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⟨1229.2⟩ MOIST HEAT STERILIZATION OF AQUEOUS LIQUIDS

INTRODUCTION

Steam sterilization of aqueous liquids (including both suspensions and emulsions with mixing), also known as sterilization of nonporous loads, is the method of choice for aqueous parenteral products, in-process aqueous liquids, laboratory media, and biological waste materials. This type of sterilization is accomplished primarily in closed containers. During steam sterilization by direct contact (also called steam sterilization of parts, hard goods, or porous items) the steam in the chamber directly contacts the surface of load items to effect sterilization (see *Steam Sterilization by Direct Contact* ⟨1229.1⟩). In contrast, sterilization of liquids in containers is accomplished by application of heat to the container, heating of the container wall, and ultimately heating of the internal liquid volume. This can be accomplished using steam, superheated water, and air in various combinations. Some aqueous liquids are susceptible to over-processing that could render them unfit for their intended use. Manufacturers should consider the influence of these differences when they establish a suitable process.

During the sterilization of liquid-filled containers, differential pressures between the interior of the containers and the sterilization chamber may potentially impact container integrity. Air over-pressure is used to minimize the pressure differential between the container and the sterilizer to protect the integrity of the container, especially prefilled syringes and plastic containers. Before sterilization of product containers, manufacturers should consider the potential adverse consequences of excess heat on the materials. In order to ensure sterility as well as functionality, the process definition and validation method used must incorporate both lower and upper temperature and time limits on the process conditions.

When the overkill method can be used for sterilization of sealed liquid containers, it is the preferred method and is described in ⟨1229.1⟩. When product quality attributes can be impaired by excessive heat, the sterilization process should use less time or a lower temperature to minimize the adverse effect on the materials. Sterilization time–temperature or F_0 conditions (F_0 is defined as the equivalent sterilization time relative to a base temperature of 121°) include both lower (sterility-related) and upper (stability-related) limits.[1] Manufacturers commonly employ the bioburden/biological indicator (BB/BI) or bioburden methods when constraints on the material's ability to withstand the process require the use of less aggressive conditions. This approach requires appropriate controls on presterilization bioburden and/or product-related *D*-values in conjunction with bioindicators of lower spore count or resistance to ensure sterilization.

Terminal Sterilization of Products

The maintenance of product attributes may require the use of sterilizing conditions that are less aggressive and sterilization equipment, cycles, and validation methods adapted to these more restricted circumstances. The substantial variations in equipment designs and methods for terminal sterilization preclude a singular description of a typical cycle. All terminal sterilizers heat the load, but they accomplish this in varying ways: saturated steam; steam–air mixtures, steam–air–water mixtures, and superheated water. Air over-pressure for maintenance of container integrity and cooling containers and water for heating/cooling of the load may be present depending upon the autoclave size, throughput expectations, and container.

In-Process Fluids

In-process fluids are used for pH adjustment, dilution to a specified volume, lubrication, and other purposes. In many instances these liquids are sterilized in conjunction with items that must be sterilized by direct steam contact, and the sterilization process must ensure that all items are adequately sterilized.

Laboratory Media

Laboratory media often are sterilized in standard steam autoclaves with minor adaptations. Provision for slow exhaust (to reduce stress on container integrity and minimize boil over) and jacket cooling can help improve the basic steam sterilizer design and operation to better accommodate the materials. The sterilization process may be specific for media containers or a combination of both liquid-filled containers and hard goods. This process may resemble the methods used for terminally sterilized products (see above). The sterilization of laboratory media may entail the processing of a number of different containers that contain different materials. Manufacturers should be aware of the potential for under- and over-processing across the load and must consider container size, container contents, and position. When liquid-filled containers are combined in the same load with hard goods, manufacturers must consider the unique concerns of each to ensure all items are properly sterilized. Because laboratory media are considered self-indicating with respect to sterility, the use of internal biological indicators during validation is not required.

Biowaste Sterilization

The sterilization of biowaste in sealed containers from laboratory or production use is similar to parts sterilization. The process is defined to ensure a minimum time–temperature exposure or attainment of a specified F_0 value throughout all items of the load. Depending on the

potential contaminants present, the autoclave design may incorporate condensate collection/sterilization or sterilizable exhaust filters to ensure that pathogens are adequately contained. Because the objective of biowaste sterilization is to render the materials safe for contact and disposal, the overkill method described in ⟨1229.1⟩ is employed.

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BIOBURDEN/BIOLOGICAL INDICATOR METHOD

Application of the BB/BI method requires a thorough understanding of the bioburden type, population, and resistance typically present in the presterilized product-filled container. The method relies on substantial differences between moist heat resistance and the population of the bioburden present and the biological indicator used during validation (*Figure 1*).

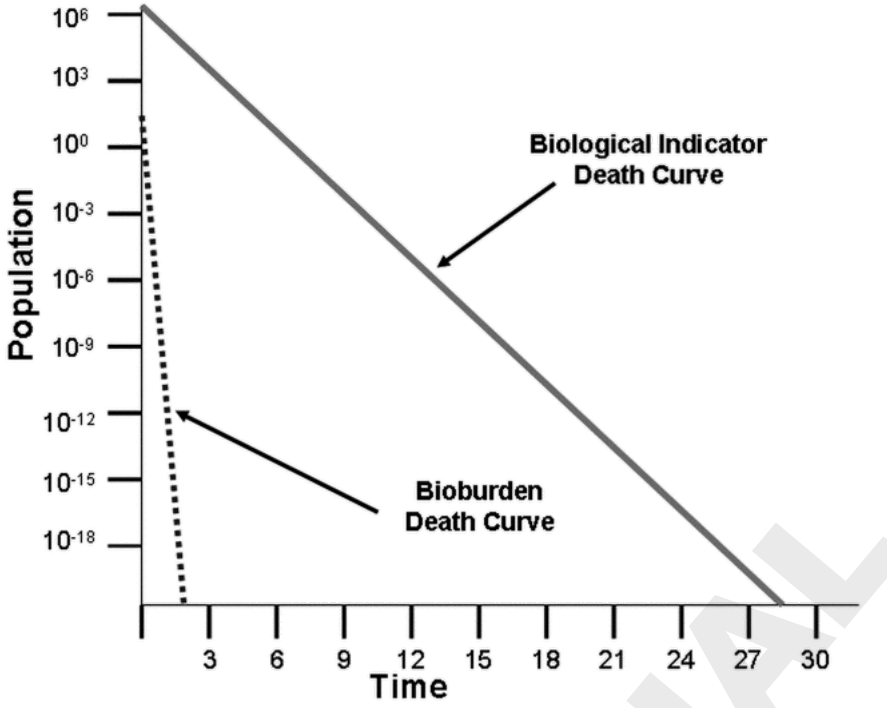

Figure 1. Relative resistance and population of typical bioburden and biological indicator microorganisms.

BB/BI is a method in which the incomplete destruction (or destruction of a modest population) of a resistant biological indicator can be used to demonstrate the capability of the process to reliably destroy any bioburden. This is accomplished using detailed knowledge of the BB and BI populations and their relative resistance.

Typical BB microorganisms have only minimal resistance in comparison to BIs, and this can be confirmed by heat screening of BB isolates. The BB population is controlled by filtration steps for the fluid, process time limits, environmental controls, gowning systems, and other means. The conventional BIs for terminal sterilization using the BB/BI method are *Clostridium sporogenes* ATCC 7955 and *Bacillus subtilis* ATCC 5230, although other strains can be used. The use of *Geobacillus stearothermophilus* for terminal sterilization is uncommon with the BB/BI method because the organism's strong resistance to moist heat makes it poorly suited for this application.

Confirmation of an acceptable probability of a nonsterile unit (PNSU) can be accomplished using physical measurements and BI response (which define the lethality of the process) in conjunction with processing limits for the BB population and resistance (which define the N_0 and D-value). D-value is the time (customarily in minutes) required to reduce the microbial population by 90% or 1 $\log_{10}$ cycle (i.e., to a surviving fraction of 1/10) and must be associated with the specific lethal conditions at which it was determined. For example, D_{121} is the D-value at 121°. Articles intended to be sterile must attain a $\leq 10^{-6}$ PNSU, i.e., less than or equal to 1 chance in 1 million that viable bioburden microorganisms are present. The PNSU can be determined from *Equation 1*.

$$\log N_u = -F/D + \log N_0 \qquad [1]$$

N_u = PNSU

F_0 = F_0-value of the process (lethality)

D = D-value of the natural bioburden

N_0 = bioburden population per container

The following example indicates the resulting PNSU under the defined conditions of validation and routine operation (*Table 1*).

Table 1. Examples of PNSU Calculation

Validation	Routine Usage
F_0 = 8.0 min	F_0 = 8.0 min
D_{121} of BI = 0.5 min	D_{121} of bioburden = 0.005 min
N_0 of BI = 10^6	N_0 of bioburden = 100 (or 10^2)
PNSU for BI = 10^{-10}	PNSU for BB = 10^{-1598}

Determining the resistance of the bioburden is accomplished using a heat-screening process during which a pure culture (a laboratory culture containing a single species of organism spores with minimal vegetative cells) is boiled at 100° for various periods. If the bioburden microorganism is viable after exposure, its resistance at 121° can be estimated for use in the PNSU calculation (▲see *Methods for Determination of Resistance of Microorganisms to Sterilization Processes ⟨56⟩*▲ (USP 1-Aug-2022)). ▲▲ (USP 1-Aug-2022)

Bioburden Method

In most respects the BB method is similar to the BB/BI indicator method. The difference lies in the isolation and characterization of the most-resistant bioburden microorganism. The worst-case isolate is used as the biological indicator in the evaluation of the process. For use in this manner, it must be cultured to produce a suitable challenge population. When this method is used, the bioburden of each process cycle must be closely controlled with respect to population and must be monitored for resistance.

Sterilization Cycle Control

Process equipment for terminal sterilization typically is controlled by calibrated and pressure sensors in/on the chamber/equipment. During the exposure portions of the cycle, attainment of a minimum dwell time at a predefined temperature is used to support process lethality. Cycle lethality for terminal sterilization customarily is measured using F_0, which is defined as an actual exposure time at a variable process temperature that is equivalent to an exposure at 121° based on an ideal microorganism with a *z*-value of 10°. This can include lethality delivered during the heat-up and cooling phases of the sterilization process. A *z*-value is defined as the number of degrees of temperature change necessary to change the *D*-value by a factor of 10. The F_0 approach is used to evaluate to a single standard sterilization processes that are operated at varying temperature conditions. The process lethality at temperatures other than 121° can be calculated to determine lethality equivalent to that provided at 121°. Sterilizer control systems for terminal sterilization deliver conditions within a predefined time–temperature or F_0 range to avoid over-processing.

Simple mathematics can be used to calculate the total lethality over the course of the process. For the specific reference temperature of 121° and a *z*-value of 10.0°, the F_0 calculation can be determined by *Equation 2*:

$$F_0 = \int_{t_1}^{t_s} 10\left(\frac{T-121}{10}\right) dt = \sum_{t_1}^{t_s} 10\left(\frac{T-121}{10}\right) \Delta t \qquad [2]$$

t = time
T = temperature

Summing the instantaneous lethality contributions over the entire sterilization process allows the calculation of the overall process lethality or F_0 delivered over the course of the entire process at varying conditions.

Validation of Liquid-Filled Container Sterilization

As previously noted, the preferred method for steam sterilization is the overkill method as defined in *Sterilization of Compendial Articles ⟨1229⟩* and *⟨1229.1⟩*. However, when the processed materials are susceptible to damage by moist heat at the overkill conditions, the BB/BI method is better suited because it results in reduced heat input while affording the same degree of process efficacy but with different controls. As noted above, terminal sterilization processes require greater consideration of the effects of the treatment on material properties. This has implications for many of the elements of the qualification and validation exercises as indicated below. The validation requirements for the BB/BI and BB methods are more rigorous than those associated with the overkill method. Although the overkill method can be confidently used without detailed consideration of the presterilization bioburden, application of the BB/BI and BB methods require continued monitoring and control of the bioburden, specifically the population and resistance. This is accomplished by testing of filled containers just before sterilization and measuring the number of colony-forming units per container and confirming the absence of resistant BB isolates. When resistant isolates are found, their thermal resistance in the fluid should be determined.

EFFECT OF THE STERILIZATION PROCESS

A preliminary determination of the liquid and the container–closure system's ability to withstand the expected sterilizing conditions should be made during product development. This can be accomplished by sterilization at conditions slightly in excess of the maximum expected and evaluating the effect on the material. The evaluation should encompass the essential quality attributes with attention focused on known and potential new impurities. Appearance and other physical properties also should be evaluated as a part of this effort.

EQUIPMENT QUALIFICATION

Equipment qualification is a predefined program that confirms the equipment has been properly installed and that it operates as intended. Qualification of the sterilizing equipment provides a baseline for preventive maintenance and change control for the sterilizer. The sterilization equipment may require qualification of air, water, utility, and other systems that impact the sterilization equipment's performance.

EMPTY CHAMBER TEMPERATURE DISTRIBUTION

The dual considerations of sterility and stability commonly associated with sterilization of liquids require that equal attention be paid to potential under- and over-processing of the load. For this reason the temperature gradient across the sterilizer may require substantially tighter control than that expected in sterilization by direct contact. The objective is to minimize the time–temperature or F_0 differences across the load throughout the process. Biological indicators are not required in the evaluation of empty chamber temperature distribution.

BIOLOGICAL INDICATORS

The selection of a BI must be considered carefully because of the balance that must be maintained between attaining sterilization and maintaining the sterilized material's essential quality attributes. The biological challenge is either directly inoculated into a liquid-filled container or is introduced via self-contained units provided there is adequate correlation between their resistance and the resistance that would occur in the process fluid. The liquid can be either the product or a surrogate fluid. The resistance of the indicator in the product (and surrogate fluid, where used) must be known. The surrogate's physical properties should approximate those of the product. If there are surfaces within the container that are not presterilized, biological challenge of those surfaces may be required.

LIQUID *D*-VALUE DETERMINATION

Determination of the thermal resistance (*D*-value and *z*-value) for the biological indicator in the liquid is required. This must be performed in a Biological Indicator Evaluation Resistometer (BIER) in replicate. The thermal resistance of each BI lot in the liquid should be determined. When a surrogate liquid is used for convenience (e.g., a master solution approach) or because of microbial inhibition of the BI by the liquid, the thermal resistance in the surrogate must be determined.

CONTAINER MAPPING

Liquid-fill containers with volumes greater than or equal to 100 mL should be mapped to determine internal cold spots. The mapping should be performed with product containers oriented as they would be within the load. The temperature probes should be introduced into the containers using methods that maintain container integrity. Internal supports (of minimally heat-conductive materials) may be required to ensure proper positioning of the probe within the container. After these locations are determined, they are used as either monitoring locations or are correlated to external conditions on the container during validation and routine processing. Smaller containers (less than 100 mL) have fewer discernible cold spots, the importance of which is reduced as container size decreases. Smaller containers (less than 100 mL) can be monitored with temperature probes secured to their exterior. The “cold spot” should be considered a “region” and not a single point in the container.

LOAD POSITIONING AND MAPPING

A fixed loading position within the sterilizer may be necessary for proper sterilization to ensure uniformity of heating and cooling in routine use. Once the load is positioned properly, its size can vary within a defined range. Load-mapping studies should be performed to determine the coldest and hottest locations within the load. These locations may not be specific individual containers but rather regions. This ensures that the containers are neither under- nor over-processed in routine operation of the sterilizer. Validation of variable-size load patterns is accomplished using a bracketing approach for which success with maximum and minimum loads (avoiding both under- and over-processing) establishes the acceptability of intermediate-size loads. However, evaluation of intermediate load sizes may be beneficial. In product sterilization, only a single-size container with a single product lot is processed concurrently.

HEAT PENETRATION AND MICROBIOLOGICAL CHALLENGE

The core of the validation activity is confirmation of acceptable heat penetration using temperature measurements and microbial challenge inactivation. Temperature probes and biological indicators are placed within the load at worst-case locations (e.g., the coldest portions of the loaded chamber). Introduction of the thermocouples must not alter the integrity of the container. Biological challenges are placed in containers adjacent to those that contain heat penetration probes (or the same unit with external temperature measurement).

Proof of cycle efficacy is provided by replicate studies in which the BIs perform as expected and the physical measurements correspond to the expected values of time and temperature or F_0. If the microbial and physical measurements do not correlate, an investigation is in order, and corrective action must be taken to rectify the discrepancy. Samples from the hottest regions of the load are used for evaluation of material stability and quality.

PRODUCT QUALITY AND STABILITY EVALUATION

Manufacturers must conduct ongoing evaluation of the product's ability to withstand the routine sterilizing conditions. The evaluation should encompass the essential quality attributes with attention focused on known and potential new impurities and those materials that receive the most heat input. Manufacturers also should evaluate appearance, other physical properties, and container–closure integrity as a part of this effort. For microbiological media, the ability of the media to meet growth promotion and other requirements is required as indicated in the appropriate test chapter(s) (e.g., *Microbial Enumeration Tests* ⟨61⟩, *Tests for Specified Microorganisms* ⟨62⟩, and *Sterility Tests* ⟨71⟩).

Routine Process Control

All sterilization processes should be subject to formalized practices that maintain them in a controlled state. The practices outlined in ⟨1229⟩ include the general requirements appropriate for all sterilization systems. This is accomplished by a number of related practices that are essential for continued use of the process over an extended period of time. The practices include: calibration, physical measurements, physicochemical integrators, indicators for sterilization, monitoring of bioburden, ongoing process control, change control, preventive maintenance, periodic reassessment, and training.

The use of parametric release is common in the terminal sterilization of finished product containers. This subject is addressed in *Terminally Sterilized Pharmaceutical Products—Parametric Release* ⟨1222⟩.

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[1] Degradation kinetics may differ from those of microbial kill, and F_0 values may not be sufficient to fully evaluate “worst case” effects.

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