Designation: E1837 – 96 (Reapproved 2014)

Standard Test Method to Determine Efficacy of Disinfection Processes for Reusable Medical Devices (Simulated Use Test)[1]

This standard is issued under the fixed designation E1837; 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.

INTRODUCTION

When special tests designed to register or validate a disinfection process currently are used, the procedures, their statistical considerations (usually all negatives at a given time point), and the physical problems of applying organisms to surfaces, such as sutures and unglazed porcelain carriers, may cause inaccurate and confusing results. Practical, in-use testing of reprocessing techniques and conditions are needed. Exaggerated conditions for testing can be achieved with the use of actual instruments contaminated with high numbers of organisms. The addition of serum as an organic load or hard water minerals as an inorganic load can be made to enhance worst-case conditions. When these elements are coupled with the processing, as actually performed, the result is a structured test that is a simulated-use procedure. This test method is designed to incorporate several elements of reprocessing, including cleaning, rinsing, and disinfection (including optional treatment of the internal channels of devices, such as endoscopes) with a terminal alcohol rinse rather than examining only the effectiveness of the entire disinfection process. A simulated-use test to examine the effectiveness of reprocessing procedures is valuable because several incidents of contamination of instruments in use have been recorded with vegetative cells of bacteria, for example, *Pseudomonas* and the mycobacteria.

When this procedure is performed with a representative mycobacterial culture, it is necessary to use a nonpathogenic strain such as *Mycobacterium terrae* (isolated from soil) that can be manipulated on an open bench. This strain is used in tuberculocidal testing in Europe, and published information shows comparable resistance to antimicrobials as that displayed by human tuberculosis strains of *Mycobacterium tuberculosis.* This organism can be handled easily and grows faster than other test strains, such as *M. bovis* (**1-5**). [2]

Because contamination of the surfaces of instruments has occurred from rinsing with tap water, bacteria-free water should be used for all rinsing during reprocessing in this test procedure when a water rinse step is part of the reprocessing directions.

1. Scope

1.1 This test method is intended to describe a procedure for testing the effectiveness of a disinfection process for reprocessing reusable medical devices when it is tested with a challenge of vegetative cells including mycobacteria. Disinfection normally deals with testing activity against vegetative cells of bacteria, viruses, and fungi. Since this test method is process oriented, the user may wish to examine a variety of test organisms.

1.2 This test method is designed to provide a reproducible procedure to verify the effectiveness of a previously validated disinfectant or disinfection procedure for reusable medical instruments and devices.

1.3 This test method is not meant to define the effectiveness of or validation of the particular disinfection process used or its kinetics, but rather, it is devised to confirm the effectiveness of the disinfection process by simulating use situations with a particular test process using medical devices and instruments. Either manual or machine reprocessing can be tested.

[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.

Current edition approved Oct. 1, 2014. Published December 2014. Originally approved in 1996. Last previous edition approved in 2007 as E1837 – 96(2007). DOI: 10.1520/E1837-96R14.

[2] The boldface numbers in parentheses refer to the list of references at the end of this standard.

Copyright © ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959. United States

1.4 This test method is intended for use with reusable cleaned and previously sterilized or disinfected (high level) medical instruments and devices. Endoscopes are described in this test method as a worst-case example for contamination and sampling. The selected sterilization or disinfection processes, or both, should have been validated previously, as well as the effectiveness of rinsing for residual sterilant/disinfectant removal determined.

1.5 An inoculum with high numbers of selected microorganisms is applied to both test and control, cleaned and sterilized, or disinfected medical instruments. Strains of microorganisms with a recorded resistance to disinfectants are used to contaminate the instrument sites known or suspected to be the most difficult to reprocess.

1.6 It is impractical to test for recovery of survivors by immersion of some instruments, for example, endoscopes or some laproscopic instruments, in growth medium because of complexity, size, difficulty in long-term incubation, or deterious effects resulting from incubation. Elution of organisms from the inoculated surfaces, therefore, may be performed to estimate the number of recoverable organisms. Immersion can be used for smaller instruments.

1.7 Control instruments are inoculated in the same manner as the test instruments and elution or immersion methods are performed to determine the number of organisms recoverable from the instrument. For channeled devices, such as endoscopes testing, the number of organisms recoverable from the instrument (inside and outside) will serve as the initial control count. It is expected that some fraction of the number of organisms inoculated will be lost in the process of inoculation/drying.

1.8 A testing procedure can be performed on a complete reprocessing cycle or can be limited to just the cleaning or disinfection portions of the cycle whether reprocessing is done in a machine or manually.

1.9 After the test cycle has been completed, remaining inoculated bacteria will be recovered from test instruments using the same elution procedures as for the control instruments.

1.10 Efficacy of a disinfection cycle or reprocessing cycle, or any part thereof, may be determined by comparison of the number of microorganisms recovered from the control instrument (initial recoverable control count) to the recovery determined for the test instruments.

1.11 A knowledge of microbiological techniques is required to conduct these procedures.[3]

1.12 *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.*

[3] CDC-NIH Biosafety in Microbiological and Biomedical Laboratories, 3rd ed., U.S. Department of Health and Human Services, Washington, DC, 1993.

2. Referenced Documents

2.1 *ASTM Standards:*[4]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

3. Terminology

3.1 *Definitions:*

3.1.1 *bioburden, n*—the number and type of viable microorganisms that can be recovered from surfaces using standard recovery procedures.

3.1.2 *CFU*—colony forming units.

3.1.3 *disinfectant*—any biocidal chemical that produces materials free from vegetative microorganisms that may contaminate them and potentially cause infection.

3.1.3.1 *Discussion*—The definitions included are the traditional ones. The user may choose to conform to other criteria that specify elimination of a certain number of test microorganisms. Other definitions describe the action as application of a process resulting in elimination of microorganisms. Whatever criteria is selected, it should be stated before initiation of the test procedure.

3.1.3.2 *Discussion*—A series of three definitions devised by Spaulding **(6, 7)** separated the activity of germicides against spores and mycobacteria and non-lipid viruses and are therefore defined by activity against groups of microorganisms. These definitions are as follows:

(1) High-level disinfectants must inactivate bacteria endospores, mycobacteria, non-lipid viruses, fungi, vegetative bacteria, and lipid viruses. If exposure time is extended long enough, this type of germicide can be used as a sterilant.

(2) Medium-level disinfectants inactivate mycobacteria, vegetative bacteria, fungi including asexual spores, and lipid and non-lipid viruses.

(3) Low-level disinfection inactivate vegetative bacteria, most fungi, and lipid viruses.

3.1.4 *disinfector, n*—any device or physical process that provides a biocidal process that produces materials free from vegetative microorganisms that may contaminate them and potentially cause infection.

3.1.5 *inoculum*—the number (usually expressed in colony forming units, cfu) and type (genus and species) of viable microorganisms used to contaminate a given sample or object. Strain identification and the means used to identify the organism should be indicated.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *accessible site, n*—a location on or in a reusable medical instruments that can be contacted by bioburden and disinfectants.

3.2.2 *reusable medical device, n*—any medical instrument that is claimed at manufacture to be usable after reprocessing.

3.2.3 *worst-case, n*—the intentional exaggeration of one or more parameters of test compared to normal condition.

[4] 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.

4. Summary of Test Method

4.1 This test method is performed by contamination of accessible, interior, and exterior surfaces of instruments or devices intending to reach the sites identified as the leastaccessible or most difficult to reach sites.

4.2 The number of microorganisms contaminating the test instruments or devices prior to processing is determined by contamination and elution of at least two control unprocessed units (representing a large complex instrument). More control units of smaller instruments may be used. Contamination with an inoculum with high numbers of microorganisms to achieve at least 10^6 cfu/instrument, recoverable is required.

4.3 After inoculation, the test instrument(s) are processed according to the manufacturer's instructions for use of the reprocessing cycle, the disinfectant, or disinfector. Either the disinfectant, disinfector cycle alone, or the disinfectant (disinfector) cycle plus any cleaning, rinsing, or other contributory steps in the directions for use, may be tested.

4.4 Following processing, the test instruments are sampled using specified elution and culture techniques to determine the number of surviving bacteria in colony forming units (cfu).

5. Significance and Use

5.1 This test method is designed to demonstrate and document that reusable devices and medical instruments can be disinfected using a specified technique.

5.2 This test method can be used to verify claims of disinfection of recesses, hinged sites, lumina, or other difficult-to-reprocess areas of reusable medical devices and instruments.

5.3 This test method also can be used to document the contribution of each element of the reprocessing cycle for reusable medical devices and instruments.

5.4 The number of surviving bacteria may be assessed using swabbing and irrigation or total immersion.

5.5 This test method may be used to produce quantitative or qualitative results.

6. Apparatus

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

6.2 *Sterile Cotton,* dacron or other swabs.

6.3 *Sterile Petri Dishes.*

6.4 *Sterile Tubes,* to hold 10 mL.

6.5 *Sterile Bottles,* to hold 50 mL and sterile flasks to hold 250 to 500 mL.

6.6 *Steam,* or other type of sterilizer.

6.7 *Water Bath,* to maintain temperatures from 20 to 50 ± 2°C.

6.8 *Incubator(s),* to maintain 35 ± 2°C.

6.9 *Membrane Filters and Filter Supports,* for membrane filters.

6.10 *Colony Counter.*

6.11 *Disposable Plastic Pipettes,* various sizes.

6.12 *Medical Devices or Instruments,* cleaned in accordance with the manufacturer's direction and sterilized or disinfected (high level) prior to use.

6.13 *Devices or Apparatus Specified by the Instrument, Disinfectant or Disinfector Manufacturer.*

6.14 *Vortex Mixer or Sonicator, or Both.*

7. Reagents

7.1 *Media:*

7.1.1 Sterile buffered elution fluid containing 0.1 % Triton X-100 prepared in Type III or better ASTM water. Specific neutralizers for the test disinfectant may be added.

7.1.2 *Soybean-Casein Digest Broth, USP,* with and without appropriate neutralizers for the specific test disinfectant chemical.

7.1.3 *Soybean-Casein Digest Agar, USP,* with and without appropriate neutralizers in 10 to 50-mL tubes or bottles tempered to 50 ± 10°C.

7.1.4 *Middlebrook 7H9 Broth,* with and without appropriate neutralizers in 10 to 50-mL tubes or 250 to 500-mL flask (for mycobacteria).

7.1.5 *Middlebrook 7H11 Agar,* with and without appropriate neutralizers in 10 to 500-mL tubes or bottles tempered to 50 ± 1°C (for *Mycobacteria*).

7.1.6 *Mycophil or Potato Dextrose Agar,* with and without appropriate neutralizers in 10 to 500-mL tubes or bottles tempered to 50 ± 1°C (for fungi). Soybean-Casein Digest Broth or agar, or both, may be used for many fungi.

7.1.7 *Bacteria-Free Water* (when a water rinse step is part of the reprocessing procedures).

7.2 *Test Organisms*—Suspensions of selected test organisms, appropriate for test.

NOTE 1—Strains such as those identified in AOAC disinfectant test procedures or other publications or resistant environmental isolates that have demonstrated resistance to antimicrobials should be selected.

7.2.1 Bacterial cultures are incubated until they reach at least 1×10^8 cfu/mL. For most test strains, this can be achieved with a 48-h suspension of bacterial vegetative cells of test strains prepared in tubes or flasks of appropriate media and incubated at the appropriate optimal growth temperature, ±2°C. Strains of mycobacteria are slow growing and must be incubated for longer times depending on the strain. *M. terrae* is normally countable using magnification and lateral lighting in 21 days, although it may be counted as early as 14 days.

7.2.2 Any test strain which is easy to identify, such as *Serratia marcescens, Escherichia coli, Mycobacterium bovis or Mycobacterium terrae, Pseudomonas aeruginosa, or Staphylococcus aureus*, and has documented disinfectant resistance selected. Suspensions should be grown for sufficient time to achieve a 10^8-cfu/mL level.

7.3 *Neutralizers* (appropriate for test system)—For neutralizer selection and testing refer to Practice E1054. Neutralizers may be added to dilution fluids.

8. Procedure

8.1 Use pre-cleaned and pre-sterilized or pre-disinfected instruments or devices.

8.2 *Inoculation of Devices (Endoscopes or Other Reusable Instruments):*

8.2.1 The suspensions are used as grown for inoculation. The approximate count may be estimated spectrophotometrically or by using routine plating procedures with appropriate agar media. This is recommended to check that a high-density suspension will supply an inoculum that will allow recovery of 10^6 cfu or higher from the instrument/device or selected sites, or both prior to initiation of the test. Other bacterial species, fungi, or viruses, may be used for inoculation of instruments by selecting appropriate media and growth conditions for production of a suspension. Soybean-casein digest media are appropriate for a wide range of bacterial species including many of those previously listed. Middlebrook 7H9 is an appropriate medium for production of a suspension of most mycobacteria.

8.2.2 For endoscopes or channeled instruments, use the prepared microbial suspensions to inoculate the exterior surface with saturated swabs or a micropipette tip, or both. Inoculate the internal surfaces and lumina of endoscopes and similar instruments with a needleless hypodermic syringe using 10 mL of microbial suspension applied to the channel opening. If necessary to obtain a higher number of microorganisms adhering to the surfaces, the suspension can be recovered as it flows through the distal-tip, reconstituted with inoculum to 10 mL and applied. Replication of the inoculum procedure three times as described has been used successfully (**8**).

8.2.3 When other less-complex instruments are inoculated, inoculum should be applied to areas identified as difficult to reprocess, yet locations reachable with a liquid inoculum.

8.2.4 The location of these inoculated sites should be marked on the selected instruments under test and recorded.

8.2.5 After inoculation, instruments are held for 30 min at ambient temperature to permit some drying. Instruments with extensive tubing, such as endoscopes, should be hung so that the inoculum does not pool.

8.3 *Elution and Recovery Techniques:*

8.3.1 *Quantitation of Inoculum and Surviving Organisms:*

8.3.2 *External Surface Sites*—For accessible instrument surfaces, such as the external surfaces of endoscopes, a sterile swab moistened with elution recovery fluid is rubbed vigorously over the entire inoculated surface. This procedure then is repeated using a new swab. Both swabs are placed in 10 mL of recovery solution and mixed on a vortex mixer or sonicated for 7 min to remove the bacteria from the swab. The number of microorganisms recovered may be determined by dilution of the recovery fluid, preparation of serial 10-fold dilutions, and addition of 1-mL samples of dilutions to 20 mL of molten agar (46–50°C) poured into sterile petri plates. Triplicate plates are prepared for each dilution. The remaining elution fluid is added to an equal volume of double-strength agar and poured into petri plates. Allow agar to solidify and incubate for 48 h or the time appropriate for enumeration of the test organism.

8.3.2.1 Alternatively, the number of microorganisms recovered from the elution fluid may be assessed using membrane filters (recommended for mycobacteria). The appropriate dilution of the elution fluid is placed on a pre-wetted (with sterile saline or phosphate buffer) filter and the vacuum turned on. The filter is washed with sterile saline or phosphate buffer with appropriate neutralizers, when required. The membrane filter is placed onto the surface of the appropriate solid agar surface in a petri plate. Enumerate after incubation for the appropriate time and temperature for the specific test organism.

8.3.3 *Internal Sites*—To recover survivors from a lumen or internal recess, irrigate aseptically with a volume of elution fluid equal to at least three times the void volume. Repeat this irrigation with fresh solution three times collecting all the fluid in the same container. Mix the tube of elution fluid with a vortex mixer and prepare serial 10-fold dilutions and subculture and enumerate as previously described. Alternatively, dilutions may be cultured for survivors using membrane filtration described in 8.3.2.

8.3.4 After the incubation period, the number of colonies recovered from each instrument or test site(s) is determined by counting appropriate sets of triplicate plates, except where membrane filtration is used for recovery, and calculation of the number of colony forming units (cfu) in the original sample of elution fluid. The total number of organisms recoverable from the control instruments also is estimated with these procedures.

8.4 *Recovery Control*—The total recoverable inoculum added to the test instruments or devices is determined by using the elution, recovery, and quantitation techniques described in 8.3.2 – 8.3.4. In order for the test to be valid, an average of $\geq 10^6$ cfu/instrument must be recovered.

8.5 *Cleaning/Preparation Cycle*—The contribution of the cleaning and preparation of the test instruments and devices can be estimated by performing a reprocessing cycle on the selected instrument or device without the claimed disinfectant process as described in 8.2.

8.6 *Complete Test Cycle with Disinfectant*—If the effectiveness of a complete test cycle with all elements is required, perform that complete test cycle using the instrument manufacturer's or the disinfectant manufacturer's direction for disinfection of the instrument or devices inoculated with the selected test organism. Deviations from routine processing directions also may be tested. Elute and quantitate the surviving organisms with the preceding elution recovery techniques.

8.7 *Comparative Quantitative Data*—The effectiveness of the disinfectant/disinfector or reprocessing cycle can be evaluated by comparing the number of organisms recovered from the control instruments (8.4) and the test instruments (8.5 or 8.6). The recoverable control count in cfu should be 10^6 or greater.

8.7.1 The number of organisms surviving on the test instrument will depend on the nature of the test organism and the level of disinfection targeted in the protocol. As stated in the definitions, the user may use a variety of criteria for disinfection, but must specify the criteria when the test is initiated. Occasional survivors in a test may represent normal variation around zero.

8.8 *Replication of Test*—More than one determination of effectiveness in this test method is required. Often, if the test instruments are small and are reprocessed in a reservoir of disinfectant or similar situation, a larger number of test instruments, usually to fill the reprocessing chamber should be used. When instruments are large, complex, or intricate, the

number of test instruments and the number of replications must be adjusted. A minimum of five complex test instruments should be evaluated whether one replicate with five instruments or five replicates with one instrument are used.

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 disinfectant; disinfection processes; disinfector; elution; high-level disinfectant; recovery; reprocessing; reusable medical instrument

REFERENCES

(1) Van Klingeren, B., and Pullen, W., "Comparative Testing of Disinfectants Against *Mycobacterium tuberculosis* and *Mycobacterium terrae* in a Quantitative Suspension Test," *J. Hosp. Infect.* , 10, 1987, pp. 292–298.

(2) Collins, F. M., "A Kinetics of the Tuberculocidal Response in Solution on an Inert Surface," *Journal of Applied Bacteriology*, 6, 1986 , pp. 87–93.

(3) Carson, L. A. and Peterson, N. J., "Growth Characteristics of Atypical Mycobacteria in Water and Their Comparative Resistance to Disinfectants," *Applied and Environmental Microbiology*, 36, 1978 , pp. 839–846.

(4) Best, J., Sattar, S. A., Springthorpe, V. S., and Kennedy, M. E., "Comparative Mycobactericidal Efficacy of Chemical Disinfection in Suspension and Carrier Tests," *Applied and Environmental Microbiology*, 34, 1988, pp. 2956–2858.

(5) Collins, F. M., "Bactericidal Activity of Alkaline Glutaraldehyde Solution Against a Number of Atypical Mycobacterial Species," *Journal of Applied Bacteriology* , 61, 1986, pp. 247–251.

(6) Spaulding, E. H., Chemical Disinfection of Medial and Surgical Materials. In Lawrence, C. A. and Block, S. S., *Disinfection, Sterilization and Preservation*, Lea and Febiger, Philadelphia, PA, 1968.

(7) Alvarado, Carla J., "Revisiting the Spaulding Classification Scheme," In *Chemical Germicides in Health Care*, Ed: W. A. Rutala, Polyscience Publications, Inc. Marin Heights, P.Q. Canada, 1994.

(8) Sasa, H. and Goldstine, S. N., *Reprocessing Flexible Endoscopes*, presentation at AAMI/FDA meeting on Reprocessing Reusable Devices, Washington, DC, April 6–8, 1996.

ASTM International takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, at the address shown below.

This standard is copyrighted by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States. Individual reprints (single or multiple copies) of this standard may be obtained by contacting ASTM at the above address or at 610-832-9585 (phone), 610-832-9555 (fax), or service@astm.org (e-mail); or through the ASTM website (www.astm.org). Permission rights to photocopy the standard may also be secured from the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923, Tel: (978) 646-2600; http://www.copyright.com/