Designation: E3218 – 21

Standard Test Method for Quantitative Method for Testing Antimicrobial Agents against Spores of *C. difficile* on Hard, Nonporous Surfaces[1]

This standard is issued under the fixed designation E3218; 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 standardized approach to quantitatively determine the effectiveness of antimicrobial chemicals in treating hard, non-porous surfaces contaminated with spores of *C. difficile* (ATCC 43598) grown in accordance with Practice E2839.

1.2 This test method is based on principles established for Test Method E2197 and an Organisation for Economic Co-operation and Development Guidance Document.[2]

1.3 Training in basic microbiology and aseptic technique are required to perform this assay.

1.4 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

A967 Specification for Chemical Passivation Treatments for Stainless Steel Parts

E2197 Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E2839 Practice for Production and Storage of Spores of ***C. difficile*** for Use in Efficacy Evaluation of Antimicrobial Agents

2.2 *Other Standards*[2]

OECD Guidance Document Quantitative Method for Evaluating Bactericidal Activity of Microbicides used on Hard Non-Porous Surfaces. Dated June 21, 2013.

3. Terminology

3.1 *Definitions*—For definition of terms used in this test method refer to Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *frozen spore suspension, n*—A preparation of bacterial endospores that is maintained at -80 °C ± 5 °C.

3.2.1.1 *Discussion*—For the purposes of this test method, the definition applies to spore suspensions of *C. difficile* that have been prepared and qualified in accordance with Practice E2839.

3.2.1.2 *Discussion*—Spore suspensions of *C. difficile* used in this test method may be stored for up to 90 days under the conditions provided in the definition.

3.2.2 *final test suspension, n*—thawed frozen spore suspension (3.2.1) including the addition of a soil load.

3.2.3 *test system control, n*—a solution of 1500 ppm ± 150 ppm laboratory-grade sodium hypochlorite (NaOCl) used to validate each efficacy test.

3.3 *Acronyms:*

AISI = American Iron and Steel Institute
CFU = Colony-forming unit

4. Summary of Test Method

4.1 The method uses disks (1 cm in diameter) of brushed stainless steel to represent hard, non-porous surfaces.

4.2 Each disk receives 10 µL of the final test suspension.

[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] Available from the Organisation for Economic Co-operation (OECD) 2, rue André Pascal 75775 Paris Cedex 16, France, www.oecd.org

[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.

***A Summary of Changes section appears at the end of this standard**

4.3 The final test suspension is dried and exposed to 50 µL of the test chemical (treated carriers) or 50 µL of a control fluid (control carriers). The contact time is allowed to elapse and an appropriate neutralizer is added at the end of the contact time.

4.4 The neutralized carriers are vortexed and the resulting suspension is serially diluted and filtered to determine the presence of spores.

4.5 Based on mean $\log_{10}$ density values, the $\log_{10}$ reduction (LR) in the viability of the test organism on treated carriers is calculated in relation to the viability count on the control carriers.

4.6 With each efficacy test, three inoculated carriers are exposed to a treatment consisting of 50 µL of 1500 ppm ± 150 ppm NaOCl. The mean LR (<3.0) in the viability of the spores on test system control carriers ensures the validity of the data.

5. Significance and Use

5.1 The test method was designed to determine the LR in spores on a hard, non-porous surface after exposure to a test chemical in a closed system.

5.2 Each test includes three control carriers (exposed to phosphate buffered saline with Tween-80), three test system control carriers (exposed to 1500 ppm ± 150 ppm sodium hypochlorite), and ten treated carriers (per test chemical/concentration/contact time combination).

6. Interferences

6.1 *Clostridioides difficile* (ATCC 43598), formerly known as *Clostridium difficile*, is an obligate anaerobe and must be incubated under strict anaerobic conditions. *C. difficile* will not grow in the presence of oxygen. Verification of anaerobic conditions is required.

7. Apparatus

7.1 Carriers, flat disks (1 cm in diameter) made of AISI Type 304 Stainless Steel with 150 grit unidirectional finish on one side.

NOTE 1—The precision and bias statement provided in Section 12 was based on testing performed using AISI 430 stainless steel carriers; the precision statistics summarized in Section 12 do not apply for testing performed using other alloys.

7.1.1 The top of the carrier is brushed; only the top is visually screened and inoculated. Carriers are single-use only. See Annex A1 for carrier specifications.

7.2 *Calibrated 10 µL positive displacement pipette with corresponding 10 µL tips,* for carrier inoculation.

7.3 *Calibrated micropipettes (for example, 200 µL) with 10-100 or 20-200 µL tips,* for preparing aliquots of soil and spores; for deposition of test chemical on carrier.

7.4 *Bottle-top dispensers, squirt bottles, pre-measured volumes in tubes, or pipettes,* for rinsing vials and filters.

7.5 *Sterile forceps,* to pick up the carriers for placement in vials and to handle membrane filters.

7.6 *Filter paper,* 150 mm diameter, to line Petri plates.

7.7 *Polyethersulfone membrane filter (PES),* for recovery of test spores, 47 mm diameter and 0.2 µm pore size. Any filtration apparatus may be used including filtration units (reusable or disposable).

7.8 *Vials with lids (plastic or comparable),* sterile, flat-bottomed, wide-mouthed (at least 25 mm diameter), approximately 20 mL capacity, for holding inoculated carriers to be exposed to the test chemical and for accommodating neutralizer.

7.9 *Vortex mixer,* to vortex the fluid in the vials to ensure efficient recovery of the test organism.

7.10 *Certified timer,* readable in minutes and seconds.

7.11 *Desiccator,* (with gauge to measure vacuum) with fresh desiccant (for example, $CaCO_3$), for drying the inoculum on the carriers.

7.12 *Vacuum source,* in-house line or suitable vacuum pump (0.068 to 0.085 MPa) for drying carriers and for membrane filtration.

7.13 *Microscope,* with 10× eyepiece and 100× (oil) objective with phase contrast option. To examine spores.

7.14 *Anaerobic chamber,* supported by a gas mixture containing at least 5 % H_2 with the balance comprising any inert gas such as CO_2, N_2, or Ar; refer to chamber manufacturer's recommendations. Use to ensure anaerobic environment.

NOTE 2—An activated anaerobic jar or other chamber may be used according to manufacturer's instructions in place of the anaerobic chamber.

7.15 *Incubator,* use an incubator at 36 ± 1 °C inside an anaerobic chamber to support the growth of the organism. Alternatively, place anaerobic jars in an incubator at 36 °C ± 1 °C.

7.16 *Digital titrator kit,* to measure total chlorine and water hardness. Alternate titration methods may be used.

7.17 *Laboratory film or sterile bags (18 by 30 cm or equivalent),* to retain the moisture in plates during prolonged incubation in an anaerobic chamber.

8. Reagents and Materials

8.1 *Culture Media:*

8.1.1 *Recovery medium*—brain-heart infusion agar with yeast extract, horse blood and sodium taurocholate (BHIY-HT).[4] For enumeration of spores, commercially available as pre-reduced.

8.2 *Reagents:*

8.2.1 *Water*—all references to water as diluent or reagent shall mean de-ionized water or water of equal purity.

8.2.2 *Phosphate buffered saline (PBS)*—for use as a rinsing agent and to prepare PBS containing 0.1 % (v/v) Tween-80 (PBS-T) and PBS-T with 0.1 % (w/v) sodium thiosulfate; adjust pH to 7.0 ± 0.5 if necessary.

[4] The sole source of supply of the BHIY-HT (Cat. No. AS-6463) known to the committee at this time is Anaerobe Systems, Morgan Hill, CA. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.