



*Institute For Thermal Processing Specialists*

## **Document G.005.V1**

# **GUIDELINES FOR MICROBIOLOGICAL VALIDATION OF THE STERILIZATION OF ASEPTIC FILLING MACHINES AND PACKAGES, INCLUDING CONTAINERS AND CLOSURES**

**The following recommendations are to be considered voluntary guidelines.** This does not preclude the application of other methods and equipment for determining the validation of sterility of aseptic filling machines and packages. These guidelines have been developed by consensus of the Institute For Thermal Processing Specialists and should be given serious consideration for adoption as methodology by individuals performing studies in this area.

## **1. SCOPE**

This document provides guidance on the issues to consider and general steps to follow when conducting microbial validation of the sterilization processes of aseptic filling machines and packages. The decision to microbiologically validate and the test methodology selected will be dependent upon several factors including the aseptic filling machine and packaging being validated and the sterilization process or processes used. References for various microbiological validation methods and statistical aspects are provided to supplement this guidance. Prescriptive microbiological validation method details are not provided in this document as they are best determined based on characteristics and needs of the aseptic filler and package to be validated. This document should not be viewed as a replacement for valid, rational, sound judgment and experience in microbiological validation of an aseptic system. There may be other non-microbiological means of validating sterilization processes; however, these are out of the scope of this document. Nevertheless, a sound, science-base approach is needed to demonstrate the efficacy of any sterilization process. Acceptable procedures for installation, operation and integrity testing of filters are outside the scope of this guidance document. Examples are listed throughout this guidance when describing various methods. These lists are not meant to be exhaustive in nature.

## **2. DEFINITIONS**

- 2.1. Commercial Sterility** – Commercial sterility is defined as the condition achieved by application of heat, chemical sterilants, or other treatments that

- renders the direct and indirect product contact pathways or surfaces of equipment free of viable microorganisms having public health significance as well as microorganisms of non-health significance capable of reproducing in food under normal non-refrigerated conditions of storage and distribution.
- 2.2. **Aseptic Zone** – The aseptic zone includes all direct and indirect product contact pathways and surfaces that must be brought to a condition of commercial sterility prior to the start of filling operations. Pictures, drawings, and schematics may be used to document the aseptic zone.
  - 2.3. **Sterility Maintenance** – The aseptic zone must be maintained in its commercially sterile condition for the duration of filling operations.
  - 2.4. **Barriers** – Barriers, be they, steam, sterile condensate, sterile air, laminar flow air, unidirectional air, differential pressure, liquid sterilant media (e.g., bath), seals and even HEPA filtered air, are necessary for sterility maintenance.
  - 2.5. **Target Organism** – The target organism is the pathogenic microorganism of public health concern that is most resistant to the specific sterilization process being employed.
  - 2.6. **Test Microorganism** – A generally recognized and accepted microorganism identified and used during validation to represent the microorganism of concern from a public health point of view (Target Organism).
  - 2.7. **Logarithmic Cycle Reduction (LCR)** – A commonly used measure of the efficacy of a sterilization process, it is the decimal logarithm of the ratio of the initial count ( $N_0$ ) of a well defined micro-organism and the count of the same organism ( $N_R$ ) after the sterilization process has been run.

$$LCR = \log_{10} \frac{N_0}{N_R} \quad (1)$$

- 2.8. **Count-reduction test** – A method where a known number of microorganisms, typically resistant spores, are exposed to a treatment. After the treatment, the number of surviving microorganisms is determined. This method requires direct measurements of surviving microorganisms after treatment in order to determine LCR of the sterilization process.
- 2.9. **End-point** – A method where a known number of microorganisms, typically resistant spores, are exposed to a treatment. This method provides binomial response. After the treatment, the presence or absence of surviving microorganisms is determined by cultivation in an appropriate medium.
- 2.10. **Critical Factor** - Any property, characteristic, condition, aspect or other parameter, variation of which may affect the scheduled process and the attainment and maintenance of commercial sterility.

- 2.11. **Worst case conditions** - A set of realistic operative conditions under which the sterilization process is expected to be the lowest. Note that this may not necessarily be the minimum/maximum allowed condition for all critical factors.
- 2.12. **Design Qualification (DQ)** - A documented review of the design of new equipment and systems, at an appropriate stage in a project (pre-installation) for conformance to operational and regulatory expectations
- 2.13. **Installation Qualification (IQ)** – A documented verification that equipment meets specifications and requirements, has been properly installed and adheres to final design specifications.
- 2.14. **Operational Qualification (OQ)** - A documented verification that equipment, a system, or process, once installed, operates as intended according to functional specifications during normal operation and throughout the expected operational range and performs as specified under abnormal conditions .

### 3. OBJECTIVES

- 3.1. The objective of microbiological validation is to demonstrate and document that all areas of the aseptic zone and the product package are brought to a condition of commercial sterility before aseptic filling and sealing.

### 4. INTRODUCTION AND BACKGROUND

- 4.1. Aseptic packaging is the operation whereby a commercially sterile food product is filled into a commercially sterile container in a commercially sterile environment. The filled container is hermetically sealed in the same commercially sterile environment.
  - 4.1.1. The commercially sterile environment is commonly referred to as the “aseptic zone” of the filling machine.
    - 4.1.1.1. The aseptic zone must be clearly and accurately defined.
    - 4.1.1.2. The aseptic zone must be brought to a condition of commercial sterility prior to the start of filling operations by active treatment of the area with heat, chemicals, energy, or other means.
    - 4.1.1.3. The commercial sterility of the aseptic zone must be maintained in that condition for the duration of the entire production run.
    - 4.1.1.4. The aseptic zone includes all direct product contact pathways as well as non-direct product contact pathways, e.g., sterilizing an air/nitrogen filter housing.
    - 4.1.1.5. A sterilization process, i.e., a specific sequence of steps and operations, is used to bring the aseptic zone and the package to a condition of commercial sterility.

- 4.1.1.6. Steps must be taken to ensure that commercial sterility of the aseptic zone is maintained, i.e., non-sterile containers, closures, belts, product, non-sterile air/nitrogen, condensate, non-sterile tools during interventions, etc. are not allowed to enter the commercially sterilized aseptic zone.
- 4.2. Microbiological validation of the aseptic zone and package sterilization process is expected for each make, model and, in some cases, version of aseptic filler developed.
  - 4.2.1. Microbiological validation is an important adjunct to the characterization, definition, and control of aseptic processes.
  - 4.2.2. For a filler operating within its established control limits, the microbiological validation provides documented evidence that the sterilization process for the aseptic zone of the aseptic filler and of the package delivers microbiological inactivation in all areas of the aseptic zone and package to predefined, acceptable and safe levels.
  - 4.2.3. Microbiological validation may allow for confirmation of microbiological inactivation in locations within the aseptic zone and on packages that are not typically monitored during the sterilization process.
    - 4.2.3.1. Failure of a microbiological validation may require modifications or process augmentations, and it may be realized that it is necessary to “monitor” such locations and areas during the sterilization process.
  - 4.2.4. Microbiological validation methods may also be useful during characterization and definition of the sterilization processes and their limits of effectiveness.
  - 4.2.5. Microbiological validation is not a replacement for Operational Qualification (OQ).
  - 4.2.6. Microbiological validation is a process. Only with an implemented Management of Change Control program in place can qualification activities result in a validation.
  - 4.2.7. The validated process should be monitored during routine operation. Periodically as needed, re-qualify and recertify equipment.
- 4.3. Microbiological validation is expected for new installations, after modification, for changes in package material, and for equipment transfers and reinstallation.
  - 4.3.1. New installations.
    - 4.3.1.1. Consideration may be given to applying existing validation data from an existing, identical installation.

- 4.3.1.2. When a decision to not validate is made because microbiological validation data already exists for an identical installation, this should be documented with a supporting rationale, including verification that the new installation is identical from the process point of view to a previously validated one.
- 4.3.1.3. Altitude and environmental temperatures may impact the effect of kill kinetics (humidity, barometric pressure, environmental temperature, and dew point) for newly installed and relocated systems, even if they were validated elsewhere (e.g., tropics vs. arid or sea level vs. high altitude).
- 4.3.1.4. The establishment producing the finished product has the ultimate responsibility to ensure that the filler is operating within critical limits and has been microbiologically validated.
- 4.3.2. After a modification is made to the aseptic filler installation, for example, a packaging handling change that modified air flow patterns, sterile air handling and/or commercial sterility maintenance systems.
  - 4.3.2.1. Any deviation from the original equipment specification, including refurbishment, may constitute a system modification and additional risk analysis is required.
  - 4.3.2.2. A decision to not re-validate should be documented with supporting rationale that should include data.
  - 4.3.2.3. A Management of Change Control program is highly recommended.
    - 4.3.2.3.1. The impact of all system changes to be performed on a previously validated line on the microbiological validation should be evaluated for approval using the protocols prescribed by the Management of Change Control program. Determining whether a new microbiological validation is needed due to the change must be part of the change control procedure.
- 4.3.3. For changes in package material of construction, shape, thickness, volume
  - 4.3.3.1. Changes to package material may affect surface and thermal properties such as surface tension, thermal diffusivity and heat capacity. This is especially important when gaseous, spray or vapor phase sterilants are used.
  - 4.3.3.2. A Management of Change Control program is highly recommended.
    - 4.3.3.2.1. All changes, including package or packaging material changes, should be evaluated for approval using as prescribed by the protocols of the Management of Change Control program.

Determining whether a new microbiological validation is needed due to the change must be part of the change control procedure.

**4.3.4. Extended Idle Period, Equipment Transfers, and Reinstallation**

**4.3.4.1.** When an existing line has been taken out of operation for an extended period, the provisions of 4.3.1 to 4.3.3 apply for requalification and/or revalidation.

**4.3.4.2.** When equipment is transferred from the original installation and must be reinstalled, the provisions of 4.3.1 to 4.3.3 apply.

**5. MATERIALS, TOOLS, EQUIPMENT NEEDED and RECOMMENDED**

**5.1.** Access to a microbiology laboratory is highly recommended. Microbiological validation methods require the access to facilities and services needed to grow, identify, maintain and test for resistance of the test microorganisms. The exact tools and facilities needed are dependent on the choice of microbiological validation method.

**5.2.** In addition to standard microbiology laboratory facilities, other materials or tools needed for microbiological validation will depend to some extent upon the test methodology being used. Examples include means to measure sterilant concentration, sterilant flow, temperature distribution, air flow patterns, pressure differentials, or radiation dosimetry.

**6. METHODS**

**6.1. Pre-requisites** - The following should be completed before beginning microbiological validation.

**6.1.1.** Aseptic filling equipment must be of good hygienic design and be capable of commercially sterile operation. That is, a microbiological validation will achieve nothing if the machine was poorly designed and constructed in the first place. This should be evaluated during the Design Qualification (DQ).

**6.1.1.1.** Equipment must be constructed in a manner that makes product contact surfaces and the aseptic zone sterilizable.

**6.1.1.2.** All direct and indirect product contact pathways and surfaces that make up the aseptic zone must be cleanable.

**6.1.2.** Process Definition – Steps, sequence of events, critical parameters and applicable ranges are defined leading to a functional description.

**6.1.3.** Demonstrate that the equipment has been designed, fabricated, instrumented and is capable of operating aseptically.

- 6.1.4. Establish that the process equipment has the capability of operating within the required parameters by completing an Installation Qualification (IQ) and Operational Qualification (OQ) of the equipment installation and all computer controls.
  - 6.1.4.1. Demonstrate that the critical control equipment and instrumentation are capable of operating within the prescribed parameters for the process equipment. All critical sensors and measuring devices needed for process control must be calibrated, properly documented, and properly installed.
  - 6.1.4.2. Alarm verification completed for all critical operations. Note that alarms might have to be temporarily disabled in order to allow the filler to run under worst case conditions. In that case, care must be taken to re-enable alarms and re-verify that they are performing as intended after completing a successful microbiological validation.
- 6.2. **Additional programs important to aseptic packaging operations** - The following programs are not strict pre-requisites for a microbiological validation, but they are necessary to ensure that the microbiological validation of sterilization processes for aseptic filling machines and packages remains valid.
  - 6.2.1. A successfully completed Cleaning Validation
  - 6.2.2. An implemented and documented Management of Change Control program.
  - 6.2.3. An implemented Preventative Maintenance program
  - 6.2.4. An implemented Calibration Program - All measurement instruments, sensors and related control loops have associated with critical control points (CCP) or prerequisite / operational prerequisite programs are to be included in an instrument calibration program.
  - 6.2.5. A comprehensive Training program
- 6.3. **Outline of a Microbiological Challenge Test**
  - 6.3.1. Identify critical factors and operational ranges. (See 6.4)
  - 6.3.2. Define the worst case conditions for the sterilization process and the specific filler and package being validated. (See 6.5)
  - 6.3.3. Identify the locations/boundaries in the aseptic zone and package surface to be challenged, for example, pictures, marking a P&ID or other equipment drawing. (See 6.6-6.8)
  - 6.3.4. Develop a protocol for the validation testing (See 6.9) that addresses the following:

- 6.3.4.1. The test methodology that will be used for each sterilization process is defined. (See 6.9.1)
- 6.3.4.2. The target organism is identified and the desired LCR is established. (See 6.9.2)
- 6.3.4.3. The test microorganism (surrogate), including resistance to the specific sterilization process being validated, is characterized. (See 6.9.3)
- 6.3.4.4. The expected LCR of the test organism is defined. (See 6.9.4)
- 6.3.4.5. The suitable carrier or substrate being used is identified. Note that different carriers may be needed in some locations or due to differences in sterilization processes. (6.10.1)
- 6.3.4.6. A suitable inoculation method is developed. (See 6.10.2)
- 6.3.4.7. The appropriate inoculation load is determined. (See 6.10.3)
- 6.3.4.8. Inoculated carriers are placed in predetermined locations. (See 6.10.4)
- 6.3.4.9. Microbiological recovery methods are defined and the actual load of the test microorganism on the carrier/substrate is determined. (See 6.10.5)
- 6.3.4.10. Culture media, incubation temperature considerations are made. (6.10.6)
- 6.3.5. Set aseptic filling machine and package sterilization processes to predefined worst-case conditions for the validation test.
- 6.3.6. Execute the validation tests.
- 6.3.7. Recover the exposed carriers or packages and determine the outcome of the test based on the microbiological validation method chosen. Tailing and inactivation effects due to the presence of residual sterilant should be confirmed as not being present.
- 6.3.8. Document results.
- 6.3.9. Confirm identity of any recovered microorganisms (may be dependent on method selected).
- 6.3.10. Repeat replicate studies as defined in the protocol.
- 6.3.11. Analyze the data. (See 7.0)

#### **6.4. Identification of Critical Factors and Operational Ranges**

- 6.4.1. The performance of the sterilization process is linked to the maintenance and control of a set of defined critical factors. Critical factors may include

physical, (e.g., time, piping design), chemical (e.g., chemical sterilant concentration), mechanical (e.g., flow rate of sterilizing medium), thermal (e.g., temperature, specific heat), radiation (e.g., electromagnetic, photonic).

**6.4.2.** Parameters to evaluate as critical factors may include, but are not limited to:

- Temperature
- Pressure (e.g., air, culinary steam supply)
- Relative humidity (e.g., dew point)
- Elevation
- Chemical sterilant concentration
- Sterilant flow rate (e.g., high pressure hot water)
- Sterilant residence time/contact time
- Sterilant phase characteristics (e.g., vapor, liquid, spray, fog, or mist)
- Radiation intensity and dose
- Piping and ductwork design
- Presence of optional components or devices such as head space injection
- Activation timing of valve actuators, pumps, heating elements
- Transition to next state of operation
- Chemical sterilant removal
- Package splicing
- Surface tension
- Interruptions, short stops and jams
- Effect of concurrent practices (e.g., product path sterilization concurrent with aseptic zone sterilization)

## **6.5. Identification of Worst Case Conditions**

**6.5.1.** Microbiological validation testing is often conducted under a pre-defined set of realistic operative conditions under which the sterilization process is expected to be the lowest.

**6.5.2.** Critical factors of the sterilization process must be defined with the appropriate values or levels at which the factor is critical to the process that delivers a commercially sterile system or package.

**6.5.3.** Points to consider when establishing the worst case condition include, but are not limited to:

- Limiting values, both high and low, for identified critical factors
- Permitted variation ranges for critical factors/parameters inclusive of the procedures (e.g. calibration) and instruments used to assure that the permitted variation is not exceeded.
- Allowed manual operations or interventions
- Interactions among critical factors, other variables and conditions, resulting in a reduction in sterilization process delivery

- Interfaces between the aseptic zone and ancillary equipment
- Interfaces between different sterilization processes within the aseptic zone
- Sterilant contact time
- Loading and speed of conveying systems through the package sterilization process and into the aseptic zone of the filler
- Motion of conveying equipment
- Hot and/or cold re-start of equipment
- Ramp-up and ramp-down
- Changeovers, including product and package
- Brand new versus aged machines
- Presence of remnant containers in the aseptic zone, partial rolls
- Splicing of rolls, longitudinal seal strips
- Idling in a sterile mode – limits before re-CIP and re-SIP

**6.5.4.** The worst case condition is not necessarily setting all critical factors and other sterilization parameters to the allowed minimum (e.g., temperature) and maximum (e.g., throughput) values.

**6.5.5.** Note that the alarm structure on the filling machine under study might prevent the machine from running in the established worse case conditions. In such a case, the relevant alarms should be disabled until the validation is completed. This would require alarm verification to be conducted or repeated at the end of a successful microbiological validation.

## **6.6. Determination of Microbiological Challenge Locations for Testing Filling Machine Sterilization**

**6.6.1.** The aseptic zone is usually geometrically complex due to the variety of operations made by the filler within this space.

**6.6.2.** Some locations within the aseptic zone may receive more or less treatment during the sterilization process. Since it is impractical to submit the entire surface of the aseptic zone to microbiological challenge, it is critical to identify locations in the aseptic zone that are most likely to receive a “minimum” or “worst case” process as well as locations that are likely to be subjected to more than the minimum process.

**6.6.3.** Identification of areas suspected to be candidates for minimum process delivery must be based on an understanding of the sterilization process. When multiple sterilization processes are used, their sequence, hierarchy, interrelatedness and interactions should be taken into account.

**6.6.4.** Factors to consider include, but are limited to:

- Temperature distribution, uniformity, stratification
- Steady-state and transient contact times
- Sterilant concentration, phase, make-up rate

- Sterilant and/or gas flow
  - Radiation dose and distribution
  - Heat transfer characteristics
  - Wetting properties, e.g. surface tension
  - Presence of wells or closed ends
  - Geometrical restrictions
  - Preferential paths for sterilant flow
  - Shielding (i.e., presence of mated, occluded, or shadowed surfaces)
  - Materials of construction (e.g., areas where there may be different surrounding materials)
  - Fabrication aspects after package sterilization impacting commercial sterility maintenance (e.g. folding, erection, thermoforming)
- 6.6.5. It is important that filters be microbiologically validated to ensure that they function properly in process applications.
- 6.6.5.1. This guidance document assumes that the processor has verified the integrity of commercially sterile filters used in aseptic processing and packaging operations using standard methods, such as ASTM F 838-83.
- 6.6.5.2. Acceptable procedures for installation, operation and integrity testing of filters are outside the scope of this guidance document.
- 6.6.6. When the aseptic zone is large, consideration should be given to the distance between microbiological challenge locations.
- 6.6.7. Some locations in the aseptic zone may be difficult to access for testing or may pose limitations on the testing technique(s) selected.
- 6.6.7.1. Examples include narrow gaps, piping dead ends, pockets, metal to metal contact surfaces.
- 6.6.7.2. In principle, testing difficulties should not be used as a reason to exclude a location from testing. Rather, this is a major reason to consider these locations for testing.
- 6.6.7.3. A decision to not test a difficult location should be documented with a supporting rationale.
- 6.6.8. Although it is not in the scope of this document, many aseptic filling systems employ the use of an aseptic surge tank that is not an integral part of the aseptic filler to hold product before filling. Therefore, following points need to be considered:
- 6.6.8.1. Temperature distribution for the steam-sterilized tanks may be adequate.
- 6.6.8.2. Microbiological challenge of select locations in the aseptic tank may be necessary to ensure that the aseptic tank can be brought to a condition of commercial sterility. In these instances, the provisions of Section 6.6

apply and may be used when conducting a validation study of the surge tank.

## **6.7. Determination of Microbiological Challenge Locations for the Packaging Material Sterilization**

- 6.7.1.** Depending on the technological solution adopted by the manufacturer, the packaging material may first be sterilized and then aseptically formed into packages, the packages may be formed under non-aseptic conditions and then be sterilized, or the packaging material can be pre-sterilized.
- 6.7.1.1.** Every spot on the surface of the packaging material or a preformed package may not receive the same sterilization.
- 6.7.1.2.** There may exist one or more spots on the surface of the packaging material, the “weakest point,” that is likely to receive the least sterilization dose.
- 6.7.1.3.** With the aid of physical, chemical and geometrical considerations, including fluid-dynamic modelling, the package or packaging material should be “mapped” to determine the weakest points.
- 6.7.1.4.** In principle, the microbiological validation could concentrate only on the weakest points, but it is advisable to challenge several spots to confirm that the weakest spots have been correctly chosen.
- 6.7.2.** For filling machines that accept different shapes and sizes of bottles or packages and closures, every individual shape or size must be considered for microbiological validation.
- 6.7.2.1.** All changes, including package or packaging material changes should go through a change control procedure for approval within the Management of Change Control program. Determining whether a new microbiological validation is needed due to the change must be part of the change control procedure.
- 6.7.2.2.** Validated mathematical models, capable of predicting the physical and chemical conditions in every spot of the package or bottle during the sterilization process, can be used to reduce, or eliminate, the need for microbiological validation of individual shapes or sizes.
- 6.7.2.3.** A decision to use a validated model should be documented with supporting rationale.
- 6.7.3.** Some filling machines aseptically apply the cap or closure to the package or bottle. In these machines the caps or closures must also undergo sterilization and therefore, they too, must be microbiologically validated. The provisions of points 6.7.1 and 6.7.2 apply.
- 6.7.4.** The portion of the outer surface, the surface not in contact with the food, of the package that enters the aseptic zone should undergo microbiological validation.
- 6.7.4.1.** The portion of the outer surface of the package that enters the aseptic zone must be brought to a level of commercial sterility to prevent recontamination of the aseptic zone and of the filled product.

6.7.4.2. The locations of the outer surface to be subjected to microbiological validation should be determined by taking into account the provisions of points 6.7.1 through 6.7.3.

#### **6.8. Filling and Packaging Machines with Multiple Lanes or Multiple Heads for Sterilization, Filling or Sealing**

- 6.8.1. Sterilization of each individual lane or head should be demonstrated.
- 6.8.2. Sterilization of packages and packaging materials should be demonstrated for each individual lane or head.
- 6.8.3. Confirmation that the physical and chemical parameters of all locations are identical should be experimentally obtained prior to the start of microbiological validation.
- 6.8.4. A decision must be made regarding the need to challenge all or only some of the different lanes and/or heads of the filler.
- 6.8.5. The decision to restrict microbiological challenge locations to representative lanes or filling heads must be documented with supporting rationale for the selection.
- 6.8.6. In general, it is acceptable to omit some lanes and filling stations or heads when they are proven identical (or symmetrical) to those which undergo microbiological challenge.

#### **6.9. Develop a Protocol for Validation Testing**

##### **6.9.1. Test Methodologies for Microbiological Validation of Sterilization Processes**

- 6.9.1.1. In general, microbiological validation provides evidence that the processes applied for machine and package sterilization deliver a LCR higher than a stated target value for a suitable test organism.
- 6.9.1.2. There are a variety of test methods that may be used for microbiological validation of any sterilization process. References are available that provide more detail on these methods. Generic information is provided in this document for the most common microbiological validation test methods.
- 6.9.1.3. Count Reduction Test – The Count Reduction test is based on knowing the initial count on the inoculated carrier/substrate and then recovering and enumerating the number of microorganisms that have survived the sterilization process. This method requires the presence (recovery) and enumeration of surviving test microorganisms. The experiment should be designed so that the colony forming units are in the countable range when the target LCR is achieved. Absence of surviving organisms indicates that the target LCR has been exceeded.

6.9.1.4. **End Point Test** – The End Point test is based on exposing inoculated carriers/substrates with known initial counts to the sterilization process, incubating the carriers/substrates using appropriate methods (e.g., media and growth temperature) and observing for growth of surviving microorganisms. A binary response—growth or no growth—is obtained, where “no growth” implies sterility of the sample. Estimation of mean survivor load is done using statistical tools when several replicate samples are available, some of which show growth. This method can also be applied to a single inoculated sample; in that case, no growth (sterility) of the sample is required for the test to be considered successful though the uncertainty associated with the binary information should be taken into account.

## 6.9.2. Identifying the Target Organism and the Target Log Reduction

- 6.9.2.1. The purpose of microbiological validation is to demonstrate that commercial sterility is achieved.
- 6.9.2.2. The identity of the target organism for the specific sterilization process and the required logarithmic cycle reduction must be determined, justified and documented.
- 6.9.2.3. The target organism is the pathogenic microorganism of public health concern that is most resistant to the specific sterilization process being employed.
  - 6.9.2.3.1. *Clostridium botulinum* has historically been considered the target organism for sterilization by moist heat, dry heat, and peroxide sterilization technologies.
  - 6.9.2.3.2. For some sterilization technologies, more than one target organism may also have to be considered, for example, *Bacillus cereus*.
- 6.9.2.4. Some systems utilize multiple sterilization processes, especially for the sterilization of the aseptic zone, and it may be necessary to identify more than one target organism with respect to the processes being employed.
- 6.9.2.5. The desired log reduction for the target organism should be determined by taking into account:
  - 6.9.2.5.1. The initial bioburden of the target organism present on the surfaces within the aseptic zone or on the incoming packaging materials,
  - 6.9.2.5.2. The fraction of bioburden likely to be released into the aseptic zone from non-food contact surfaces of the package,
  - 6.9.2.5.3. The possible public health consequences of surviving target organism, and

- 6.9.2.5.4. The maximum acceptable frequency of occurrence of a package contaminated with target organism.

### **6.9.3. Selecting the Test Microorganism (Surrogate)**

- 6.9.3.1. Direct testing of the sterilization process using the pathogenic target organism is not desirable in an industrial production environment. Thus, surrogate microorganisms have historically been used as the test organisms for validation of various sterilization processes (see Table 1).
- 6.9.3.2. Selection criteria for the surrogate/test microorganism include, but are not limited to:
  - 6.9.3.2.1. The test organism must be acceptable from the plant/factory, occupational and public health perspective, i.e., safe disposal and bio-hazard handling, in the conditions and environment encountered during the challenge test.
  - 6.9.3.2.2. The test organism should have a sufficiently high resistance to the sterilization process being validated to demonstrate the desired log reduction of the target organism.
  - 6.9.3.2.3. The expected target log reduction of test organism (See section 6.9.4.)
- 6.9.3.3. Resistance of the test organism to the specific sterilization process must be characterized.
  - 6.9.3.3.1. The test organism should preferably exhibit first order (exponential) inactivation kinetics.
  - 6.9.3.3.2. At a minimum, the D-value must be determined for the specific batch/crop of test microorganism being used. A single batch/crop of test microorganism is recommended for the complete validation of a sterilization process. Ideally, the z-value should also be determined.
  - 6.9.3.3.3. Standardized methods for resistance determination should be used whenever possible.
  - 6.9.3.3.4. Organism storage stability should be demonstrated and recent resistance data should be documented and available.

Table 1. Surrogate microorganisms historically used for validating aseptic filling machines and packaging.

Sterilization Process	Common Surrogate Microorganisms	Comments
<b>Saturated Steam/ Superheated Water</b>	<ul style="list-style-type: none"> <li>- <i>Clostridium sporogenes</i></li> <li>- <i>Geobacillus stearothermophilus</i></li> </ul>	<p>This sterilization process is unlikely to apply to the aseptic filler. Product pathway is a possible exception. Use of <i>G. stearothermophilus</i>, a thermophilic microorganism, is advantageous in that it eliminates and/or reduces the need for aseptic technique when recovering exposed carriers.</p>
<b>Superheated Steam &amp; Dry Heat</b>	<ul style="list-style-type: none"> <li>- <i>Geobacillus stearothermophilus</i></li> <li>- <i>Bacillus polymyxa</i></li> <li>- <i>Bacillus atrophaeus</i><sup>†</sup></li> </ul>	<p>Microorganisms listed are used when mode of microbiological inactivation is dry heat.</p>
<b>Hydrogen Peroxide + Heat</b>	<ul style="list-style-type: none"> <li>- <i>Bacillus atrophaeus</i><sup>†</sup></li> <li>- <i>Bacillus subtilis</i></li> </ul>	
<b>Hydrogen Peroxide + UV</b>	<ul style="list-style-type: none"> <li>- <i>Bacillus atrophaeus</i><sup>†</sup></li> <li>- <i>Bacillus subtilis</i></li> </ul>	
<b>Peroxyacetic Acid</b>	<ul style="list-style-type: none"> <li>- <i>Bacillus atrophaeus</i><sup>†</sup></li> <li>- <i>Bacillus subtilis</i> SA 22</li> </ul>	<p>It is customary to use spores of these organisms when testing the effectiveness of packaging sterilization devices utilizing PAA (VDMA, 1997). No generally accepted surrogate organism has yet to be identified for compliance with US FDA regulations.</p>
<b>Filtration</b>	<ul style="list-style-type: none"> <li>- <i>Brevundimonas diminuta</i><sup>††</sup></li> </ul>	<p>For verifying integrity of filters for sterilization filtration of liquids. Not for use with HEPA, membrane or depth filters. During sterilization validation, the appropriate surrogate organism should be chosen for the sterilization process employed.</p>

<b>Heat of Formation, (e.g. extrusion, thermoforming, blow-molding)</b>	- <i>Geobacillus stearothermophilus</i>	The same comment as for Dry Heat applies.
<b>E-beam irradiation</b>	- <i>Bacillus pumilus</i>	May apply to on-line or off-site sterilization of packages, containers, and closures.
<b>Gamma irradiation</b>	- <i>Bacillus pumilus</i>	Primarily for pre-sterilization of preformed containers off-site

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<sup>†</sup> Previously named *Bacillus subtilis* var. *niger* or *Bacillus globigii*.

<sup>††</sup> Previously named *Pseudomonas diminuta*.

#### 6.9.4. Expected Log Reduction for Test Organism

- 6.9.4.1. The target log reduction on the test organism should be adequate to demonstrate that the sterilization process is sufficient to deliver the expected log reduction on the target organism.
- 6.9.4.2. The target log reduction on the test organism should be determined from the expected log reduction of the target organism and the relative resistance of the test organism ( $D_{Test}$ ) versus the target organism ( $D_{Target}$ ). If both target and test organisms show first order exponential inactivation, the target log reduction of the test organism should be

$$LCR_{Test} \geq \frac{LCR_{Target} \cdot D_{Target}}{D_{Test}} \quad (2)$$

When data available in scientific literature are insufficient, specific studies may be needed to compare the resistance of the test and of the target organisms to a specific sterilization technology.

- 6.9.4.3. The target log reduction on the test organism should also be adequate to ensure sufficient inactivation of possible non-pathogenic microorganisms. The target LCR determined according to 6.9.4.2 should be revised versus this requirement and increased if needed.

#### 6.10. Test Microorganism and Carrier/Substrate Selection

##### 6.10.1. Carrier Selection

- 6.10.1.1. The choice of carrier (i.e., substrate used to hold the test microorganism) will be dependent upon several factors:
  - 6.10.1.1.1. The sterilization process,
  - 6.10.1.1.2. The microbiological method being used, and
  - 6.10.1.1.3. Commercial availability of inoculated carriers of various types with a variety of surrogate microorganisms (Biological Indicators according to EN-ISO 11138[2006]).
- 6.10.1.2. In the case of packages, the “carrier” is usually the packaging material itself, be it the container, closure, or seal material.
- 6.10.1.3. Common carrier materials or types include paper discs or strips, metal discs or strips, self-contained ampoules, and packaging material. The material chosen to be the carrier should have similar ease/difficulty of sterilization, surface effect of sterilizing agent, and heat or mass transfer properties relative to the challenge location of the surface(s) being sterilized.

- 6.10.1.4. Specific studies may be required to support the choice of carrier material.
- 6.10.1.5. For validation of sterilization of aseptic zone, direct inoculation of the surfaces to be validated may be considered, especially if it is the most convenient or the only way to challenge one specific location. In this case, care has to be taken as to the appropriateness and the limitations of the methods applied for inoculation, recovery and enumeration.

#### 6.10.2. Inoculation Method

- 6.10.2.1. Methods such as spraying or depositing drops may be used to directly inoculate carriers and surfaces. When selecting a method, the following points should be considered:
  - 6.10.2.1.1. *Accuracy* – The method must be able to deposit the desired load of the test microorganism on the surface.
  - 6.10.2.1.2. *Precision* – The range of loads among the inoculated surfaces should be known. The impact of the range of inoculated loads on the challenge results should be documented. This requires inoculation recovery and enumeration of a suitable number of non-exposed surfaces.
  - 6.10.2.1.3. *Application* – Inoculation of the surface, by spray, spread or point application, should be conducted in a manner that allows for determination of the minimum treatment for the process, appropriate for the analysis procedure used for the data, and the ability to enumerate the surviving load.
- 6.10.2.2. Dispersion of test microorganism on the inoculated surface
  - 6.10.2.2.1. To verify delivery of sterilization conditions, it is preferable to use inoculation techniques that produce a single layer of test organisms, so that each individual cell or spore is exposed to the very same process, and the tendency of the test organism to flake away from the surface is minimized.
  - 6.10.2.2.2. To prove the effectiveness for sterilization processes, it may be useful to create multiple layers of test organisms or even to inoculate test organisms in a mixture with some supporting or shielding materials; this may be considered the only way to simulate a worst case for those sterilization techniques. In such cases, the exposure of the test organism to the sterilizing agent may be non-uniform. This could generate deviations from exponential inactivation kinetics, and thus render the concept of LCR not applicable. Also, consideration should be given to control

and reproducibility of inoculation conditions, which may be particularly critical in this case.

**6.10.2.2.3.** The inoculation method should not alter the resistance properties of the test microorganism.

**6.10.2.2.4.** The actual load on the inoculated surfaces should be determined for each batch of test microorganism/inoculated carrier/surfaces and for each statistical control sample. The actual, measured load (as opposed to the intended load) should be used in all calculations involving the initial load.

**6.10.2.3.** Direct inoculation of the surfaces of packaging materials is usually preferred.

### **6.10.3. Inoculation Load**

**6.10.3.1.** The required microbiological load on the inoculated carriers/surfaces (or other biological indicator such as self-contained ampoule) should be established based on the desired logarithmic cycle reduction (LCR), the test methodology, and recovery methods.

**6.10.3.2.** In general, a Count Reduction Test requires carriers/surface inoculated with a higher load than the target LCR because of the requirement to observe a countable number of survivors at the target LCR.

**6.10.3.3.** For the End Point Test, when a sufficient number of replicates are available, the test load should be high enough to obtain a fraction of the samples as “positive” for the target LCR.

**6.10.3.3.1.** When only one sample is available for analysis, the inoculation load should be high enough to demonstrate that the target LCR has been achieved if the sample is found to be absent of growth (“negative”) after sterilization treatment.

**6.10.3.4.** The use of more than one inoculated load (i.e., challenge titer or level) is beneficial. In this case, runs with inoculated loads 1 log lower and 1 log higher than the expected LCR are typically added.

### **6.10.4. Placement of Inoculated Carriers**

**6.10.4.1.** The inoculated carriers should be placed in the challenge locations in a manner that does not alter the sterilization process. For example, placement of the carriers should not obstruct pipes, ducts, or otherwise hinder or alter the flow of the sterilizing medium.

**6.10.4.2.** Where applicable, placement of inoculated carriers should not hinder or alter removal of condensate, exhausted sterilizing medium, air or other non-condensable and non-miscible gases.

6.10.4.3. As package surfaces are typically directly inoculated, the placement of the inoculum may be dictated by the need to eliminate machine contact and allow for recovery.

**6.10.5. Recovery and Estimation of Challenge and Survivor Levels**

6.10.5.1. Once the sterilization process has been completed, the number or presence of surviving microorganisms must be determined.

6.10.5.2. Actual recovery and estimation methods will be dependent upon the test methodology /test microorganism used and should always separate out any residual kill effects as a result of the test, e.g., due to residual peroxide.

6.10.5.3. Recovered/surviving microorganisms should be identified to confirm that they are the test microorganisms.

6.10.5.4. Confirmation of the identity of surviving microorganisms may also be necessary to demonstrate lack of contamination.

**6.10.6. Growth Promotion Characteristics of Culture Media and Incubation temperatures**

6.10.6.1. The suitability of media and incubation conditions should be demonstrated and documented. This also applies to the filled product being used as a culture medium for the validation, as may happen with End Point testing of package sterilization.

6.10.6.2. The culture media and the incubation time and temperature should be selected to promote growth of a single viable cell or spore of the test organism, even if injured by the sterilization process.

6.10.7. Standard procedures for handling the test organism including how to store, dilute, inoculate, cultivate, enumerate, and determine the load on inoculated carriers should be followed.

6.10.8. Involvement of a skilled microbiologist with access to a microbiology laboratory capable of proficiently conducting quantitative microbiological sterilization validation studies is highly recommended.

**7. DATA ANALYSES**

7.1. Generally recognized methods, appropriate for the test procedure, must be selected for data analysis.

7.2. Involvement of a skilled statistician with access to statistical software not only capable of analyzing data, but also with the ability to verify assumptions and the applicability of the analysis method chosen is highly recommended.

7.3. Document all deviation from the written validation protocol. Include supporting rationale on why the deviations were or were not acceptable.

#### **7.4. Equations**

7.4.1. Relevant equations and correlations should accompany all reports developed to document the results of the validation study.

7.4.2. Appropriate statistical analyses should be used where the underlying assumptions have been validated for the test procedure.

#### **7.5. Operating Conditions**

7.5.1. Document allowed operating conditions that are supported by the data.

7.5.2. Construct a table listing critical factors associated with bringing the filling machine and package material to a condition of commercial sterility and for maintaining sterility for the entire production run. Also specify the minimum/maximum value or acceptable range for critical factors and the corrective action necessary when a deviation occurs.

#### **7.6. Discussion of Results**

7.6.1. Clearly document a discussion of the data recorded, how the data was analyzed and the justification for the conclusions drawn. This documentation is also important to the success of the Management of Change Control program and for determining the impact of changes and repairs.

### **8. RISKS, ISSUES and OTHER CONSIDERATIONS**

8.1. Microorganisms are living entities and do not always behave in the expected manner. Elimination of any test result must be carefully considered. Unexpected results may require additional testing. Unexpected results must be documented and included in the final report. Microbiological validation methods each have associated risks, issues, and other considerations. Users should familiarize themselves with the advantages and disadvantages of the different methodologies when selecting the method to use for the sterilization process validation.

8.2. A challenge for any microbiological method is the ability to recover and propagate microorganisms that have been stressed. Recovery of damaged or stressed microbial cells must be taken into consideration when analyzing results. Careful attention must be paid to recommendations provided by suppliers regarding handling of the test microorganism both prior to and after exposure to the sterilization process.

8.3. Partial challenge location studies where a limited number of challenge locations are selected for a test due to an unexpected result or procedural error should be used judiciously and sparingly. A decision to conduct a partial

challenge study as part of the validation testing should be documented with a supporting rationale.

- 8.4. A rationale should be provided for the chosen confidence level for statistical treatment of the LCR survivor data.
- 8.5. A rationale should be provided for the chosen number of test repetitions or other acceptance criteria for a validation approach.

## 9. DOCUMENTATION

- 9.1. Documentation needs have been mentioned in several locations in the body of this document. A final report detailing the testing and results should be written. The final report documents that validation of the aseptic filling system was completed and specifies the conditions under which the validation was originally conducted.

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Institute For Thermal Processing Specialists  
304 Stone Rd. W., Ste. 301, Guelph. ON N1G 4W4  
P: +1 519 824 6774 F: +1 519 824 6642 info@iftps.org  
www.iftps.org