Designation: E3160 – 18

Standard Test Method for Quantitative Evaluation of the Antibacterial Properties of Porous Antibacterial Treated Articles[1]

This standard is issued under the fixed designation E3160; 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 To determine the bactericidal or bacteriostatic properties of porous articles treated with an active biocidal agent, samples of porous treated materials, such as textiles or paper, are inoculated with a defined suspension of microorganisms and then incubated. The changes in numbers of the bacterial populations on the treated article are compared with untreated articles either over designated time or they are compared to the initial bacterial population at "zero time" for the treated article to measure antibacterial properties.

1.2 This test method is used for measuring the quantitative antibacterial activity of porous materials that have been treated with a biocide to inhibit the growth of bacteria on the treated materials. This method may also be used to measure the ability of the treated material to inhibit the growth of a microorganism. It can measure both bactericidal and bacteriostatic activity.

1.3 This test method shall be performed by individuals experienced and adept in microbiological procedures and in facilities suitable for the handling of the microorganisms under test.

1.4 This test method may involve hazardous materials, operations, and equipment. *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.*

1.5 *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:*[2]

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2180 Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials

E2149 Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E2922 Guide for The Use of Standard Test Methods and Practices for Evaluating Antibacterial Activity on Textiles

2.2 *AATCC (American Association of Textile Chemists and Colorists) Documents:*[3]

AATCC TM100 : Antibacterial Finishes on Textile Materials: Assessment of:

2.3 *ISO (International Organization for Standardization) Documents:*[4]

ISO 22196 Plastics – Measurement of Antibacterial Action on Plastic Surfaces.

ISO 20743 Textiles – Determination of Antibacterial Activity of Antibacterial Finished Products

2.4 *IBRG (International Biodeterioration Research Group) Documents*[5]

IBRG TEX13/005/1.0 Quantitative Method for Evaluating Bacterial Activity of Textiles and Porous Material and Articles

[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] 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] Available from American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709-2215, http://www.aatcc.org.

[4] Available from International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

[5] Available from IBRG, Pale Lane, Hartley Wintney, Hants, UK RG27 8DH, http://www.ibrg.org.

3. Terminology

3.1 *Defintions*—For definition of terms used in this test method, refer to, E2756 Standard Terminology Relating to Antimicrobial and Antiviral Agents.

4. Summary of Test Method

4.1 A liquid suspension of bacteria is applied to porous materials both untreated and treated with antimicrobial finishes.

4.2 Samples of each treated or untreated material are inoculated with a specified concentration of bacteria suspended in a solution containing a defined concentration of nutrients.

4.3 The inoculated materials are then incubated under conditions of controlled temperature and humidity for a specified period of time.

4.4 After incubation, the samples are immersed in a neutralizer suitable for deactivating the active substance(s) used to produce the intended antimicrobial effect, and agitated to remove surviving organisms.

4.5 The number of colony forming units present in the resulting suspension is then determined using standard plate counting techniques.

4.6 Changes in the number of the test organism are then calculated in relation to the numbers present on the untreated materials after a specific contact time or in relation to the numbers applied (inoculum count), or both.

5. Significance and Use

5.1 Porous articles (often textiles) are often treated with antimicrobial agents to reduce the growth of microorganisms during use, in storage, or while waiting to be laundered, or both. Additionally, antimicrobial agents are added to reduce or control the overall microbial growth on porous articles that may affect the material's odor, visual, chemical or physical integrity, or both.

5.2 Antimicrobial textile test methods that measure the antimicrobial behavior of treated textiles do exist but they are often specific for one type of antimicrobial agent or are designed to or may artificially (not expected in real life) promote the release of some specific antibacterial agents over others. This test method is designed to be able to measure the antimicrobial activity from all common antimicrobial agents used to treat porous articles, including textiles, without giving either positive or negative bias to one type of chemistry or product over another.

5.3 In an effort to avoid excessive use or abuse of antimicrobial agents in the environment, it is important to understand if untreated porous articles are susceptible to microbial contamination and growth. In this test method, a small amount of nutrients is added to each test sample in order to promote some microbial growth on susceptible test samples but not enough to overwhelm potential antimicrobial agents that may be effective in real life situations. Furthermore, low levels of nutrients allow investigators to add soiling agents that may be more reflective of a specific treated product's end use or expected performance.

5.4 Very specific parameters are identified within this method to limit any variability that may be seen between laboratories. Identifying and clarifying potential variables found in other guides or methods used in the industry will allow for better reproducibility and repeatability between and within laboratories.

5.5 This test method provides the foundation for conducting tests on porous antibacterial treated articles. Modifications of this method that simulate intended use, durability and compatibility of the treated article should be outlined to ensure an accurate assessment of antimicrobial activity with each particular biocide that substantiates end use claims made for the article. A list of these typical modifications and current test methods for textiles can be found in Guide E2922.

5.6 This test method is appropriate for porous materials such as textiles, paper, or similar porous materials. It is intended to measure the antibacterial properties of such materials. In most instances, further studies will be required to support and substantiate actual claims being made for the performance of treated materials in practice or as part of a regulatory process.

5.7 This test method or indicated modifications may be used to determine antimicrobial activity as indicated in 5.6 or may be used as a routine bioassay in standard quality control programs.

6. Apparatus

6.1 *analytical balance*—with capacity and precision of 0.01 to 10 g ±0.001, respectively, to weigh chemicals and to calibrate inoculum delivery volumes by pipettes.

6.2 *biological safety cabinet*—suitable for the containment of the test organisms used.

6.3 *Bunsen burner*—with a gas source and flame igniter.

6.4 *colony counter*—(optional.)

6.5 *dispensers*—for dispensing sterile 10-ml aliquots of diluent/neutralizer.

6.6 *forceps*—sterile for handling treated articles.

6.7 *freezers*—a freezer at -20 ±2 °C for the storage of media and additives. A second freezer at -70 °C or lower to store the stocks of test organisms (optional).

6.8 *glass rods*—sterile. for use in holding porous samples in place. Rods should be no more than 40 mm in length.

6.9 *gloves*—sterile, disposable, for handling test items.

6.10 *hot air oven*—an oven at 60 ± 2 °C to dry clean and wrapped sterile glassware.

6.11 *incubator*—an incubator to maintain a temperature of 35 ± 2 °C and maintain a relative humidity of not less than 80 %.

6.12 *inoculating loops*—Sterile plastic or sterilizable metal inoculating loops (10-µl).

6.13 *magnetic stir plate and stir bars*—large enough for a 5-L beaker or Erlenmeyer flask for preparing culture media or other solutions.

6.14 *sterile (plastic) or sterilizable petri dishes*—100 × 15 mm for microbial growth and recovery media.

6.15 *pH meter*—having an accuracy of ±0.1 pH units to measure pH of media and suspensions. A puncture electrode or a flat membrane electrode should be used for measuring the pH of agar media.

6.16 *sterile or sterilizable pipette and tips (electronic or non-electronic positive displacement)*—100 to 1000-µl pipette and appropriate pipette tips fitted with "plungers" that can dispense accurately 200-µl.

6.17 *refrigerator*—4 ± 2 °C; for storage of media, culture plates and reagents.

6.18 *sterile or sterilizable serological pipettes*—reusable or single-use pipettes of 1.0, 5.0 and 10.0-ml capacity (optional).

6.19 *sterilizer (autoclave)*—any steam sterilizer suitable for processing culture media, reagents and labware; the steam supplied to the sterilizer should be free from additives toxic to the test organisms.

6.20 *sterile or sterilizable vials or tubes for dilution*—suitable to hold 30-ml easily.

6.21 *sterile containers for sample inoculation and incubation*—4 oz, screw-on lid, sterile, disposable and individually sealed specimen cups with a bottom surface diameter of 45 mm have been shown to be suitable.

6.22 *vortex mixer*—to mix the cell suspensions and neutralizer suspensions to ensure efficient recovery of the test organism(s).

6.23 *water bath*—capable of reaching and maintaining a temperature of 45 ± 2 ºC to keep agar media from solidifying when making culture plates.

6.24 *incubator shaker*—an orbital incubator shaker capable of agitating broth cultures of bacteria and able to maintain a temperature of 35 ± 2 °C.

6.25 *test validity control substrate*—a suitable control material has been found to be a cellulosic filter paper such as Whatman #4 or any textile substrate shown to promote at least 1 log CFU/g of bacterial growth under the parameters outlined below.

NOTE 1—Sterilize all laboratory ware and equipment as appropriate. Sterilization can be achieved by moist heat in an autoclave, by dry heat in a hot-air oven or other appropriate, validated sterilization process. Many of the consumable items used in this guideline can be purchased pre-sterilized and ready for use. Sterility of all ware and equipment should be confirmed prior to use.

7. Reagents and Materials[6]

7.1 *Sterile Tryptic Soy Broth,* (TSB).

7.2 *Sterile Tryptic Soy Agar,* (TSA).

7.3 *Neutralizing Broth,* appropriate for the antimicrobial compound tested (See Practice E1054).

7.4 *Suspension Medium,* 1/500 TSB plus wetting agent (1/500 TSB). Dilute the TSB (7.1) 1:500 with distilled or deionized water plus 0.05 % v/v Triton X-100 and then adjust the pH to a value between 6.8 and 7.2 with either sodium hydroxide or hydrochloric acid. Sterilize by autoclaving at 121 ±2 ºC. If it is not used immediately after preparation, store it at 4 ± 2 °C.

7.5 *Sterile Distilled or Deionized Water.*

7.6 *Ethyl Alcohol,* for forcep sterilization.

8. Test Organism

8.1 *Escherichia coli*, American Type Culture Collection (ATCC) No. 25922.

8.1.1 Cultures of the test organism should be maintained according to good microbiological practice and checked for purity on a routine basis. Consistent and accurate testing requires maintenance of a pure, uncontaminated test culture. Avoid contamination by use of good sterile technique in plating and transferring. Avoid mutation or reversion by strict adherence to monthly stock transfers. Check culture purity by making streak plates periodically, observing for colonies characteristic of *Escherichia coli*, Gram-staining or performing other forms of microbial identification.

NOTE 2—This method was developed and validated using ATCC No. 25922 as the test organism. If an alternative culture is used, the results must be reported as having been obtained using a *modified* test method. *E. coli* was chosen for this method due to its proven reproducibility in initial laboratory ring tests. If the test method is modified in any way, the report must also include a detailed description of all modifications made, including, but not limited to: test organisms, media, buffer, bacterial concentration, etc.

9. Preparation of Bacterial Inoculum

9.1 Grow a fresh overnight (18-24 h) culture of *E. coli* in sterile 100 % TSB at 35 ±2 °C prior to performing the test on an orbital incubator shaker allowing for maximum aeration of culture. This culture should originate from an 18-24 h growth coming from stock culture plates or growth on agar slants.

9.2 The fresh overnight culture is then diluted with suspension medium (7.4), as appropriate to obtain a bacterial concentration that is between 2.5×10^5 colony forming units (CFUs)/ml and 1.0×10^6 CFUs/mL, with a target concentration of 6×10^5 CFUs/mL. This suspension is used as the test inoculum. The test inoculum shall be used within 2 h of preparation. The number of colony forming units in the initial inoculum can be estimated using direct microscopic observation and a counting chamber or another appropriate method (for example, spectrophotometrically). Actual viable bacterial count is verified by standard plate count.

NOTE 3—In some instances, the performance intended might require the use of elevated concentrations of nutrients (for example, 1/20 TSB in place of the 1/500 dilution specified) or an alternative suspending medium (for example, phosphate buffered saline, bovine serum albumin, yeast extract). The use of these modifications might be required in order to more closely simulate end use conditions. When any such modifications of the method are made, the guidance provided in Note 2 applies.

[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 *Annual Standards for Laboratory Chemicals,* BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary,* U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.