Designation: E3227 – 20

Standard Test Practice for Qualitative Assessment of Antifungal Activity on Textiles[1]

This standard is issued under the fixed designation E3227; 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 practice determines the relative fungal growth inhibition properties of materials treated with an active biocidal agent. Samples of porous treated materials, such as textiles, are inoculated with a defined suspension of fungal conidia or spores and then incubated. The inhibition of growth or visible growth present on treated compared with identical untreated materials is used to measure relative antifungal properties of the treated identical materials.

1.2 This test practice must be performed by individuals experienced and adept in microbiological procedures and in facilities suitable for the handling of the species under test.

1.3 *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.*

1.4 *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]

E2722 Test Method for Using Seeded-Agar for the Screening Assessment of Antimicrobial Activity in Fabric and Air Filter Media

E3152 Guide for Standard Test Methods and Practices Available for Determining Antifungal Activity on Natural or Synthetic Substrates Treated with Antimicrobial Agents

2.2 *AATCC Standard:*[3]

AATCC Test Method 30 – Antifungal Activity, Assessment on Textile Materials: Mildew and Rot Resistance of Textile Materials

3. Significance and Use

3.1 Textiles are often treated with antimicrobial agents to reduce the growth of odor-causing organisms during use, in storage, or while waiting to be laundered, or both . Additionally, antimicrobial agents are used to reduce or control microbial growth on the textile that may affect the material's visual, chemical or physical integrity, or both.

3.2 Anti-fungal test methods that measure antimicrobial behavior on treated textiles or other porous or non-porous substrates do exist (Guide E3152, Test Method E2722, AATCC TM30), but they were developed for either specific types of antimicrobial agents or put under unrealistic conditions such that other agents are disadvantaged or end-use conditions exaggerated.

3.3 This test practice is designed to measure relative antimicrobial activity of all common antimicrobial agents used to treat porous materials such as textiles without positive or negative bias for one type of chemistry or product over another. The practice is designed to more closely simulate conditions that might be experienced in the actual end-use of the porous treated materials (for example, low initial fungal spore exposure and limited available nutrients but with ideal conditions to grow). This practice is designed to demonstrate a significant reduction in visible surface fungal growth on a porous treated material (such as textiles) relative to an identical untreated control.

4. Summary of Practice

4.1 This test practice outlines a procedure for *(1)* preparing a suitable specimen for testing, *(2)* inoculating the specimen with the proper fungal species, *(3)* exposing the inoculated samples under the appropriate conditions for growth, and *(4)* providing a schedule and guideline for visually rating growth. This test practice is not designed to include all the procedures

[1] This test practice 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 Dec. 1, 2020. Published February 2021. DOI: 10.1520/E3227–20

[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.

necessary to maintaining the proper microbiological techniques required for providing the most accurate results.

5. Apparatus

5.1 *Balance,* capable of weighing to 0.10 g.

5.2 *Incubator,* or other device capable of maintaining a constant temperature of 30 °C ± 2 °C and relative humidity of ≥85 %.

5.3 *Refrigerator,* or other device capable of maintaining a temperature of 4 ± 2 °C.

5.4 *Autoclave,* capable of producing 103 kPa (15 psi) of steam pressure at 121 °C and maintaining it for a minimum of 15 min. An autoclave is not necessary if prepared media plates are used.

5.5 *pH meter,* having an accuracy of calibration of no more than ± 0.1 pH units to measure pH of buffers, eluents and test substance.

NOTE 1—A puncture electrode or a flat membrane electrode should be used for measuring the pH of the agar media.

5.6 *Substrate,* Filter Paper Whatman #2 or equivalent.

5.7 *Water Bath,* capable of achieving and maintaining a temperature of 45 ± 2 ºC to prevent agar media from solidifying when making culture plates.

5.8 *20×-40× stereoscope,* (Optional).

5.9 *Counting Chamber,* (Hemocytometer).

6. Materials

6.1 *Dilution tubes, sterile or sterilizable micropipette and tips, sterile or sterilizable serological pipettes, Pasteur pipets, Glass Erlenmeyer Flasks,* and other routine microbiological equipment.

6.2 *Sterile containers for sample inoculation and incubation.* 150 mm × 25 mm petri dishes have been shown to be suitable.

6.3 *Nontoxic wetting agent*—such as sodium dioctylsulfosuccinate (Docusate) or Triton X-100.

6.4 *Sabouraud Dextrose Broth* (SDB).

6.5 *Sabouraud Dextrose Agar* (SDA).

6.6 *Glass wool.*

6.7 *Test Viability control substrate*—a suitable control material has been found to be a cellulosic filter paper or 100 % cotton fabric.

NOTE 2—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.

NOTE 3—Whatman #2 filter paper and 100 % Cotton fabric from the International Antimicrobial council have been shown to be suitable commercially available viability control substrates.

7. Reagents[4]

7.1 *Purity of Reagents*—Reagent grade chemicals should be used in all tests. Unless otherwise indicated, all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided they are first ascertained to be of sufficiently high purity to permit use without decreasing the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water are understood to mean distilled water or water of equal or higher purity.

7.3 SDA plates and SDB tubes can be purchased prepared or the SDA and SDB powders can be purchased and prepared according to the instructions using standard microbiological techniques and equipment.

7.4 *Spore Diluent Solution*—sterile SDB containing 0.05 g⁄L of a nontoxic wetting agent such as dioctyl sodium sulfosuccinate.

8. Test Organism

8.1 *Aspergillus niger,* American Type Culture Collection No. 6275.

8.2 Maintain stock cultures of this fungus on an appropriate medium, such as Sabouraud dextrose agar plates or slants. Stock cultures can be stored for not more than 4 months at approximately 3 to 10 °C (37 to 50 °F). Prior to each experiment, subculture fungus onto slants or plates and incubate for 7 to 20 days at 28 to 30 °C (82 to 86 °F) until conidiation is considered adequate. Use these subcultures in preparing the spore suspension.

8.3 Prepare a spore suspension of the test fungus by pouring into the subculture container 10 mL of sterile water or a sterile 0.05 g/L solution of a nontoxic wetting agent such as sodium dioctylsulfosuccinate. Swirl or gently agitate the slant or plate to loosen the conidia and carefully aspirate the suspension with a sterile Pasteur pipet, avoiding mycelial fragments as much as possible.

8.4 Filter the shaken or ground suspension through a thin layer of sterile glass wool in a glass funnel into a sterile flask in order to remove mycelial fragments.

8.5 The conidial titer of the stock suspension should then be determined using a counting chamber.

8.6 Dilute the stock conidial suspension with diluent solution (6.4) such that the resultant suspension contains 0.8 to 1.2 × 10^5 conidia/mL.

8.7 The stock suspensions can be held in the refrigerator at 3 to 10 °C (37 to 50 °F) for not more than fourteen days.

[4] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, 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. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.