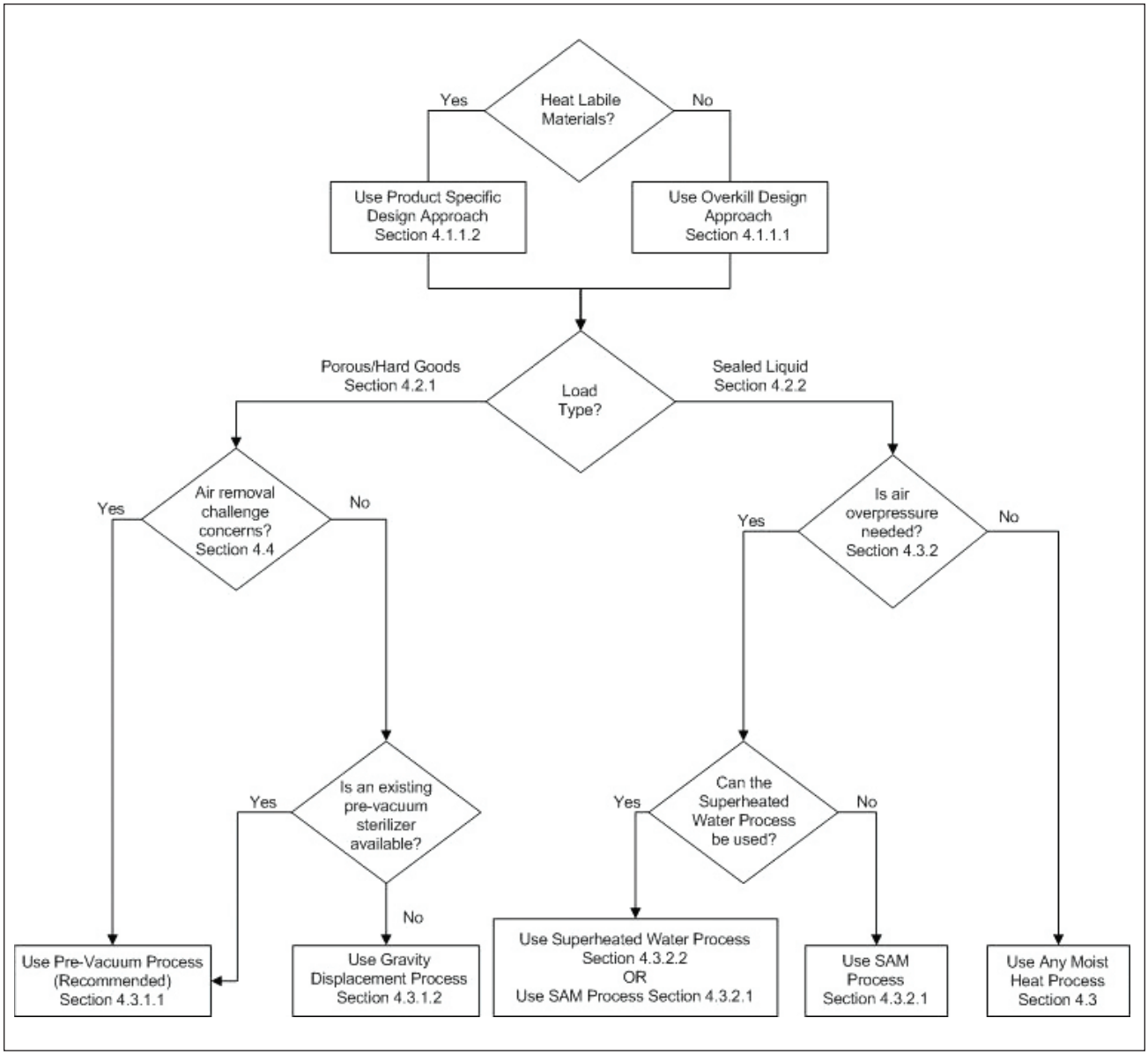
The objective of sterilization process development is to develop a process that fulfills the design requirements. This section progresses from establishing design requirements and determining load types to process selection and parameter determination.

A decision tree has been developed to guide the user of this document in the selection of a cycle design approach and sterilization process development (**Figure 4.0-1**). The decision tree summarizes key product and process considerations and provides recommendations regarding recommended cycle design approaches and process development steps based on these considerations.

4.1 Design Approaches

Two principal sterilization cycle design approaches that may be used for the development of sterilization processes are the overkill design approach and the product-specific design approach. Both of these approaches are able to provide the same level of sterility assurance to the product or

Figure 4.0–1 ■ Moist Heat Sterilization Process Decision Tree

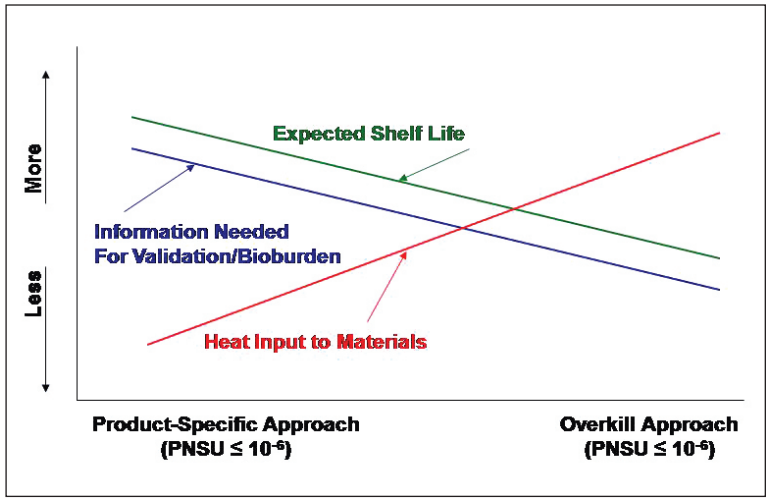


materials being sterilized. This section discusses use of the semilogarithmic model to calculate the desired process lethality using one of the two design approaches.

In the design of sterilization cycles, the choice between the two design approaches is largely based on the thermal stability of the product or materials being sterilized.

The overkill design approach requires less initial and ongoing information on the bioburden of the materials being sterilized than the product-specific design approach, as depicted in **Figure 4.1–1**. It requires a greater heat input, and consequently has a greater potential to degrade the items being sterilized.

Figure 4.1–1 ■ Comparison of Sterilization Approaches



The product-specific design approach requires a greater amount of initial and continuing information on the items being sterilized, the indicator organisms (those test organisms shown to be most resistant to the sterilization process), and the bioburden levels than the overkill design approach. The accumulation of this information provides confidence in the values determined in development to use a lower thermal input than required for the overkill design approach.

Using a lower thermal input also has the added benefit of providing greater stability of the materials being sterilized, potentially increasing their shelf life. For this reason, the product-specific design approach is more appropriate for the terminal sterilization of heat-labile formulations in their final containers.

In **Section 5.0**, the semilogarithmic model is used to determine the appropriate biological challenge needed to demonstrate that the desired lethality has been delivered to the product. **Section 5.2** discusses qualification approaches to verify that the design criteria for both physical and biological lethality (F_{PHY} and F_{BIO}) are delivered to the items being sterilized.

4.1.1 Use of Survivor Curve in Cycle Design Approaches

The following semilogarithmic survivor curve equation is used in both cycle design approaches to determine the necessary lethal dose requirements to achieve the desired endpoint (N_F) – a PNSU of 10^{-6}.

$$\text{Log } N_F = -F_{(T,z)}/D_T + \log N_0 \qquad \text{[Equation 1]}$$

Rearranging for F

$$F_{(T,z)} = (\text{Log } N_0 - \text{Log } N_F) \times D_T$$

From a design standpoint, the overkill and product-specific design approaches differ in the values that are used for D and N_0.

4.1.1.1 Overkill Design Approach

The objective of the overkill design approach is to assure a level of sterility assurance regardless of the number and heat resistance of the actual bioburden organisms in the load. The assumed product bioburden values for population and resistance are set at the following levels:

$$\begin{aligned} N_0 &= 10^6 \\ D_{121^\circ C} &= 1.0 \text{ minute} \\ z &= 10^\circ C \end{aligned}$$

And, to achieve the necessary PNSU,

$$N_F = 10^{-6}$$

Using the above values, the design requirements for delivered lethality, F_{PHY} and F_{BIO}, can be calculated as follows.

$$\begin{aligned} F_0 &= D_{121^\circ C} \times (\text{Log } N_0 - \text{Log } N_F) \\ F_0 &= 1.0 \text{ minute} \times (\text{Log } 10^6 - \text{Log } 10^{-6}) \\ &= 12 \text{ minutes} \end{aligned}$$

Thus, using the semilogarithmic model and the assumptions outlined above, a cycle designed with the overkill design approach can be defined as a sterilization cycle that is demonstrated to deliver an F_{PHY} and F_{BIO} of at least 12 minutes to the items being sterilized. **Sections 5.1** and **5.2** discuss how this design objective is qualified both physically and biologically.

EU regulations for terminally sterilized dosage forms defines overkill as sterilization by moist heat at 121°C for 15 minutes. (34)

Very few naturally occurring microorganisms have been found to have a $D_{121^\circ C}$-value greater than 0.5 minutes. The overkill design approach assumes both a higher bioburden population and resistance than would be expected. Most microorganisms have lower heat resistances; therefore, a cycle designed using the overkill design approach will inactivate them and provide a significant level of sterility assurance. Because worst-case assumptions are made for the bioburden population and resistance with this design approach, there is little scientific necessity for routine bioburden monitoring of the load items.

4.1.1.2 Product-Specific Design Approach

An overkill design approach cannot ordinarily be used when sterilizing heat-labile products or items. This situation is often the case in the terminal sterilization of drug products. A cycle must be developed which will adequately destroy the microbial load but will not result in unacceptable product degradation. The cycle is dependent on studies to determine the number and heat resistance of microorganisms in the product. Once the heat resistance and population of the bioburden organisms is characterized, a cycle can be designed that will result in a PNSU of 10^{-6}.

For design purposes, the values selected for N_0 and D_T are based on values determined by bioburden analysis plus additional safety margins that are based on: 1) professional judgment 2) the extent of the bioburden data and 3) the degree of product bioburden testing that will be conducted on an ongoing basis.

Actual bioburden population for products made under CGMPs should be very low, 1.0–100 cfu per container. Generally, only spore-forming environmental or product isolates need be subjected to *D*-value determination. Heat-shocking the product for 10–15 minutes at 80–100°C can screen out less resistant organisms. The value selected for D_T should include a safety margin over the most resistant organisms detected in bioburden studies. The safety margin selected inversely correlates to

the frequency and magnitude of ongoing tests conducted for bioburden population and resistance. For example, if the observed worst-case product bioburden resistance is 0.3 minutes and a D_T-value 0.4 minutes is selected, then extensive ongoing bioburden resistance testing would be necessary. On the other hand, if a D_T-value with the resistance of 1.0 minute (similar to the assumption for D_T using the overkill design approach) is selected, then there is little scientific necessity for routine bioburden monitoring of the load items. Additional examples of the product-specific design approach are provided in the call-out box.

Product-Specific Design Approach Examples

Example 1

a) bioburden testing of product

$N_0 < 10^1$ resistant microorganisms per unit of product

$D_{121°C} < 0.25$ minutes

b) values used for process design

$N_0 = 10^2$ microorganisms

$N_F = 10^{-6}$ (PNSU)

$D_{121°C} = 0.4$ minutes

c) calculated minimum lethality to achieve a PNSU of less than 10^{-6}

$F_{121°C} = (\text{Log } N_0 - \text{Log } N_F) \times D_T$

$(\text{Log } 10^2 - \text{Log } 10^{-6}) \times 0.4 \text{ minute} = 3.2 \text{ minutes}$

In this example, a minimum F_0 of 3.2 minutes would achieve an acceptable product PNSU. Since the design value for the resistance ($D_T = 0.4$ minute) is only slightly higher than the heat resistance of microorganisms found in the product, ongoing monitoring of bioburden population should be conducted to ensure no drift in population magnitude or resistance occurs over time.

Example 2

a) bioburden testing of product

$N_0 < 10^1$ microorganisms per unit of product

$D_{121°C} < 0.25$ minutes

b) values used for process design

$N_0 = 10^2$ microorganisms

$N_F = 10^{-6}$ (PNSU)

$D_{121°C} = 1.0$ minute

c) calculated minimum lethality to achieve a PNSU of 10^{-6}

$F_{121°C} = (\text{Log } N_0 - \text{Log } N_F) \times D_T$

$(\text{Log } 10^2 - \text{Log } 10^{-6}) \times 1.0 \text{ minute} = 8.0 \text{ minutes}$

In this example, a minimum F_0 of 8.0 minutes would achieve an acceptable product PNSU. Since the design value selected for heat resistance is very conservative ($D_{121°C} = 1.0$ minute), the need for ongoing product bioburden heat resistance testing is significantly reduced, but should still be monitored periodically.

When multiple products are processed using the same cycle, a minimum lethality to be delivered for product-specific loads (e.g., an F_0 of 8.0 minutes) may be specified, regardless of the results of bioburden testing for individual product. In this case, there is typically a safety margin built into the minimum F_0 requirements. Ongoing bioburden monitoring is then conducted to assure that the specified cycle continues to be appropriate. The qualification activities outlined in **Section 5** describe how to assure that these lethality requirements are consistently delivered to the load.

4.2 Load Types

The next step in sterilization process development is to determine the exact physical nature of each discrete container, package or other item that collectively constitute the load to be sterilized. By first understanding the physical characteristics of each item in the load, such as permeability to steam, the load can be further characterized (or "typed") as porous/hard goods or liquid. Based on this determination, it is possible to select an appropriate sterilization process (**Figure 4.0–1**).

4.2.1 Defining Porous/Hard Goods Loads

Porous/hard goods loads contain items where sterilization is achieved through direct contact with saturated steam. Heat is transferred when steam condenses directly on the surface of the items being sterilized. (By contrast, energy from moist heat is transferred to the contents of liquid-filled containers through conduction and/or convection.)

Porous/hard goods loads, as used in the pharmaceutical industry, encompass true porous items (such as cartridge filters and packed fabric) and hard items (such as stainless-steel vessels and filling machine parts). It is not common practice to develop specific sterilization cycles for each item type. It is common to have standard cycles that are capable of providing the same minimum level of sterility assurance, irrespective of load content.

Examples of porous/hard goods items include but are not restricted to:
- Filters* (disc membranes, cartridge membranes, and depth filters)
- Stoppers and other polymeric closure materials
- Tubing and hoses
- Garments
- Cleaning equipment
- Machine change parts

* Filters should be sterilized according to manufacturers' recommendations.

4.2.2 Defining Liquid Loads

Sealed liquid-filled containers in a production setting are often homogeneous, comprised of containers of a single size, single fill-volume, and are from a single lot. Liquid load cycles are usually developed and validated using the product-specific design approach, although the overkill design approach may also be used. If the product is not an aqueous solution (e.g., some oil-based products), special consideration should be made in order to ensure it is suitable for moist heat sterilization.

Examples of liquid-filled containers include, but are not restricted to:
- Formulations (solutions, suspensions and/or emulsions) in their final product container (e.g., vial, bag, bottle, syringe or ampoules)
- Post-test or post-process waste fluids containing potentially pathogenic microorganisms

4.3 Sterilization Processes

For moist heat sterilization, two major types of processes are typically used: saturated steam sterilization processes and air overpressure sterilization processes. Saturated steam processes are

typically used for sterilization of porous/hard goods loads, while air overpressure processes are typically used for liquid product loads. The following is an overview of these process types.

4.3.1 Saturated Steam Processes

There are two main types of saturated steam sterilization processes; prevacuum and gravity displacement. The principles of saturated steam are discussed in **Section 3.3.3**.

4.3.1.1 Prevacuum Process

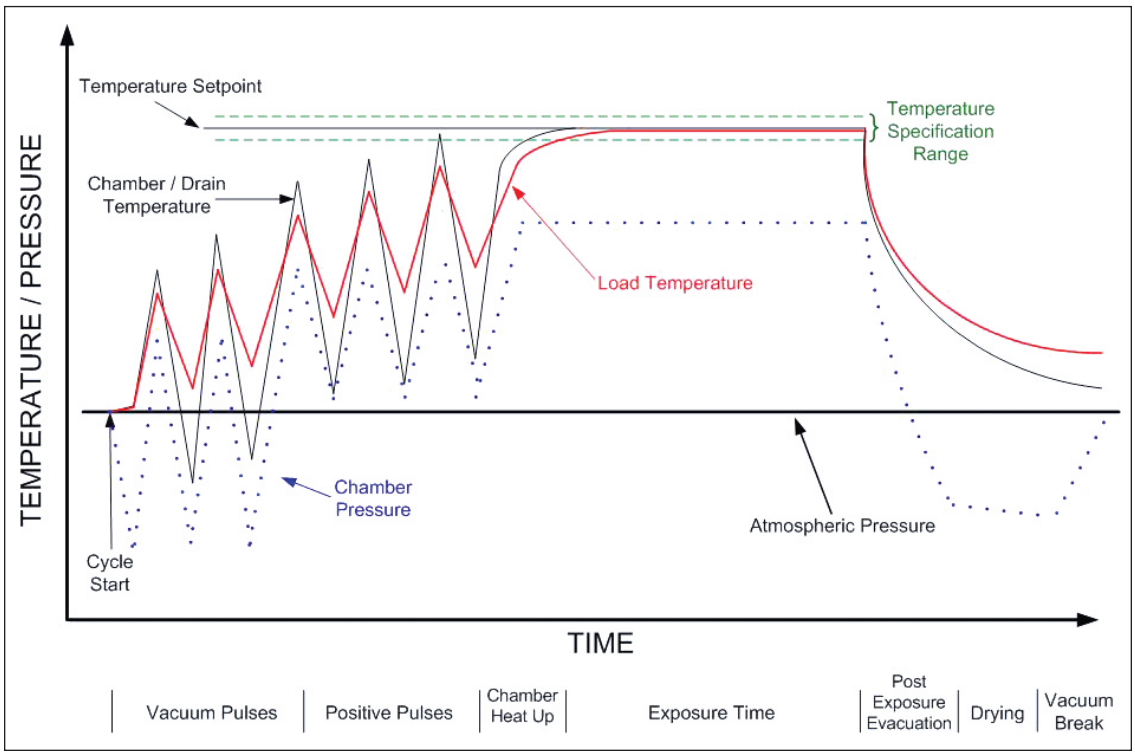
The most commonly used saturated steam process is the prevacuum process. This method removes air from the chamber using a mechanical vacuum pump or steam eductor before the sterilization phase of the process begins. The prevacuum process is particularly suited for load items that can trap air, such as tubing, filters and filling machine assemblies. Vacuum pulsing processes are used frequently in the pharmaceutical industry to sterilize porous/hard goods loads that contain items from which air removal can be difficult.

Treatment of the load prior to the start of the sterilization exposure phase is important. If each vacuum pull is to 0.1 atmosphere, then each pulse will reduce the air in the sterilizer by 90%, or one log. Three vacuum pulses (three vacuum pulls with corresponding steam charges) create a 3-log reduction, effectively removing 99.9% of the air (**Figure 4.3.1.1–1**). Additional positive pulses (steam charges with corresponding evacuations above atmospheric pressure to avoid air leaking into the chamber) may be added to condition the load. Through this approach, air removal efficiency can be increased, leading to shorter equilibration times (**Section 4.4.1.4**). The precise number and type of pulses should be determined during the development of the cycle.

4.3.1.2 Gravity Displacement Process

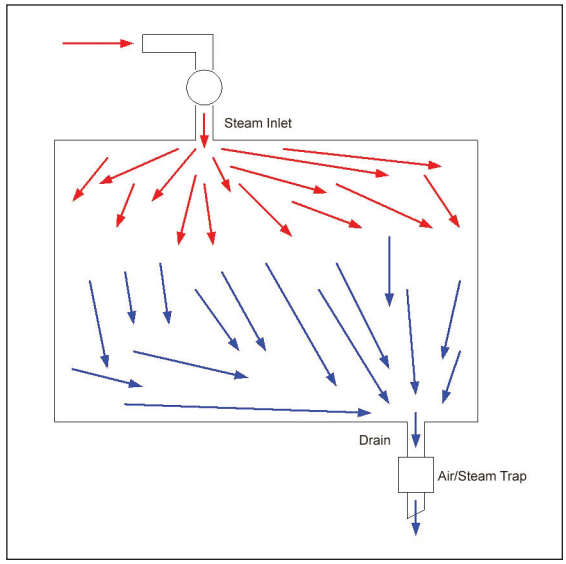
The typical gravity displacement process is based on the principle that cold air within the chamber is heavier than the steam entering and will sink to the bottom of the chamber. As the steam enters

Figure 4.3.1.1–1 ■ Prevacuum Process Cycle Example



the chamber, air is pushed out of the drain at the bottom of the chamber and exits (with the condensate) through the steam trap. The success of the process in removing air depends on the correct operation of the trap and the proper distribution of steam. Steam is injected into the sterilizer chamber through a baffle or spreader bar (such as a perforated pipe). If steam is added too rapidly or not distributed properly, air pockets may be trapped near the top of the load. If steam is added too slowly, the air can be heated, diffuse into the steam, and become more difficult to remove.

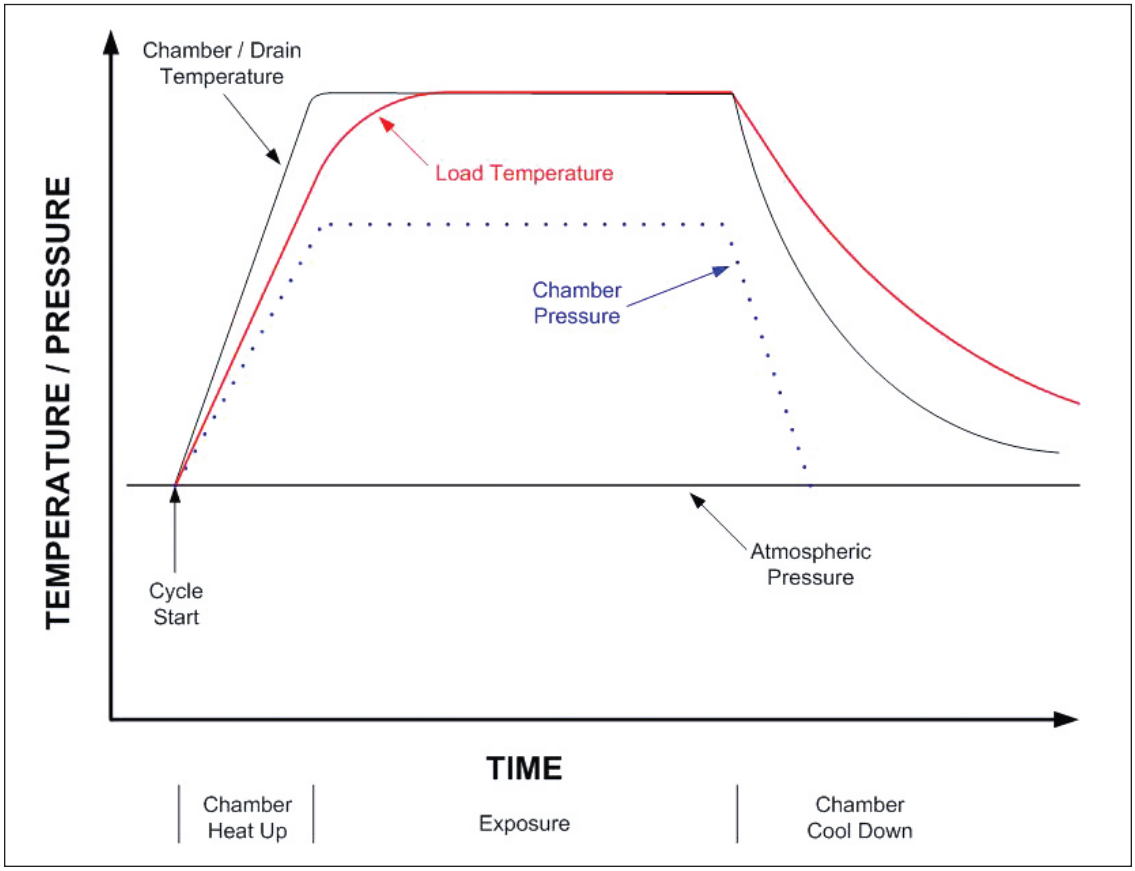
Figure 4.3.1.2–1 ■ Gravity Displacement Sterilizer



Gravity displacement sterilizers are less efficient in air removal than other designs and are not recommended for items with air removal challenges. **Figure 4.3.1.2 –1** depicts a gravity displacement sterilizer. The red arrows represent steam entry through the steam inlet, displacing the air downward through the air/steam trap (drain), as shown by the blue arrows.

An example of a typical gravity displacement process is depicted in **Figure 4.3.1.2–2**.

Figure 4.3.1.2–2 ■ Gravity Displacement Process Cycle Example



4.3.2 Air Overpressure Processes

In nearly all liquid containers, there is gas (air, nitrogen, or other inert gas) in the headspace above the liquid. (35, 36, 37) As the liquid is heated, the headspace gas expands, and pressure within the container increases.

For most liquid load applications, such as prefilled syringes, some glass bottles or vials, plastic bags and semirigid containers, the chamber pressure needs to be increased to minimize the differential pressure (between the internal container pressure and the chamber), maintaining the container shape and closure integrity, and in the case of syringes, stopper position. The air overpressure needed to compensate for internal container pressure may vary significantly, depending on the item type (i.e., glass bottles versus plastic bags).

It is common to use oil free compressed air for air overpressure processes. The quality of the air is dependent on the application. In some cases, a microbial-retentive air filter may be necessary in the air supply line.

The following sections summarize the two common air overpressure process types.

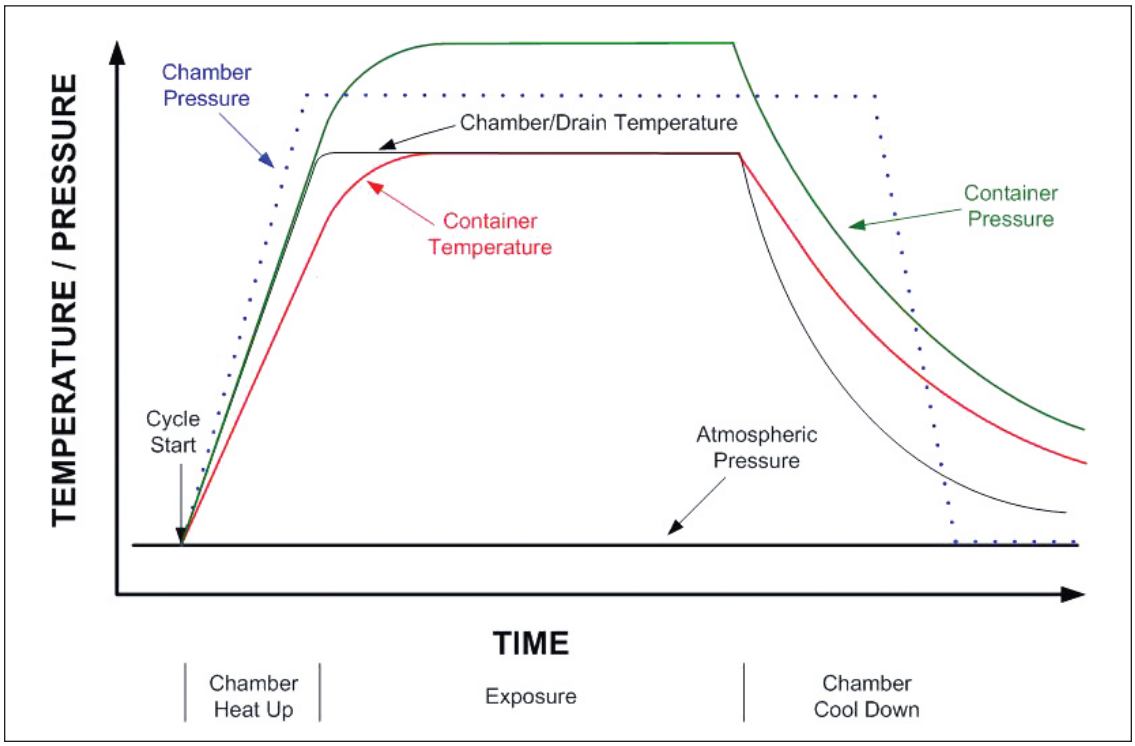
4.3.2.1 Steam-Air Mixture (SAM) Process

When air is added to steam to create a total pressure above the saturation pressure of the steam (at the specified temperature), it is called a steam-air mixture process. The presence of air, although necessary, reduces the heat transfer rate when compared to saturated steam. The steam-air mixture process must continuously circulate the steam and air to:

- Prevent stratification and formation of cold spots within the load
- Reduce depletion of steam in the steam-air mixture next to the cold container

Fans are typically used to circulate the steam-air mixture. **Figure 4.3.2.1–1** illustrates an example of this process cycle. Internal container pressure and temperature dynamics are a function of the container type (e.g., rigid or nonrigid), fill volume, head space volume, and chamber temperature.

Figure 4.3.2.1–1 ■ Steam-Air Mixture Process Cycle Example



The steam-air mixture process can use various methods to cool the product after the exposure phase. The most common method is to cool the recirculating air by adding chilled water to the sterilizer jacket or to coils within the sterilizer. Some steam-air sterilizers reduce the product temperature by spraying cooling water over the product.

4.3.2.2 Superheated Water Process

Sterilization of liquid-filled containers with recirculating superheated water is an efficient way to sterilize some products. There are many types of recirculating superheated water processes, the most common of which uses a pump to continuously recirculate water from the bottom of the sterilizer (below the load zone) to spray nozzles above the load zone. A slight modification to this process is the use of a water distribution pan to create a water cascade in lieu of spray nozzles.

The recirculating water can be heated and cooled, either directly by the injection of steam and cooling water or indirectly via heat exchanger(s). When using the indirect heating and cooling method (via a sanitary heat exchanger), almost any type of steam or water can be used on the non-sanitary side of the heat exchanger. Use of an indirect cooling method is preferred, because the cooling water that directly contacts the sealed containers has been sterilized along with the product.

Another type of recirculating superheated water process involves the complete submersion of the product in water. Most superheated water processes are conducted in batch sterilizers, but continuous sterilizers can also be used.

All of these recirculating superheated water processes use air overpressure that can be controlled during the sterilization process. The minimum overpressure is determined by the temperature being used, the pressure needed to maintain desired product characteristics, and the required overpressure needed to keep the recirculation pump primed.

One of the primary advantages of the recirculating water process over other steam sterilization methods is that the heat-up and cool-down rates are easier to control and, if set up properly, are not influenced by variations in product load and utility supply. An example of the superheated water process is depicted in **Figure 4.3.2.2–1**.

The primary quality attribute of the water used for a superheated water process is the microbial content of the water. The water may be sterilized in the chamber with the load, sterilized in a separate vessel, maintained at elevated temperatures, or chemically treated to maintain the desired low microbial content. Internal container pressure and temperature dynamics are a function of the container type (e.g., rigid or nonrigid), fill volume, head space volume, and chamber temperature.

The use of recirculating water provides efficient cooling of terminally sterilized products thereby increasing sterilizer throughput. This rapid cooling also might be necessary to help ensure product stability.

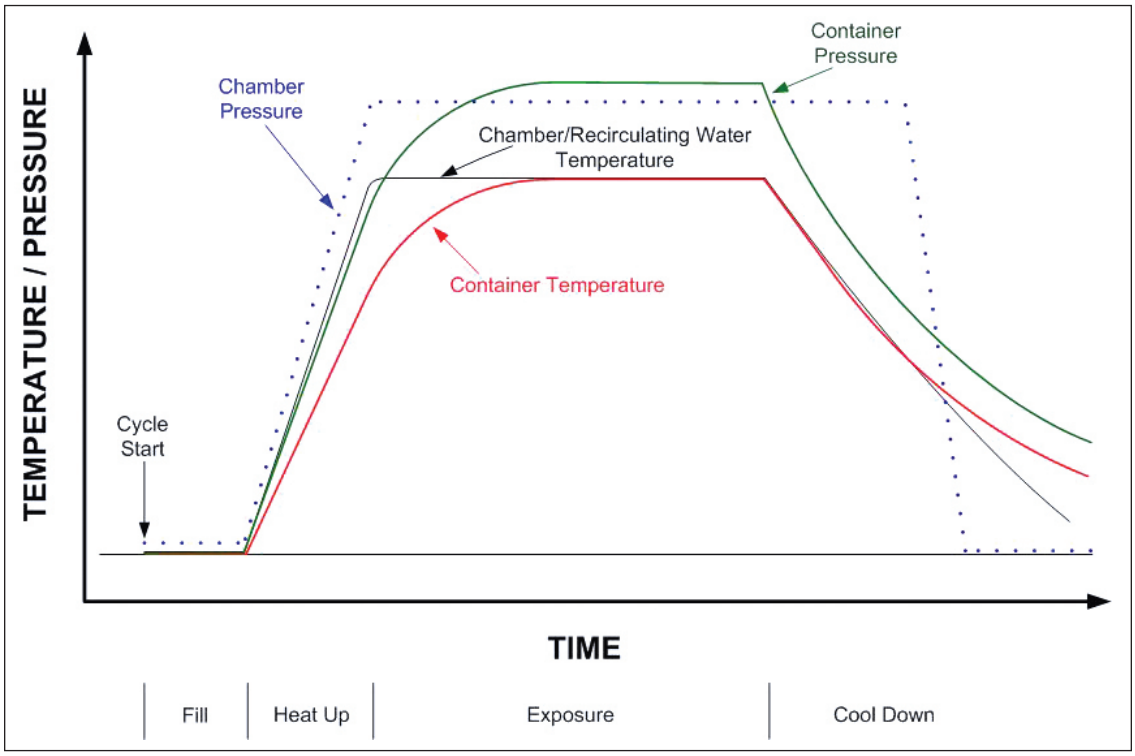
4.4 Cycle Development

Cycle development is the process of determining the physical parameters of the sterilization cycle that will be used to sterilize the items in a defined load pattern. The goal of cycle development is to identify critical and key operating parameters that will result in a product or material that is both sterile and functional after being sterilized. This development process is often documented in a formalized development plan.

4.4.1 Porous/Hard Goods Cycle Development

The greatest obstacle to achieving repeatable and predictable assurances of sterility for porous/hard goods loads is the potential presence of air within the individual items. It is important to ensure that

Figure 4.3.2.2–1 ■ Superheated Water Process Cycle Example



sufficient air is removed from the sterilizer chamber and items prior to the exposure phase of the cycle. A supply of saturated, dry steam to the sterilizer is a specific requirement for porous/hard goods items and may not be essential for sterilization of liquid filled containers.

Biological indicators and air removal test kits may be useful in cycle development. Development studies may vary according to prior knowledge of the sterilization process and of sterilization of similar loads.

It may be beneficial to use already developed sterilization cycles to minimize the total number of different cycles used in a company in order to reduce the ongoing costs of qualification and requalification. In this type of strategy, a company analyzes whether using an existing cycle provides sufficient assurance that the newly identified load will achieve the desired PNSU without compromising quality.

4.4.1.1 Slowest-to-Heat Location on an Item

Before loaded-chamber heat penetration studies are performed, item-mapping studies may be necessary to identify appropriate monitoring location(s) within individual load items. This may be referred to as item-temperature mapping because it is done to determine the location within the item or package that is the most difficult to heat.

Item temperature mapping should be conducted on the more difficult to heat items (e.g., large mass, potential for trapped air, hoses with longest lengths or any combination of these issues). When conducting item temperature mapping, it is important to consider the types of challenges the item may represent (e.g., air removal versus significant mass) and to position the temperature probes in slowest-to-heat locations.

Item mapping studies can be conducted in a sterilizer (including laboratory sterilizers) other than the one to be used in commercial production, provided that the sterilizing conditions and cycles are similar. Multiple temperature sensors may be needed to adequately measure the penetration of steam in large items. During mapping studies, the items should be prepared according to established procedures. Care should be taken when introducing temperature sensors into the item, as they may artificially facilitate or obstruct steam entry into the item.

4.4.1.2 Item Preparation

Porous/hard goods items may be prepared for sterilization in a variety of ways. Examples include, but are not limited to:

- Items contained in steam and air permeable wrappings (e.g., paper or other polymeric wrapping materials, nonshedding fabric or combinations)
- Items in closed, but not sealed, boxes (these may be stainless steel or anodized aluminum that are perforated to allow steam penetration, air removal and drainage of any condensate)
- Items placed on open trays (with or without steam and air-permeable wrapping)
- Items in static or rotating drum containers (e.g., stoppers)

Items for use in aseptic processing must be packaged in order to maintain sterility prior to use. In most instances, this is to allow storage and handling in ISO Class 5 (Grade A) environments. A careful balance needs to be achieved between the steam permeability of wrapping materials to ensure adequate air and condensate removal with the need to provide a non-shedding microbial-protective barrier.

Preparation methods should be well defined in operating procedures. Strict adherence to these procedures is important to assure proper sterilization. Item preparation may include: cleaning, rinsing, drying, wrapping, and storage. Wrapped articles should be covered with only enough wrapping material to protect critical surfaces (e.g., product contact surfaces), while allowing free transfer of saturated steam and air through the material. Use of sterilization tape should be minimal.

Wrapping materials or containers used in sterilization should be constructed of nonshedding material. Aluminum foil, glassine paper and other non-permeable materials should not be used for wrapping items to be sterilized by saturated steam. Changes in wrapping material, orientation in the sterilizer, or use of racks may affect the penetration of steam into, and condensate drainage from, the item.

Metal containers should be of stainless steel or anodized aluminum. Plain aluminum is a source of particulate matter and should not be used. When process equipment includes vents or filters, they should be designed to ensure rapid equilibration of pressure during the sterilization cycle. Prior to sterilization, it is important to confirm that vents are in the open position and that any wrapping materials used to protect items are not likely to block them during air removal.

4.4.1.3 Porous/Hard Goods Load Patterns

After the operational qualification and prior to beginning the performance qualification, load types and patterns need to be determined and documented. The following considerations should be given to sterilization effectiveness and production efficiency:

- Load items should not come into contact with the interior surfaces of the chamber.
- Contact between flat surfaces of metal boxes and trays may be minimized by use of racks with perforated, and if necessary, adjustable shelving.
- Item orientation should be well defined to facilitate air removal, condensate drainage and steam penetration (e.g., buckets should be sterilized upside down), and should be documented.
- Largest mass items should be placed on the lower shelves of the sterilizer to minimize wetting by condensate. (38)

Table 4.4.1.4–1 ■ Typical Operating Parameter Considerations for Porous/Hard Goods Loads

Phase	Parameter*	Considerations
All	Jacket Temperature and/or Pressure	The temperature of the jacket should not exceed or be significantly less than the sterilizing temperature in the chamber. The temperature should be controlled to avoid superheating or excessive condensation. Typically a key parameter.
Heat-Up	Number, Range and Hold Duration (if applicable) of Vacuum/Pulses	Used as needed to remove air from porous items and to achieve appropriate equilibration time. Typically a critical parameter.
	Number, Range and Hold Duration (if applicable) of Positive Pulses	The use of positive pressure pulses can be an effective means of conditioning the load prior to exposure. Typically a key parameter.
	Chamber Heat-Up Time	For saturated steam sterilizers, this operation is a function of the steam supply; alarm limits can be applied to indicate a nonroutine heat-up time.
Exposure	Exposure Time	This is typically a critical parameter that is confirmed during validation and monitored/recorded for every cycle.
	Exposure Phase Temperature Set Point	This is a critical control set point that is confirmed during validation.
	Independent Drain or Chamber Temperature During Exposure	This is typically a critical parameter that is confirmed during validation and monitored/recorded for every cycle.
	Load Probe Temperature(s)	This is typically not a control parameter and is not extensively used in porous/hard goods sterilization.
	Chamber Pressure During Exposure	For saturated steam cycles, this can be used to confirm saturated steam conditions. Depending on control system, potentially a critical parameter.
	Minimum Load Probe Accumulated F_0	This is typically a critical parameter if load probes are used.
Cool-Down	Drying Time	The following may be considered to increase drying efficiency: heating, deep vacuum, pulsing or a combination of these processes. Typically a key parameter for load items with specific drying needs.
	Vacuum Break Rate	May be set to protect integrity of packaging and filters; however, not typically used. Potentially a key parameter.
*The list of parameters is not all-inclusive and may not be applicable or available to all cycles or sterilizers.		

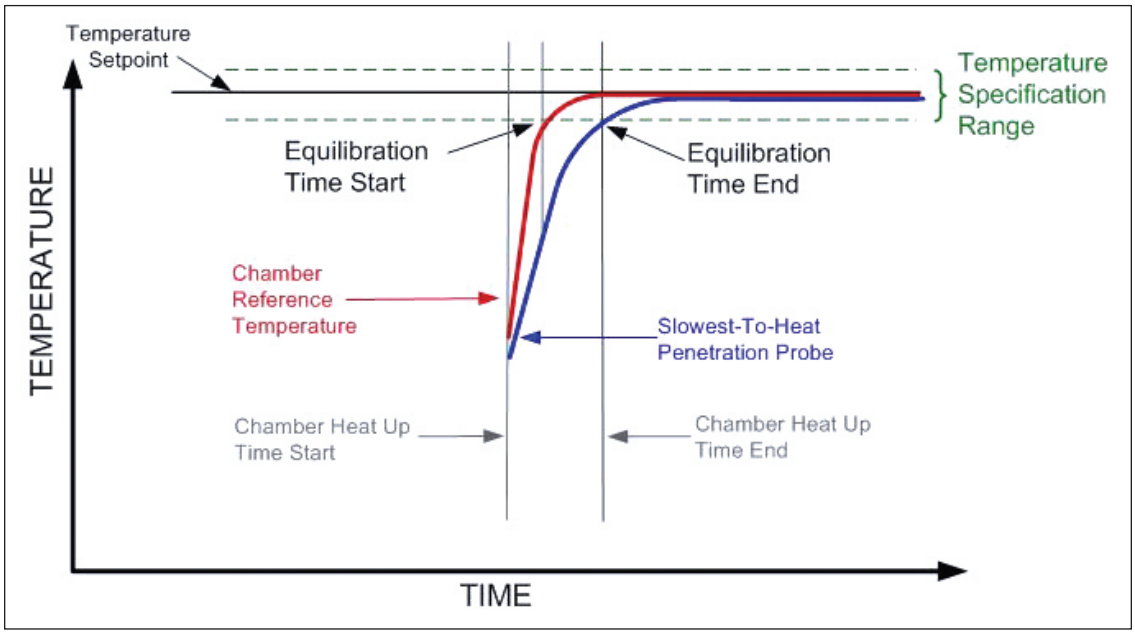
- An important consideration for porous/hard goods loads is control over the number of articles in the sterilizer. In the event the load size is expected to vary, minimum and maximum loads should be identified. A sound bracketing approach to qualifying intermediate loads should include the most-difficult-to-sterilize load item(s) in the minimum load.
- Variable loading patterns may be possible if qualification studies demonstrate item position does not affect sterilization efficacy.
- Loading instructions should be documented and readily available for operator reference.

4.4.1.4 Porous/Hard Goods Operating Parameter Determination

One of the more crucial aspects of cycle development is to identify operating parameters in order to meet the process design objectives and to determine if they are critical or key parameters. **Table 4.4.1.4–1** lists various considerations for process parameters.

Critical parameters are linked to safety and efficacy of the product. Failure to meet a critical parameter should result in rejection of the load. Key parameters assure the ongoing "state of control" of sterilization runs. The failure to meet a key process parameter should result in an investigation with a documented rationale for the disposition of the load.

Figure 4.4.1.5–1 ■ Equilibration Time



4.4.1.5 Equilibration Time

Equilibration time is an important function of conditioning porous/hard goods loads that includes the number and depth of prevacuum and positive pulses. **Figure 4.4.1.5–1** graphically depicts equilibration and chamber heat-up time.

The equilibration time is the period that elapses between attainment of the minimum specified sterilizing temperature in the chamber (chamber reference temperature – typically in the drain) and attainment of the minimum specified sterilization temperature in the load, as measured by the slowest-to-heat penetration probe. This period is an indication of the ability to properly condition the load through air removal and load heating.

Extended equilibration times can be indicative of inadequate air removal or heating, even if the desired temperature is eventually achieved. When developing a cycle, it is important to take practical precautions to minimize equilibration time. The following options can be used to reduce equilibration time:

- Assure loads are oriented for efficient air removal (e.g., hoses not pinched)
- Increase number of vacuum or positive steam pulses
- Add hold steps during vacuum and/or steam pulses
- Increase depth of vacuum pulses
- Optimize steam exposure to load items

If none of the above actions are successful, then consideration may be given to modifying the configuration of items (e.g., shortening hose length). However, this should only be done after giving full consideration to all other risks that may compromise sterility (e.g., modifications that increase the complexity and number of aseptic assembly steps or interventions).

4.4.1.6 Evaluating $F_{Physical}$ and $F_{Biological}$ Agreement

For a sterilization cycle, when the F_{BIO} of a BI is measurable, the F_{BIO} and F_{PHY}, measured at the same location, should be equal. The BI inactivation requirements of the qualification sterilization

cycle are for BIs to be negative; this requires a large F_{PHY}. At this F_{PHY}-delivered condition, it will not be possible to measure an F_{BIO} since this BI condition is outside the measurable quantal area. The heat input necessary to achieve kill of BIs can be calculated. If the actual lethality (F_{PHY}-delivered) is substantially less than the required (F_{PHY}-required), the test cycle will result in BIs that are positive for growth.

The example below is a simple illustration of how the semilogarithmic model can be used to evaluate the agreement between F_{PHY} and F_{BIO}.

Example 1

If a biological indicator has the following characteristics:

$$\begin{aligned} D_T &= 2.5 \text{ minutes} \\ &\quad (\text{maximum BI resistance expected}) \\ N_0 &= 3 \times 10^6 \\ F_{BIO} &= D_T \times LR \\ &= 2.5 \text{ minutes} \times \text{Log } N_0 \\ &= 2.5 \text{ minutes} \times 6.47 \\ &= 16.2 \text{ minutes} \end{aligned}$$

Equation 1 can be rearranged to determine the lethality (F_T) requirement to kill the BI to a probability of nonsterility (PNSU) of 10^{-2} ($N_F = 10^{-2}$).*

$$\begin{aligned} F_T &= (\text{Log } N_0 - \text{Log } N_F) \times D_T \\ &= [\text{Log}(3 \times 10^6) - \text{Log}(1 \times 10^{-2})] \times 2.5 \text{ minutes} \\ &= 21.2 \text{ minutes} \end{aligned}$$

A test cycle that delivers an approximate F_{PHY} of 21.2 minutes should kill the BIs to a PNSU of 1.0 in 100. If the actual biological lethality delivered is less than this predicted lethality based on physical measurements, then BIs placed in the load should be positive for growth at a rate higher than 1.0 in 100. This would indicate potential problems with the delivery of the steam, and efforts should be considered to understand and correct the cause of this divergence.

Note that if this evaluation is conducted during cycle development, the F_{BIO} of the BI does not necessarily have to be derived from the cycle lethality requirements determined during design (**Section 4.1**). If the evaluation of F_{BIO} versus F_{PHY} is made during qualification studies (**Section 5.2**), then the study design becomes more complex because two objectives are being evaluated simultaneously: 1) F_{BIO} equals or exceeds the design requirements, and 2) there is general agreement between F_{PHY} and F_{BIO}.

While there is no standard approach to designing studies to evaluate the agreement of F_{BIO} and F_{PHY}, several approaches have been detailed in literature. (*39*, 40) It is important to note that while this evaluation provides a higher degree of process understanding, many successful cycles have been developed and qualified without this evaluation. One of the goals of this technical report is to promote this cycle development objective and to stimulate additional exploration into appropriate methods for its evaluation.

**For the purposes of this evaluation, it is important to select an N_F for the BI that is sensitive enough to detect potential problems with the delivery of the lethal agent, while also resulting in a high probability of all BIs negative when the cycle performs as expected. An N_F of 10^{-2} to 10^{-3} seems an optimum range for balancing these two criteria.*

4.4.2 Liquid Load Cycle Development

The sterilization of sealed container contents is achieved through transfer of energy from the heating medium to the aqueous liquid within. The water content of the liquid product provides the moisture needed for sterilization within the container. For sterilization of aqueous suspensions and emulsions, the load may have to be kept in motion (i.e., tumbled) to facilitate internal heat circulation. The steam in the chamber may be combined with (or in immersion sterilizers even completely replaced with) superheated water and compressed air. These cycles are routinely accomplished without air removal from the chamber but generally require forced circulation of the heating/cooling medium to aid heat transfer during heating and/or cooling of the load.

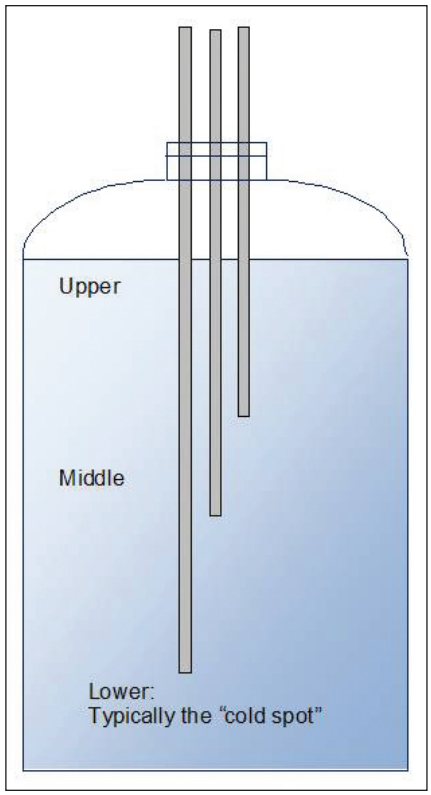
The major concern in the terminal sterilization of product formulations is the development of a sterilization cycle that ensures that sufficient lethality has been provided to the cold spot of the coldest container within the load, while at the same time making certain that the hottest container in the load retains its product quality attributes. Due to these factors, care must be taken to ensure that:

- The load is in the same position during validation and routine processing
- The heat input to the load is uniform to avoid inadvertent over- or under-processing
- The bioburden of the filled containers meets established limits
- The air overpressure (where utilized) is sufficient to minimize the breakage or distortion of containers

The following factors should be considered when developing sterilization cycles for liquid-filled container loads:

- Efficient heating of the exterior surfaces of the fluid containers by steam, steam-air or superheated water, as needed, to attain uniform sterilizing conditions across the entire load
- Allowance for efficient cooling of the load postexposure to protect product quality attributes
- Product stability
- Container/closure integrity and minimization of container breakage or container deformation through appropriate pressure balance
- Sterilization of fluid path (product contact) closure interface
- Temperature mapping within the container
- Biological indicator resistance in product formulation
- Sterilizer racks/stacks/trays should be well designed for the type of heating medium (saturated steam, steam-air mixture or superheated water) and the type of fluid containers (e.g., glass, flexible bags and plastic bottles) to be sterilized

Figure 4.4.2.1–1 ■ Example of Probes in a Liquid Container



4.4.2.1 Container Cold-Spot Mapping

The container cold spot is the location in the sealed liquid container that achieves the lowest process lethality (F_0) during the process. Use of a cold spot is a conservative approach for cycle development, because it assumes all of the microorganisms in a container exist at the cold spot and are only exposed to the temperatures achieved at that location.

For large-volume parenteral loads (LVPs) (>100mL), the cold spot is typically located between the geometric center of the product and the bottom of the product along the vertical axis (**Figure 4.4.2.1–1**) and should be confirmed. Cold-spot

mapping is typically not determined in small-volume parenteral (SVPs) (≤100 mL), since the solution heats at almost the same rate as the sterilizer.

Container orientation can also affect the cold-spot location. When the container is rotated or tumbled during the process, there may be no discernable cold spot.

4.4.2.2 Liquid Load Patterns

The following factors should be taken into account when assembling sealed, liquid-filled container loads for steam sterilization:

- Efficient penetration of the load by steam, steam-air or superheated water as needed to attain uniform sterilizing conditions across the entire load
- Allowance for efficient cooling of the load poststerilization to protect product quality attributes and/or growth promotion properties
- Minimization of container breakage/deformation through appropriate pressure balance
- If the load size is expected to vary, minimum and maximum loads should be identified

Cool and hot zones for the load should be established by heat penetration mapping for each of the container and load sizes to be processed in the sterilizer. This is accomplished through multiple runs of various load and container sizes using sealed, liquid-filled containers. During these trials, load density, rack position, tray height and other parameters should be clearly defined for the loading patterns to be tested during the challenge studies.

At the conclusion of these trials, load patterns and qualification monitoring locations should be established and documented. Successful sterilization of load patterns consisting of different sized containers (or different liquid volumes in the same sized container) is possible but rarely undertaken in terminal sterilization.

4.4.2.3 Liquid Load Operating Parameter Determination

An important aspect of cycle development is to identify operating parameters in order to meet process design objectives and to determine if they are critical or key parameters. Critical parameters are linked to safety and efficacy of product. Failure to meet a critical parameter should result in rejection of the load. Key parameters assure the on-going "state of control" of sterilization runs. The failure to meet a key process parameter should result in an investigation with documented rationale for the disposition of the load. **Table 4.4.2.3–1** lists various parameter considerations.

4.5 Stability Studies

Stability studies for terminally sterilized products are required regardless of the design approach used. Product characteristics studied to determine the impact of the terminal sterilization cycle on product stability may include: product degradation, assay values, pH, color, buffering capacity and product-specific quality attributes.

Sterilization and degradation reactions are cumulative over both time and temperature. This means that heat-up and cool-down variations will affect stability as well as lethality. Therefore, stability studies should expose product to worst-case heat input conditions.

When cycles are controlled to obtain the same total accumulated product F_0, higher temperature cycles and shorter times are believed to have a less adverse affect on products, but the actual impact of the selected cycle should be evaluated.

Table 4.4.2.3–1 ■ Typical Operating Parameter Considerations for Liquid Loads

Phase	Parameter*	Considerations
All	Jacket Temp and/or Pressure	Jackets are not typically used for superheated water cycles. If used, the temperature of the jacket should not exceed the sterilizing temperature in the chamber.
	Fan rotations per minutes (RPM) for the SAM Process	At a minimum, a fan failure should activate an alarm. The fan operation should be considered a key parameter.
	Agitation/Rotation Rate (e.g., RPM)	If required, an agitation/rotation failure should activate an alarm at a minimum. The agitation/rotation operation should be considered a key parameter.
	Superheated Water Recirculation Flow Rate	At a minimum, a pump failure should activate an alarm. The pump operation should be considered a key parameter.
Heat-Up	Chamber Water Level (for the Superheated Water Process)	Minimum levels should be defined and typically alarmed. Potentially a key parameter.
	Chamber Heat-Up Time	For saturated steam sterilizers, this is a function of the steam supply. Alarm limits can be applied to indicate a non-routine heat up time. Potentially a key parameter for SAM and superheated water processes.
	Chamber Heat-Up Rate (e.g., °C/minute)	A controller function typically defined for SAM and superheated water processes to obtain a repeatable heat-up time and temperature profile under any loading condition. Rate determination should consider worst case BTU requirements for the load and available utilities. Potentially a key parameter.
	Pressure Increase Rate	Specific rates are needed for some products using SAM or superheated water processes to maintain specific container attributes (e.g. shape, stopper position in syringes). Potentially a key parameter for container integrity.
Exposure	Temperature Set Point	This is a critical control set point confirmed during validation.
	Exposure Time	This is a critical parameter if not using load probes. This variable is confirmed during validation and monitored/recorded for every cycle.
	Chamber Pressure During Exposure	Can be used to confirm saturated steam conditions and should be considered a key parameter. For air overpressure cycles this would be a user-defined parameter. Depending on control system used, potentially a critical parameter for saturated steam processes.
	Independent Heating Media Temperature During Exposure	This is a critical parameter if not using load probes. This temperature is monitored and recorded for every cycle.
	Load Probe Time Above a Specified Minimum Temperature	May be applicable for products that have specific time/temperature requirement in lieu of specific F_0 requirements. Potentially a key or critical parameter.
	Minimum Load Probe Accumulated F_0	This is typically a control parameter when load probes are used.
Cool Down	Minimum Load Probe Accumulated F_0	Typically a critical parameter if load probes are used.
	Maximum Load Probe Accumulated F_0	Potentially a key parameter if load probes are used.
	Temperature Decrease Rate (e.g., °C/minute)	A controller function typically defined during development of SAM and superheated water cycles.
	Pressure Decrease Rate	Specific rates are needed for some products using SAM or superheated water processes to maintain specific container attributes (e.g., shape, stopper position in syringes). Potentially a key parameter for container integrity.
	Load Cool-Down Time	As appropriate, time to obtain desired product temperature for post sterilization processing/handling (e.g., labeling, case pack). Not typically a key or critical parameter.
*The list of parameters is not all inclusive and may not be applicable or available to all cycles or sterilizers.		

5.0 PROCESS PERFORMANCE QUALIFICATION

Process performance qualification is conducted to demonstrate that the sterilization process consistently meets the design criteria determined for the cycle (**Section 4.1**). As noted in **Figure 1.1–1**, performance qualification follows parameter development as an integral part of the life cycle approach to sterilization process validation and consists of the following two elements:

- Physical qualification, which includes temperature distribution and heat penetration runs, confirms that the desired F_{PHY} is consistently delivered throughout the load.
- Biological qualification with appropriate microbiological challenges confirms that the required F_{BIO} is consistently achieved throughout the load by the developed cycle.

Prior to performance qualification, the following qualification and development activities should be completed and documented in accordance with company sterilization policy and current regulatory expectations:

- Qualification of utilities [e.g., steam with appropriate quality testing (**Section 3.3.3**), compressed air, or coolants] as needed for proper functioning of the sterilizer in relation to the type of loads to be sterilized
- Qualification of the sterilizer [Design Qualification (DQ), Installation Qualification (IQ), Operational Qualification (OQ) or Commissioning], and calibration of critical instrumentation (control systems, monitoring devices and alarms)
- Development of parameters for each phase of the cycle (**Section 4.4**)
- Definition of loads, loading patterns and determination of which loads will be used in the qualification studies. Minimum and maximum loads and difficult-to-sterilize items should be considered in this determination
- Temperature mapping of the chamber and load items, as applicable, to identify appropriate locations for physical and biological evaluation

Consistency between physical and microbiological results is central to sterilization validation. Physical data taken from temperature and pressure measurements cannot alone provide confirmation that specified conditions required for lethality have been achieved in items where steam penetration or heat penetration may be difficult. For instance, the proper sterilization of items (e.g., syringe lumens, needle shields, or filter membranes) cannot be assured entirely from physical measurements. Likewise, the destruction of a biological indicator without consideration of the physical parameters needed to kill the BI is not sufficient evidence of the suitability of a cycle. Biological challenge results should be in general agreement with the physical data, and vice versa.

The minimum acceptable cycle (MAC), in terms of delivered lethality, should be both physically and biologically qualified. In order to assure consistent delivery of the minimum acceptable cycles, routine operational cycles generally include a safety margin through the use of higher temperatures and/or exposure times.

For initial qualification, replicate runs should be conducted to confirm repeatability of the sterilization process. Typical qualifications include three consecutive acceptable runs for each load configuration evaluated. Subsequent to the initial, successful qualification of a process, ongoing event-based requalification (**Section 6.3**) and time-based requalification (**Section 6.4**) should be conducted.

5.1 Physical Qualification

The primary objective of the physical component of cycle qualification is to obtain physical data confirming that the developed cycle consistently achieves the heat penetration requirements established during cycle design.

5.1.1 Temperature Distribution

The primary purpose of temperature distribution qualification is to verify uniform distribution of the heating medium across the load zone. For measurements of temperature distribution, probe sensors should be placed in the load zone but should not be in contact with the load items or sterilizer hardware (e.g., carts, shelves, trays). Diagrams detailing specific temperature sensor locations for each load should be provided.

During performance of the temperature distribution qualification runs, critical and key operating parameters should be confirmed and documented. Acceptance criteria throughout the exposure phase of the cycle may include a maximum:

- Variation in the temperature measured by each probe
- Variation in the temperature measured from probe to probe
- Difference in temperature between the probes and the set temperature

5.1.2 Heat Penetration

Heat penetration qualification demonstrates that the desired amount of energy has been transferred to the materials (e.g., liquids) or surfaces of the items within the load. Heat penetration data is used to calculate F_{PHY}.

Heat penetration temperature probes should be positioned in the cold spot within containers of liquid or in the slowest-to-heat locations of porous/hard goods items determined to be most difficult to sterilize.

Additionally, for liquid loads, probes are typically placed in containers throughout the load in a randomly generated or geometric pattern, as well as in any cold or hot zones within the chamber that may have been identified.

For porous/hard goods loads consisting of items that may have different heat penetration characteristics (frequently referred to as a "mixed loads"), probes should be placed in representative items of each item type. For porous/hard goods loads consisting of only one item type (e.g., stoppers), the same approach used for liquid loads should be adopted. Temperature sensor locations for each qualification load and the rationale for selecting each location should be documented.

5.2 Biological Qualification

The objective of the biological component of cycle qualification is to obtain biological data confirming that the developed cycle achieves the actual biological lethality requirements established during cycle design.

Biological qualification using microbiological challenges follows a straightforward sequence:

- An appropriate microbial challenge system is devised based on the desired lethality (*F*-value) determined during the design of the process, as discussed in **Section 3.2.1**.
- The load is exposed to minimal or subminimal sterilization conditions.
- After completion of the cycle, the microbiological challenges are retrieved.
- Each microbiological challenge is individually incubated in appropriate media and conditions for growth of survivors.
- The results are evaluated to ensure that the spore log reductions achieved for the microbiological challenges meet predetermined acceptance criteria.
- Growth of the microbiological challenge organism is required in positive controls.

5.2.1 Biological Indicator Challenge Systems

In order to assess whether the cycle delivers sufficient lethality to meet the design requirements determined during development (**Section 4.1**) an appropriate microbiological challenge should

be selected to give meaningful results. In order to do this, the microbiological challenge should be presented in an appropriate fashion (the biological indicator challenge system) that may differ for the various types of load, and should have a resistance and challenge size appropriate for its purpose. This biological qualification data is used to calculate the F_{BIO} for the cycle.

5.2.1.1 Determination of Population and Resistance of BI Challenge Systems

The semilogarithmic model can be used to determine the attributes (population, resistance) of the BI challenge system used during biological qualification. The required delivered lethality is determined during process design. Using Equation 1, a BI challenge system can be designed to demonstrate that the required lethality actually has been delivered. Note that this is a separate exercise from using the model to determine the desired delivered lethality for product safety, as discussed in **Section 4.1**.

$$\text{Log } N_F = -F_{(T,z)}/D_T + \text{Log } N_0 \qquad \text{[Equation 1]}$$

Where,

F = the desired lethality determined during process design

D = resistance of the biological challenge

N_0 = starting population of the biological challenge

N_F = the population of the biological challenge after exposure. For calculation purposes, if the biological challenge is killed, then it can be assumed that there is less than one surviving microorganism, which is depicted as $N_F = 10^0$ in this equation.

Equation 1 can be rearranged to determine the minimum starting population of the BI necessary to qualify the delivery of the desired biological lethality (F_{BIO}).

$$\text{Log } N_0 = \text{Log } N_F + F/D$$

The process qualification examples provided in the call-out box on the following page depict the relationship between lethality (F), BI challenge population (N_0), BI challenge surviving population (N_F), and BI challenge D-value (D) in calculations to determine a theoretical BI challenge system that could be used to qualify the process. These calculations assume the sterilization process is delivered under ideal circumstances (i.e., complete air removal, pure saturated steam, and no BI population/resistance variability). See **Section 4.4.1.6** for a discussion of the agreement that should typically be established during development as a precursor to these qualification runs. It is important to note that the lethality (F-value) used in these calculations is the minimum lethality required for the process, and a greater F-value is typically delivered to the product in the minimum acceptable cycle (MAC).

5.2.2 Use and Placement of Biological Indicators

For cycles designed using the overkill design approach, the challenge system is typically spores of *Geobacillus stearothermophilus* either inoculated on the actual items to be sterilized, on paper or other appropriate substrate, or as a commercial biological indicator.

Lower levels of thermal input may be delivered for the product-specific design approach. Consequently, the challenge organism used in qualification is often less resistant than spores of *G. stearothermophilus*. In **Section 3.2.1**, test microorganisms that are widely used in moist heat sterilization qualification are discussed. The use of the semilogarithmic model to determine the characteristics of the BI challenge system also may be used to calculate the appropriate challenge to biologically qualify a cycle, regardless of the resistance of the challenge organism selected.

Process Qualification Examples

Example 1

The desired minimum lethality (F) determined during process design using the product-specific design approach is 6.0 minutes; and for this qualification protocol, the total kill ($N_F = 10^0$) of a BI with a D-value of 1.2 minutes is expected. The minimum population (N_0) for the challenge BI is calculated as follows:

$$\begin{aligned} \text{Log } N_0 &= \text{Log } N_F + F/D \\ &= \text{Log } 10^0 + 6.0/1.2 \\ \text{Log } N_0 &= 5 \\ N_0 &= 1 \times 10^5 \end{aligned}$$

Therefore, in order to assure that the design requirement $F_{BIO} \geq 6.0$ minutes is achieved, the MAC should completely kill a BI with an $N_0 = 105$ and a D-value of 1.2 minutes. Since an F_0 of 6.0 minutes has been established as the minimum lethality for product safety, the MAC will likely exceed this minimum F_{PHY}-value.

Example 2

For the overkill design approach, the desired lethality, F_{PHY} and F_{BIO}, is greater than, or equal to, 12 minutes. If a BI with a resistance of 2.1 minutes is used in the qualification study, then the minimum challenge BI population (N_0) to be completely inactivated is calculated as follows:

$$\begin{aligned} \text{Log } N_0 &= \text{Log } N_F + F/D \\ &= \text{Log } 10^0 + 12/2.1 \\ \text{Log } N_0 &= 5.71 \\ N_0 &= 5.2 \times 10^5 \end{aligned}$$

Therefore, a MAC that inactivates a BI challenge with an $N_0 = 5.2 \times 10^5$ and a D-value of 2.1 minutes has been biologically qualified as an overkill cycle ($F_{BIO} \geq 12$ minutes).

Example 3

A qualification study can be designed where the endpoint (N_F) is not necessarily total kill of the BI. If the design requirement is an F_0 of 8.0 minutes and the BI has a starting population (N_0) of 2.0×10^6 and a D-value of 2.1 minutes, then the maximum endpoint (N_F) can be calculated that still assures that the desired F_{BIO} has been delivered by the process.

$$\begin{aligned} \text{Log } N_F &= \text{Log } N_0 - F/D \\ &= \text{Log } 2.0 \times 10^6 - 8.0/2.1 \\ \text{Log } N_F &= 6.3 - 3.8 = 2.5 \\ N_F &= 3.2 \times 10^2 \end{aligned}$$

Therefore, if the MAC reduces the BI challenge (D-value = 2.1 minutes) from $N_0 = 2.0 \times 10^6$ to an endpoint population (N_F) below 3.2×10^2, then an $F_{BIO} \geq 8.0$ minutes has been demonstrated.

Example 4

The half-cycle qualification method may also be used. The use of this method for an overkill design approach necessitates that a minimum F_{BIO} of 6.0 minutes be delivered to product/items using a cycle with one-half of the MAC. The minimum population to be inactivated for the challenge-BI with a resistance of 1.0 minute is calculated as follows:

$$\begin{aligned} \text{Log } N_0 &= \text{Log } N_F + 0.5(F/D) \\ &= \text{Log } 10^0 + 0.5(12/1.0) \\ \text{Log } N_0 &= 6.0 \\ N_0 &= 1.0 \times 10^6 \end{aligned}$$

Therefore, if a cycle with *half* the exposure time of the MAC is used in this qualification run, a BI with a D-value of 1.0 and a population of 10^6 must be killed to demonstrate an $F_{BIO} \geq 12$ minutes.

5.2.2.1 Liquid Load Cycle Qualification

For biological qualification of liquid loads, sealed, liquid-filled containers and closures are inoculated with appropriate microorganisms. The liquid medium may be the product or an appropriate surrogate. It may be necessary to use a surrogate fluid as the suspending medium if the liquid product contains preservatives or other antimicrobial agents that demonstrate growth inhibition. The decision to use the product as the suspending medium should be supported by studies that verify microbial growth is not inhibited.

The microbiological challenge should be placed in containers throughout the load in a random or geometric pattern, as well as in any cold zones that may have been identified in development. Heat penetration probes should be placed in the container adjacent to the inoculated containers to monitor the heat input of the cycle.

5.2.2.2 Porous/Hard Goods Cycle Qualification

Biological indicators used for biological qualification of porous/hard goods loads are typically obtained from commercial sources and may be mounted on paper, stainless steel, aluminum or other substrates. Alternatively, spores may be inoculated on designated test-piece items. The use of sealed ampoule BIs may not represent the actual conditions of a porous/hard goods load.

BI challenge systems are placed in slowest-to-heat locations in items that are considered most difficult to sterilize. These may be, for instance, within the pleats of cartridge filters or at the center of a length of tubing where there is the potential for air to be trapped, or on rubber stoppers where it may be difficult for steam to penetrate.

To evaluate the correlation between F_{PHY} and F_{BIO}, biological indicators should be placed near thermometric probes. When positioning the probes and indicators, care should be taken to not artificially increase or decrease air removal or steam penetration to a particular area. It may be necessary to place duplicate items in the load – where one item contains a thermal probe and the other a biological indicator – to obtain representative results.

5.3 Process Performance Acceptance Criteria

Acceptance criteria for physical and biological qualification should be clearly defined in test protocols. These criteria should be based on the type of items to be sterilized, the sterilization method being applied, applicable regulatory expectations, and the operating parameters determined in cycle development.

Following are examples of typical physical qualification acceptance criteria:

- Minimum and maximum time above a specified minimum temperature measured with heat penetration probes
- Minimum and maximum total accumulated F_0
- Minimum F_0 at end of the exposure phase
- Minimum and maximum pressure during exposure
- Correlation of temperature and pressure for saturated steam
- Minimum and maximum chamber temperature during exposure
- Maximum temperature or F_0 variation between heat penetration probes
- Maximum temperature variation in temperature distribution probes
- Maximum equilibration time
- Minimum number of properly functioning probes

Following are examples of typical biological qualification acceptance criteria:

- Microbial challenge spore log reduction achieved meets the predetermined acceptance criteria
- Positive and negative controls function as specified

In addition to meeting the above acceptance criteria, the overall qualification should demonstrate general agreement between F_{PHY} and F_{BIO} if it is not demonstrated during development.

5.4 Sterilizer Equivalence

Where two or more sterilizers are of similar design (including utilities supplied to them), it may be possible to establish their operational equivalence and reduce the amount of process qualification testing. A robust-risk management process is recommended to justify making this determination. For example, sterilization processes that have more parameters that are difficult to control (e.g., prevacuum processes) may be more difficult to establish equivalency than those with parameters that are easier to control. Regulatory approval may be required to support a reduction in qualification testing. Initially, all sterilizers must be qualified and meet operating parameter acceptance criteria in order to demonstrate equivalency between sterilizers.

Operational equivalence between sterilizers can be established by using the same sterilization parameters and load configuration (product, formulation, container size, fill volume, stoppers, equipment, or loading pattern) and by comparing their performance to one another. Comparable criteria to be demonstrated should include critical and key parameters, temperature distribution, heat penetration, F_0 range and microbiological inactivation.

5.5 Bracketing

Bracketing of product formulation, container size, fill volume, items or loads may be performed to reduce the amount of qualification work. Bracketing requires identification of the worst-case challenge (single-ended bracket) or the use of configurations that represent the range of items (two-ended bracket) to be qualified. Selection of worst-case challenge(s) is conducted using scientific rationale and/or determined through heat penetration or microbiological inactivation studies.

5.5.1 Product Formulation Bracketing

For biological qualification, a master solution approach may be used to bracket product formulations. The product formulation with the greatest resistance to the challenge microorganism is used as the master solution to represent product formulations in which challenge microorganisms have lower resistances. Typically a resistometer is used to determine resistance. See **Section 3.1.1** for further discussion of resistance determination (*D*-value).

For physical qualification, a master solution approach may also be used to bracket product formulations. The product formulation with the greatest viscosity can be used to represent product formulations with lower viscosities.

5.5.2 Container Size/Fill-Volume Bracketing

Bracketing of similarly designed liquid load containers of different sizes and different fill volumes can be established if using the same sterilization parameters. When using this approach, the largest container with the largest fill and the smallest container with the smallest fill would be qualified to bracket intermediate sizes and fills.

5.5.3 Item Bracketing

Bracketing of porous/hard goods items may be considered. The item that represents the greatest sterilization challenge is used to qualify similar items that represent lesser sterilization challenges. For example, if differing lengths of tubing with the same material, diameter, wall thickness, orientation and packaging are to be qualified, the longest length of tubing is used to qualify the shorter lengths of tubing.

5.5.4 Load Bracketing

An approach for bracketing porous/hard goods loads requires the use of items that represent the greatest heat-up (mass) and/or air-removal challenges in the minimum and maximum load qualification. For the minimum load, the greatest heat-up and/or air-removal item(s) may be the only item(s) present in the chamber during the qualification.

In the event that load size is expected to vary for operational flexibility, minimum and maximum loads should be identified and qualified. At a minimum, three consecutive acceptable biological qualification runs should be achieved for maximum loads in each sterilizer. For sterilization cycles that do not have fixed heat-up and cool-down rates/times, the minimum load typically receives lower thermal heat input than maximum loads and should also be challenged. Where risk has been assessed and deemed to be acceptable, the number of replicate runs may be reduced.

6.0 ONGOING PROCESS CONTROL

After completion of sterilization cycle development and performance qualification, monitoring of the sterilization process is conducted in order to assure state of control. Important elements of the ongoing sterilization program include: review of critical and key operating parameters during routine sterilization cycles, confirmation of sterilizer suitability, deployment of an effective change control program, as well as calibration, maintenance, and requalification of the process.

6.1 Routine Release

Release of sterile loads is accomplished by demonstrating that qualified critical and key operating parameters were met during routine sterilization cycles. Additionally, compendial sterility tests may be required to support product release.

Section 4.4 discusses critical and key operating parameters that are defined during cycle development and qualified during operational qualification and performance qualification in order to establish a baseline of sterilizer performance. The parameters for routine operational cycles should be evaluated to ensure they meet qualified ranges. All critical parameters must be met for load release. Key parameters, which are more indicative of the state of control, should also be evaluated. A scientifically sound rationale must be developed to support the release of a load if a key parameter is not met. Operational parameter review is essential, regardless of whether the materials processed in the sterilizer are being parametrically released for distribution, subject to subsequent sterility testing, or sterilized to support further processing.

6.2 Sterilizer System Suitability

System suitability evaluations should be incorporated into a load-release program; however, the need and frequency of these tests is determined by the end-user, according to internal and possible regulatory requirements. Examples of system suitability evaluations include:

- Air Removal Test: Demonstration that air removal meets applicable requirements for porous loads sterilized with a saturated steam prevacuum process.
- Chamber Leak Test: Demonstration that the chamber leak rate is below a stated maximum rate. This test is recommended for sterilizers using a saturated steam prevacuum process for sterilization of porous loads.
- Air Detector Device: Acceptable air detector results (supported by testing performed during cycle development and/or qualification) can be used to support the release of porous/hard goods loads using a saturated steam prevacuum process. The use of a properly qualified air-detector device may reduce the need to perform chamber leak tests and air removal tests. This device is not available on all sterilizers. This device is typically installed near the drain in the sterilizer chamber.
- Chemical Indicator/Integrator Results: Acceptable results demonstrate that the load was processed and in some cases can measure specific time and/or temperature conditions in the sterilizer.

6.3 Change Control

In order to maintain the state of control of a sterilization system, a change control program should be in place. This program should document sterilization system or product changes and include documentation of any testing required to ensure the qualified state of control.

Changes involving modifications of the sterilizer chamber, product carrier/tray design, load arrangement, sterilization medium supply/distribution systems, or the sterilizer operation/control mode may necessitate temperature distribution, heat penetration, and/or microbiological challenge studies. Replacement of "like-for-like" sterilizer equipment system parts is generally not considered a major change, provided that it is demonstrated that sterilizer performance is not affected.

Changes to product, including design, materials of construction, item or product tolerances, mass, venting, formulation or packaging, may require temperature distribution, heat penetration and/or microbiological requalification.

Requalification of the sterilization process should be performed whenever there is a major modification to the sterilization system (including prior-to-sterilizer decommissioning) or product that has the potential to affect process efficacy.

A change control package should identify qualification documents that are affected by the change and should include:

- A description of the proposed change
- A documented reason/rationale for the proposed change
- A description of the tests needed to qualify the sterilization process after the change is made, or a technical rationale supporting that the change has no impact on the sterilization process efficacy (minor change)
- Supporting documentation for tests performed, interpretation of results and conclusions
- Confirmation that documents affected have been updated
- Approval of the change control package by the quality unit and other management representatives

6.4 Periodic Requalification

Requalification should be performed on a regular basis (typically every 12 months) to ensure there has not been an undetected change in product or process.

Requalification should be performed using the same operational parameters and acceptance criteria as the original qualification runs. Supporting documentation for tests performed under the PQ program should include, as applicable, information outlined in the original qualification effort. Verification of acceptable steam quality for porous/hard goods load sterilization should also be performed at this time. Results of the requalification study should demonstrate that the sterilizer's performance has not changed since the original effort.

The most difficult-to-sterilize load(s) should be included in requalification runs. Where equivalence has been established between sterilizers (**Section 5.4**), a reduction in requalification activity may be considered. For example, in a facility in which multiple product formulations are sterilized using equivalent sterilizers, it may not be necessary to requalify each product in each sterilizer.

Requalification should also include review of performance data from various monitoring sources (e.g., engineering, maintenance and calibration data) of sterilizers and supporting equipment to verify that there have been no adverse trends or drifts away from the baseline performance established during validation. A review of change control documentation should be conducted as part of the requalification. Refer to **Section 6.3** for information regarding change control.

7.0 REFERENCES

1. ISO/TS 11139:2006, Sterilization of Health Care Products – Vocabulary. Definition 2.3
2. ISO/TS 11139:2006, Sterilization of Health Care Products – Vocabulary. Definition 2.6
3. Derived from ISO/TS 11139:2006, Sterilization of Health Care Products – Vocabulary. Definition 2.6
4. USP 30, NF-25, <1231> Water for Pharmaceutical Purposes (2007) pp 687–706
5. ISO 18472:2006, Sterilization of Health Care Products – Biological and Chemical Indicators – Test Equipment, Definition 3.11
6. ISO/TS 11139:2006, Sterilization of Health Care Products – Vocabulary. Definition 2.46
7. ISO/TS 11139:2006, Sterilization of Health Care Products – Vocabulary. Definition 2.51
8. ISO/TS 11139:2006, Sterilization of Health Care Products – Vocabulary. Definition 2.52
9. Rahn, Injury and Death of Bacteria by Chemical Agents, *Biodynamica*, Normandy, Mo., (1945)
10. Pflug, I. J. and Holcomb, R. G., Gomez, M., Chapter 6, Principles of the Thermal Destruction of Microorganisms, *Disinfection, Sterilization, and Preservation*, Fifth Edition, S. S. Block, Editor, Lippincott Williams & Wilkins (2000) pp 79–129
11. USP 30, NF-25 <55> Biological Indicators-Resistance Performance Tests (2007) pp 81–83
12. ISO 11138-1:2006 Sterilization of Health Care Products – Biological Indicators
13. Pflug, I.J., Chapter 7, Analysis of Microbial-Survivor Data, *Microbiology and Engineering of Sterilization Processes*, 12th Edition, Parenteral Drug Association (2007)
14. Stumbo, C.R., Murphy, J.R., Cochran, J., Nature of Thermal Death Time Curves for P.A. 3679 and *Clostridium botulinum*. *Food Res.* (3), (1950) pp 323–346
15. Halvorson, H.O., Ziegler, N.R., Applications of Statistics to Problems in Bacteriology, *Journal Bacteriology* 25, (1933) pp 101–121
16. Pflug, I.J., *Microbiology and Engineering of Sterilization Processes*, 12th Edition, Parenteral Drug Association (2007)
17. Pflug, I.J., Chapter 6, Gathering Experimental Microbial-Kill Data, *Microbiology and Engineering of Sterilization Processes*, 12th Edition, Parenteral Drug Association (2007)
18. Fundamental Process Control Theory, Instrumentation Society of America (1981)
19. Kemper, C. and Harper, G., Probe Induced Errors, *Pharmaceutical Technology* (1978)
20. Reed, R.P., Thermoelectric in Homogeneity – Obscure Obstacle to Quality, National Conference of Standards Laboratories, Albuquerque, N. Mex. (1998)
21. Pflug, I.J., Chapter 10, General Method of Heat-Process Evaluation, *Microbiology and Engineering of Sterilization Processes*, 12th Edition, Parenteral Drug Association (2007)
22. USP 30, NF-25, <1035> Biological Indicators for Sterilization (2007)
23. ISO 18472:2006, Sterilization of Health Care Products – Biological and Chemical Indicators – Test Equipment
24. FDA, Guidance for Industry, Sterile Drug Products Produced by Aseptic Processing – Current Good Manufacturing Practice (2004)
25. Berger, T.J., Nelson, P.A., The Effect of Formulation of Parenteral Solutions on Microbial Growth – Measurement of *D*- and *z*-values, *PDA Journal of Pharmaceutical Science and Technology* 49 (1), (1995) pp 32–41
26. Russell, Allan D., Chapter 3, Principles of Antimicrobial Activity and Resistance, *Disinfection, Sterilization and Preservation*, Fifth Edition, S. S. Block, Editor, Lippincott Williams & Wilkins (2000) pp 31–55

27. Derived from: IAPWS-IF97, SI units, Chapter 3, ASME International Steam Tables for Industrial Use, The American Society of Mechanical Engineers, 58 (2000) page 16

28. Derived from: IAPWS-IF97, SI units, Chapter 3, ASME International Steam Tables for Industrial Use, The American Society of Mechanical Engineers, 58 (2000), page 16

29. Derived from: IAPWS-IF97, SI units, Chapter 3, ASME International Steam Tables for Industrial Use, The American Society of Mechanical Engineers, Volume 58 (2000), page 16

30. Pflug, I.J., *Procedures for Carrying Out Heat Penetration Tests and Analysis of the Resulting Data, Department of Food, Science and Nutrition*, University of Minnesota, Minneapolis, Minn. (1975)

31. USP 30, NF-25, <1231> Water for Pharmaceutical Purposes (2007)

32. EN 285, Sterilization – Steam Sterilizers – Large Sterilizers, CEN European Committee for Standardisation (1996)

33. Shuttleworth, K., Application of Steam Quality Test Limits – *European Journal of Parenteral Sciences,* 4 (109) (2000)

34. Decision Tree for the Selection of Sterilisation Methods, Annex to Note for Guidance on Development Pharmaceutics (CPMP/QWP/054/98 Corr), EMEA, April 2000

35. Joyce, M.A. and Lorenz, J.W., Internal Pressure of Sealed Containers During Autoclaving, *PDA Journal of Pharmaceutical Science and Technology* 44 (6) (1990) pp 320–323

36. Allwood, M.C., Hambleton, R. and Beverley, S., Pressure Changes in Bottles During Sterilization by Autoclaving, *PDA Journal of Pharmaceutical Science and Technology* 64 (2), (1975) pp 333–334

37. Halls, N.A., *Achieving Sterility in Medical and Pharmaceutical Products*, New York: Marcel Dekker, Inc. (1994)

38. Agalloco, J., The Validation of Life Cycle, *PDA Journal of Pharmaceutical Science and Technology*, 47 (3) (1993)

39. Evans, K., Pflug, I.J., Carrying Out Biological Qualification, The Control Operation of Moist-Heat (Steam Sterilization) Processes for Producing Sterile Pharmaceuticals and Medical Devices, *PDA Journal of Pharmaceutical Science and Technology,* 54 (2) (2000) pp 117–135

40. Pflug, I.J., Chapter 17B, Microbial Control in Pharmaceuticals and Medical Devices using Moist Heat (Steam Autoclave), *Microbiology and Engineering of Sterilization Processes*, 12th Edition, Parenteral Drug Association (2007)

NOTES

NOTES

NOTES

PDA Journal of Pharmaceutical Science and Technology

EDITOR
Lee Kirsch, Ph.D.
c/o The University of Iowa
Pharmacy Building, S233
115 S. Grand Avenue
Iowa City, IA 52242
Tel: +1 (319)384-4408
Fax: +1 (319)384-4409
pda-journal@uiowa.edu
Editorial Assistant: Salil Desai

PDA Journal of Pharmaceutical Science and Technology (ISSN 1079-7440) is published biomonthly by the PDA, Inc., Bethesda Towers, 4350 East West Hwy., Suite 200, Bethesda, MD 20814.

Subscriptions—PDA membership dues include an annual subscription to the *PDA Journal of Pharmaceutical Science and Technology*. For an application form and information regarding membership, address the Association. Industrial, university, and public libraries, as well as government agencies, may subscribe at the rate of $270 (U.S.) per year. Back issues are available from the Association at the rate of $55 members/$100 nonmembers plus shipping. Copies of individual articles are available at a cost of $20 members/$40 nonmembers, plus shipping (please specify volume number, issue, and title of article: this information may be referenced at *www.pda.org*).

Claims—Issues lost in transit will not be replaced if claim is received more than 90 days from date of issue or if loss was due to failure to give notice of change of address. The Association cannot accept responsibility for delivery outside the United States when shipment has been made by first-class mail.

Periodicals postage paid at Bethesda, Maryland and additional mailing offices. Postmaster: Send address changes to the *PDA Journal of Pharmaceutical Science and Technology,* Bethesda Towers, 4350 East West Hwy., Suite 200, Bethesda, MD 20814.

Circulation
PDA
Bethesda Towers
4350 East West Highway, Suite 200
Bethesda, MD 20814
+1(301) 656-5900

Advertising
Cindy Tabb
+1(301) 656-5900 x222
tabb@pda.org

Printed in the USA.

ISSN 1079-7440
ISBN 0-939459-15-9

Copyediting services provided by
G.I. Lovitt-Wood, G.I. Lovitt & Associates
Walter Morris, PDA
Lindsay Donofrio, PDA
Evelyn Heitman, Consultant

Design and desktop publishing services provided by:
Anita Dahlman, Dahlman Creative LLC

Bethesda Towers
4350 East West Highway
Suite 200
Bethesda, MD 20814 USA
Tel: +1 (301) 656-5900
Fax: +1 (301) 986-0296
E-mail: info@pda.org
Web site: www.pda.org