Designation: E1766 – 15

Standard Test Method for Determination of Effectiveness of Sterilization Processes for Reusable Medical Devices[1]

This standard is issued under the fixed designation E1766; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers a reproducible procedure for testing processes used to sterilize reusable medical devices (instruments). This test method is not designed to validate a sterilization process, but tests an established sterilization cycle or process. It is a practical test of the effectiveness of a sterilization process applied to reusable medical devices. Bacterial spores more resistant to the test sterilant than the natural bioburden of the instrument are used as the test organisms. Commercially available liquid suspensions of bacterial spores are used to inoculate the instruments.

1.2 This test method is intended for reusable medical devices cleaned in accordance with the device manufacturer's instructions and prepared for sterilization in accordance with the instructions for the sterilization process being used.

1.3 This test method assumes that cleaned, reusable medical devices will be free of visible soil but may have remaining adherent bioburden. A worst-case bioburden can be represented by suspensions of bacterial endospores, which are commercially available for monitoring chemical or physical sterilization processes. These endospores should have a verifiable resistance (D value) to the specific process and sterilant being evaluated.[2]

1.4 It is impractical to test for the sterility of some devices by immersion in growth medium because of their complexity, size, and availability (for long-term incubation) or adverse effects on the devices from long-term immersion. Therefore, elution, rinsing, or swabbing techniques are used to recover test organisms from inoculated devices.

1.5 A recovery control will be included by inoculation of a test device and use of the elution methods without applying the sterilization process being tested. A minimal recovery of 10^6 colony-forming unit (CFU)/mL per device is required for the recovery control.

1.6 Results of the recovery control and process test cycle are compared to determine the effectiveness of the sterilization process.

1.7 Results of the recovery control and applied inoculum are compared to determine the recovery efficiency, if desired.

1.8 The procedure should reveal that tested devices are free of recoverable microorganisms when five or more consecutive tests are conducted.

1.9 A knowledge of microbiological techniques is required to conduct these procedures.

1.10 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.11 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1193 Specification for Reagent Water

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

2.2 *Other:*

ASTM Poster Presentation : "Use Verification of a Proposed Draft ASTM Standard to Determine the Efficacy of Sterilization Techniques for Reusable Medical Instruments" Presented at the E35.15 Subcommittee meeting in Montreal, Canada

3. Terminology

3.1 *Definitions:*

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] Oxborrow, G. S., and Berube, R., "Sterility Testing—Validation of Sterilization Processes, and Sporicide Testing," *Disinfection, Sterilization, and Preservation*, Block, S. S., 4th Edition, Lea and Febiger, Philadelphia, PA, 1991, pp. 1047–1058.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3.1.1 *bioburden*—the number and types of viable microorganisms that contaminate a device.

3.1.2 *CFU*—colony-forming unit.

3.1.3 *inoculum*—the number (usually specified as CFUs) and type (genus and species) of viable microorganisms used to contaminate a given sample or device.

3.1.4 *sporicidal agent*—any chemical or physical agent that kills spores.

3.1.5 *sterilant*—any sterilizing agent.

3.1.6 *sterile*—a state of being free of living organisms.

3.1.7 *sterilization cycle or process*—a physical or chemical process that has been demonstrated to meet applicable criteria for sterilization as defined by AAMI.[4]

3.1.8 *sterilizer*—any device using a chemical or physical process that produces sterile materials.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *applied inoculum*—the estimated count of the suspension of bacterial spores expressed as CFU/mL used to inoculate the test devices. This value may be used if the efficiencies of the recovery methods are determined.

3.2.2 *process test cycle*—a complete sterilization cycle that uses all parameters of the sterilization process as dictated by the manufacturer.

3.2.3 *recovery control*—the CFU recoverable from a device following inoculation and optional drying of the spore suspension in or on the unprocessed device. The recovery of $\geq 10^6$ CFUs per device is required.

3.2.4 *recovery efficiency*—a measure of the recovery of inoculated organisms from a device may be determined when necessary. The recovery efficiency may be expressed as the ratio of the CFU from the recovery control compared to the CFU of the applied inoculum. This value is multiplied by 100 to express efficiency as a percent. It is recommended that a minimum of three tests be performed when estimating recovery efficiency.

3.2.5 *reusable medical devices*—any medical device that is claimed to be usable after reprocessing.

3.2.6 *spore*—a bacterial endospore. (Strain identification and the means used to identify whether the vegetative or spore state is present should be indicated.)

3.2.7 *worst-case*—the intentional exaggeration of one or more parameters of a test compared to normal clinical conditions.

4. Summary of Test Method

4.1 Percent recovery of inoculum may be used to ensure reproducible inoculation and recovery techniques.

4.2 The test method is performed by contaminating the cleaned reusable medical device with a bacterial endospore suspension.

4.3 After inoculation, and drying, if required, the device is prepared and processed according to the sterilant or sterilizer manufacturer's instructions.

4.4 Following the sterilization process, the test devices are sampled using specified elution techniques to recover any surviving spores.

5. Significance and Use

5.1 The test method is designed to demonstrate that all accessible surfaces and internal recesses or lumina of previously cleaned, reusable medical devices can be rendered free of recoverable microorganisms when processed in a specified sterilizer cycle.

5.2 Surviving spores are recovered by swabbing, brushing, or irrigating with an elution fluid. Recovery methods may be enhanced by mechanical action, sonication, and repeated flushing with elution fluid.

NOTE 1—The spore inoculation technique described in this test method is only one of the available procedures for testing the sterilization of devices. Spores on paper strips (biological indocators) are a traditional tool used to develop and monitor sterilization cycles and are also appropriate for the evaluation of sterilization of medical devices.[5]

6. Apparatus

6.1 *Syringes,* 10 to 50 mL, sterile.

6.2 *Sterile Cotton Swabs.*

6.3 *Sterile Petri Dishes.*

6.4 *Sterile Test Tubes,* to hold 10 mL.

6.5 *Sterile Glass Bottles,* to hold 50 mL.

6.6 *Steam Sterilizer.*

6.7 *Water Bath,* 48 ± 2°C.

6.8 *Incubator(s),* 35 ± 2°C and 55 ± 2°C.

6.9 *Colony Counter.*

6.10 *Medical Device,* precleaned in accordance with the manufacturer's instructions.

6.11 *Disposable or Reusable Membrane Filter Apparatus,* sterile, 0.45-μm pore size.

6.12 *Micropipette,* calibrated to dispense 5 to 20 μL.

6.13 Other devices or apparatus specified by the sterilant, medical device, or sterilizer manufacturer.

7. Reagents

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where

[4] See "Guideline for Industrial Ethylene Oxide Sterilization of Medical Devices" (ST27), AAMI, Arlington, VA, 1992, for typical criteria.

[5] *United States Pharmacopeia*, XXIII, or current edition, Rand McNally, Taunton, MA, 1995, pp. 200–206.

such specifications are available.[6] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type III of Specification D1193.

7.3 *Media:*

7.3.1 *Type III or Better ASTM Water,* for making broths and elution fluids.

7.3.2 *Sterile USP Fluid D*[7]—(elution fluid), containing polysorbate 80 or stripping solution[8] containing 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, and 1.0 g.

7.3.3 *Isooctylphenoxypolyethoxy Ethanol* (Triton X-100), in 1-L distilled water adjusted to pH 7.8.

7.3.4 *Soybean-Casein Digest Broth,* USP, single or double strength, with neutralizers for the test sterilant, as appropriate, and a volume acceptable for each test.

7.3.5 *Soybean-Casein Digest Agar,* USP, single or double strength, with neutralizers for the test sterilant, if appropriate; 10 to 50 mL in tubes or bottles, tempered to 48 ± 2°C.

7.4 *Test Organisms/Spore Suspension:*

7.4.1 For moist heat sterilization, standardized spore suspensions of *Geobacillus stearothermophilus* containing nominally 10^8 CFU/mL and meeting USP resistance criteria for steam sterilization should be used.[9]

7.4.2 For chemical or dry heat sterilization, standardized spore suspensions of *Bacillus atrophaeus* containing nominally 10^8 CFU/mL and meeting USP resistance criteria for ethylene oxide or dry heat sterilization should be used.[8]

7.4.3 For sterilants in which either of the above organisms may be inappropriate, other indicator organisms may be substituted, provided there is substantial evidence, and experimental data to indicate that they are more appropriate.

7.4.4 The origin of the spore strain, production, storage, and expiration dates should be identified.

7.5 *Neutralizers* (as appropriate)—See Practices E1054 for recommended neutralizers.

8. Procedure

8.1 Select the devices to be evaluated.

8.2 Read the cleaning instructions for each medical device to be tested, and ensure that all required accessories are available. Clean and dry the device according to the device manufacturer's instructions.

8.3 Inoculate the device and include the sites most difficult to sterilize. The number of sites may vary with the complexity of the medical device. The rationale used to identify and verify the most difficult to sterilize sites must be documented. Inoculation procedures producing a recoverable count of 10^6 CFU spores per instrument should be used.

8.4 Specific procedures are used for inoculation, elution, control testing, neutralization, growth promotion, and sterility testing.

8.5 *Inoculation of Devices to Determine the Applied Inoculum*—These paragraphs describe the steps for enumerating the CFU of spores inoculated onto the medical device being tested. The spores are not subjected to drying or other treatments, as they might be when determining the recovery control (see 8.6).

8.5.1 *Surface Site Inoculation:*

8.5.1.1 *Micropipetter Method*—Inoculate the surface directly by introducing a volume of spore suspension containing≥ 10^7 spores to the site(s), and distribute over the inoculated site(s) with the tip of the pipet. Immerse the device in elution fluid or rinse immediately, or swab the inoculum into a test tube or other sterile container. Alternatively, recovery can be determined by micropipetting aliquots of suspension directly into tubes of elution fluid and enumerating the mixture. Mix thoroughly by vortexing. Make serial dilutions, and add 1.0 mL of each sample (the original eluate as well as each dilution) to individual tubes containing 20 mL of molten (46 to 50°C) agar, mix, and pour into sterile petri plate. Allow the agar to solidify and incubate at a temperature optimal for spore outgrowth. Examine plates for colonies at 48 h, and re-examine daily for up to 7 days. Determine the mean number of CFU recovered from each device by counting the appropriate plates, and calculate the total CFU recovered from the inoculated device using the dilution factor.

8.5.1.2 *Swab Method*—Moisten a sterile swab with spore suspension, and swab the selected site. The number of spores applied onto the surface should be measured by immediately eluting and swabbing the inoculum from the device. Enumerate the eluate using standard dilution and plating techniques, as noted in 8.5.1.1.

8.5.2 *Internal Site Inoculation*—As appropriate, connect any cleaning or irrigation attachment(s) recommended by the medical device manufacturer, and inoculate by irrigating the internal lumina or recesses with inoculum. Using a sterile irrigation device (syringe, pump, etc.), elute the suspension from the device aseptically by irrigating the internal lumina or recesses with several volumes of elution fluid. Collect all elution fluid, mix thoroughly, and determine the CFU using standard dilution and plating techniques, as noted in 8.5.1.1.

8.6 *Inoculation of Devices to Determine the Recovery Control*—These paragraphs describe the steps for enumerating the CFU of spores recoverable from the medical device after it has been inoculated but just prior to being subjected to a process test cycle.

8.6.1 *Surface Site Inoculation:*

[6] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

[7] *United States Pharmacopeia XXII*, or current edition, "Sterility Tests, Diluting and Rinsing Fluids," Rand McNally, Taunton, MA, 1990, p. 1484.

[8] Williamson, P., "Quantitative Estimation of Cutaneous Bacteria," *Skin Bacteria and Their Role in Infection*, Maibach, H. I., and Hildick-Smith, G., eds., McGraw-Hill, New York, NY, 1965.

[9] *United States Pharmacopeia XXII*, or current edition, "Sterilized Instruments," pp. 1486–1487; "Biological Indicator for Ethylene Oxide Sterilization, Paper Strip," pp. 171–173; and "Biological Indicator for Steam Sterilization, Paper Strip," pp. 173–175, Rand McNally, Taunton, MA.

8.6.1.1 *Micropipetter Inoculation*—The same inoculum and technique(s) used to determine the applied inoculum (see 8.5.1.1) should be used to inoculate the medical device. Dry the inoculum if specified by the manufacturer's instructions for preparation of the instrument prior to sterile processing. Conduct elution and enumeration procedures aseptically, as noted in 8.5.1.1. Average all replicate tests.

8.6.1.2 *Swab Inoculation*—The same inoculum and technique used to determine the applied inoculum (see 8.5.1.2) should be used to inoculate the surface site. Dry the inoculum if specified by the manufacturer's instructions for preparation of the instrument prior to sterile processing. The instrument is evaluated in the condition that the specific process dictates. Conduct elution and enumeration procedures aseptically, as noted in 8.5.1.1. Average all replicate tests.

8.6.2 *Internal Sites*—The same inoculum and technique used to determine the applied inoculum (see 8.5.2) shall be used to inoculate the surface site. Conduct elution and enumeration procedures aseptically, as noted in 8.5.1.1. Average all replicate tests.

NOTE 2—**Caution:** When drying is indicated, small lumina may be difficult to dry.

8.6.3 A minimum of 10^6 recoverable microorganisms from the device being tested is required for the test to be valid.

8.7 *Growth Promotion and Neutralization Controls*—Tests shall be conducted to demonstrate that any chemical inactivators (neutralizers) that may be used to stop the antimicrobial action of the sterilant are not inhibitory to the germination or outgrowth of the test spores. Evaluate using the standard practices set forth in Practices E1054.

8.8 *Process Test Cycle:*

8.8.1 Repeat the steps described in 8.6.1.1, 8.6.1.2, or 8.6.2, without performing any elution steps, and subject the device to a process test cycle. Following the instructions of the sterilant or sterilizer manufacturer, place inoculated instruments in the processing chamber and process.

8.8.2 *Sterility Testing of Processed Devices—Elution, Recovery, and Culturing of Survivors:*

8.8.2.1 Some directly inoculated devices may lend themselves to immersion into growth media. For these devices, aseptically transfer the device directly into growth media, incubate, and examine daily for seven days for evidence of microbial growth.

8.8.2.2 The elution techniques used for determining the recovery control (see 8.6) should be used for recovering any viable spores after processing in the test cycle. The sterility test elution process must be conducted under strict aseptic conditions. All elution fluid is to be cultured. Small volumes, up to approximately 50 mL, can be cultured by adding the eluate to an equal volume of double-strength broth. Larger volumes, such as those generated from the irrigation of devices with internal lumina, should be filtered aseptically through membrane filters and the filters transferred to bottles of appropriate liquid growth medium adequate to immerse the filter. All containers should be incubated at a temperature optimal for the test organism and examined daily for seven days for evidence of microbial growth.

8.8.2.3 If the inoculated site is to be sampled with a swab, moisten a sterile swab with elution fluid and swab the entire inoculated site rigorously. Using aseptic technique, break the swab tip off into 10-mL tube of growth media. Sonicate or vortex to dislodge spores trapped in the swab matrix. Incubate the tubes for seven days, and examine daily for evidence of microbial growth.

8.8.3 Repeat the process test cycle with a minimum of five devices or five times with a single device. Clean, dry (when indicated), and reinoculate the device after each test.

8.8.4 Devices not producing any recoverable challenge microorganisms after seven days of incubation shall be recorded as sterile. If a minimum of five replicate or consecutive tests have not produced growth (are sterile), a device will be considered sterile. Tubes showing growth (non-sterile) should be cultured to identify the test organism before being recorded as non-sterile.

8.8.5 Devices producing one or more sterility failures may not be tested repeatedly until a sequence of five sterility tests is obtained. When a failure occurs, the cause of the failure should be identified, documented, corrected, and then retested for at least five consecutive replicates with no failures.

9. Precision and Bias

9.1 A precision and bias statement cannot be made for this test method at this time.

10. Keywords

10.1 elution; recovery; reprocessing of medical devices; reusable medical device; sterilant; sterilization processes; sterilizer