Designation: E2722 – 14

# Standard Test Method for Using Seeded-Agar for the Screening Assessment of Antimicrobial Activity in Fabric and Air Filter Media[1]

This standard is issued under the fixed designation E2722; 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

Fabrics (woven, non-woven) and filters often incorporate a topical or polymer incorporated antimicrobial agent to protect them from mold and bacteria. The American Association of Textile Colorists and Chemists (AATCC) Method AATCC 147–2004 and AATCC 100–2004 permit qualitative and quantitative (respectively) assessment of fabric for antibacterial activity. AATCC 30–2004 is an established antifungal method, qualitative only, of antimicrobial treatments in or on fabric. However, these methods are not well suited for rapid screening of antimicrobials low in water solubility or that have slow diffusion rates when incorporated into a fabric back-coating layer. The standard method described here provides a rapid screen of antimicrobial activity in or on fabric and filter media and does not depend on a zone of inhibition to demonstrate a surface protective effect.

## 1. Scope

1.1 This test method is designed to evaluate qualitatively the presence of antibacterial and antifungal activity in or on fabrics or air filter media.

1.2 Use half-strength (nutrient and agar) tryptic soy agar as the inoculum vehicle for bacteria and half-strength potato dextrose agar as the inoculum vehicle for mold conidia. Use of half-strength agars may reduce undue neutralization of an antimicrobial due to excessive organic load.

1.3 This test method permits evaluation, both visually and stereomicroscopically, of the antimicrobial activity of fabric or filter media.

1.4 Use this test method to assess the durability of the antimicrobial treatments on new fabric or filter media, and on those repeatedly laundered or exposed to in-use conditions.

1.5 This test method may not be suited for covalently bonded (that is, silane-modified quaternary ammonium compounds) or actives with limited migration or solubility.

1.6 Knowledge of microbiological techniques is required for the practice of this test method.

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

1.8 *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:*[2]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *AATCC Documents:*[3]

AATCC 30–2004 Antifungal Activity, Assessment on Textile Materials: Mildew and Rot Resistance of Textile Materials

AATCC 100–2004 Antibacterial Finishes on Textile Materials: Assessment of

AATCC 147–2004 Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method

## 3. Terminology

3.1 For definitions of terms used in this test method refer to Terminology E2756.

[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 Aug. 1, 2014. Published September 2014. Originally approved in 2009. last previous edition approved in 2009 as E2722–09. DOI: 10.1520/E2722–14.

[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, http://www.aatcc.org.

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3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *back-coating, n*—a film (typically synthetic latex) applied to the back side of certain textiles to provide dimensional stability.

3.2.2 *inoculum vehicle, n*—carrier solution used to transport bacterial cells or mold conidia to the test substrate.

3.2.3 *seeded agar, n*—molten (liquid) microbiological agar containing either bacterial cells or mold conidia (spores) used to challenge a test substrate.

3.2.4 *swatch, n*—a small sample of fabric of a defined size.

## 4. Summary of Test Method

4.1 Using flame-sterilized scissors, cut fabric or media samples into 9 $cm^2$ swatches. Arrange the swatches in sterile petri dishes. Cool molten agars to 45 ± 2°C and inoculate with the challenge bacteria or mold conidia. Following wrist-action mixing, immerse swatches into the seeded-molten agar, allow excess agar to drain from the sample, and then place the swatch into a petri dish. Pipette 2 mL of seeded agar at the perimeter of the petri dish. This will be used as a viability control. Incubate the petri dish for 48 to 72 h at 30 ± 2°C. Visually and microscopically examine at the surface of the fabric swatch for inhibition of the challenge microorganisms. Report the presence and degree of fabric surface inhibition.

## 5. Significance and Use

5.1 This test method provides for rapid screening of antimicrobial treatments located in or on fabrics and air filter media.

5.2 This test method simulates actual use conditions that may occur on fabrics, for example, food and beverage spills; soiling from body contact, that is, body oils, skin cells; prolonged moisture exposure.

5.3 This test method provides a means to screen for activity and durability of an antimicrobial treatment under conditions of organic loading.

5.4 This test method provides for the simultaneous assessment of multiple fabric components, for example, fabric, component fibers with polymer incorporated treatments, and back coating if present, for antimicrobial activity.

5.5 Fabrics or filter media may be cleaned prior to testing with this method in order to assess the durability of the antimicrobial effect.

## 6. Apparatus

6.1 *Stereomicroscope,* (10× to 70× objectives).

6.2 *Erlenmeyer flasks,* 250 mL.

6.3 *Petri dishes,* 150 mm, sterile.

6.4 *Incubators,* set at required temperatures (30 ± 2ºC and 36 ± 1°C)

6.5 *Autoclave.*

6.6 *Water bath,* capable of maintaining water at 45 ± 2°C

6.7 *Test tubes,* 16 by 100 mm.

6.8 *Hot Plate,* with stirrer.

6.9 *Spectrophotometer.*

6.10 *Sterile cuvettes.*

6.11 *Test fabric.*

6.12 *Flame-sterilized scissors.*

6.13 *Petri dishes,* 100 mm, sterile.

6.14 *Sterile funnel,* with a glass wool plug.

6.15 *Counting chamber (hemocytometer).*

6.16 *Light microscope,* (10× and 40× objectives).

6.17 *Disposable latex examination gloves.*

6.18 *Flame-sterilized forceps or hemostats.*

## 7. Reagents and Materials

7.1 *Media:*

7.1.1 Tryptic soy broth or nutrient broth.

7.1.2 Tryptic soy agar or nutrient agar.

7.1.3 Potato dextrose agar.

7.1.4 Sterile 0.85 % saline with 0.1 % Tween 80.

7.2 *Test Organisms*—Specific species are recommended, however, other microorganisms may be used to mimic those found in a specific environment, or those expected contaminants which may be present where the fabric is expected to perform.

7.2.1 Gram-positive species *Staphylococcus aureus* ATCC 6538.

7.2.2 Gram-negative species *Serratia marcescens* ATCC 14756.

7.2.3 Fungus: *Aspergillus brasiliensis* ATCC 9642 (deposited as *Aspergillus niger*).

## 8. Procedure

8.1 Grow 18 h tryptic soy broth cultures of *Staphylococcus aureus* at 36 ±1°C and *Serratia marcescens* at 30 ± 2°C. These cultures should originate from 18 to 24 h growth stock culture plates or agar slants.

8.2 Prepare a suspension of fungal conidia by harvesting conidia from a 2-week-old stock culture plate or slant incubated at 30 ± 2°C. Pour sterile 0.85 % saline with 0.1 % Tween 80 (see Note 1) over the fungal mat, agitate the liquid with a sterile glass rod, and filter out hyphal fragments by pouring the suspension through a sterile funnel plugged with glass wool.

NOTE 1—Other surfactant agents may be chosen provided that they are non-damaging to the fungal conidia, and that they do not chemically neutralize the antimicrobial of interest. Test Methods E1054 may be used to assess for neutralization potential.

8.3 Prepare 200 mL lots of half-strength tryptic soy (20 g/L), or nutrient, and half-strength potato dextrose agars (19.5 g/L) in 250 mL erlenmeyer flasks and autoclave. Cool the molten agars to 45 ± 2°C in a water bath. To minimize cross-contamination and antimicrobial treatment leachate, separate agar lots should be prepared for each fabric type or fabric treatment tested.

8.4 Cut fabric or filter media samples to form 3.0 by 3.0 cm swatches. A minimum of triplicate swatches should be used for each challenge organism.

8.5 Place samples into 150 mm petri dishes such that they do not touch one another. Each dish should contain replicates of the same sample and a control (if used).

8.6 Standardize the bacterial inoculum to 1-2×$10^7$ CFU/mL.

8.7 Standardize the suspension of fungal conidia to 1-2×$10^6$ CFU/mL.

8.8 Inoculate 200 mL lots of cooled, half-strength (45 ± 2°C) tryptic soy agar, or nutrient agar, with 2.0 mL of standardized bacterial inoculum (final cell density 1-2×$10^5$ CFU/mL). Wrist-action mix (manually) the agar for 30 s.

8.9 Inoculate 200 mL lots of cooled, half-strength (45 ± 2°C) potato dextrose agar with 2.0 mL of fungal conidia suspension (final conidial density 1-2×$10^4$ CFU/mL). Wrist-action mix the agar for 30 s.

8.10 Immerse each sample into the half-strength seeded agar using flame sterilized forceps. Allow excess agar to drain from the swatch or wring out the swatch on the inner neck of the flask. Then place each sample into the petri dish as described in 8.5. Fabric samples with varying concentrations of the same antimicrobial treatment should be immersed from low to high concentration to minimize carry over and buildup in the agar.

8.11 Drop 2 mL of seeded agar at the perimeter of the petri dish using a sterile pipette and pipette tip. This is to be used as the viability control.

8.12 Allow the seeded agar to gel at the perimeter of the petri dishes (10 min).

8.13 Incubate all samples at 30 ± 2ºC for 48 to 72 h. An open 35 mm diameter tissue culture dish containing sterile deionized water may be placed beside the test specimens (inside the 150 mm dish) in order to maintain relative humidity during the test.

## 9. Report

9.1 The report shall contain the following elements:

9.1.1 Report gross examination of the fabric for direct surface inhibition at 48 and 72 h.

9.1.2 Report the results of a stereo-microscopic (10 to 30× magnification) inspection. Examine the surface of the fabric. Compare the observations to a non-treated control fabric or the viability control area located at the perimeter of the petri dish.

9.1.3 Key for reporting the presence and degree of bacteria or mold inhibition by the treated fabric samples is as follows:

9.1.3.1 NI = bacterial or fungal growth on the sample; no inhibition when compared to controls.

9.1.3.2 CI = no bacterial or fungal growth directly on the surface on the sample; complete inhibition of the challenge microorganism when compared to controls.

9.1.3.3 PI = partial inhibition of the bacterial or fungal growth directly on the sample. Partial inhibition at 72 h is rated qualitatively as:

| | |
|---|---|
| Low | >50 but less than 100 % coverage of the sample |
| Medium | 10 to 50 % coverage of the sample |
| High | <10 % coverage of the sample |

9.1.4 Morphological confirmation of challenge mold via tape mount and examination with light microscopy at 400× magnification is useful in the case of non-sterile samples.

## 10. Precision and Bias

10.1 Highly lofted fabrics or filter media or those constructed of wool, cotton, and hemp may absorb an excess volume of the seeded agar making them less likely to demonstrate meaningful surface layer inhibition.

10.2 Natural fiber fabrics may have inherent bioburdens and may influence results obtained for the recommended challenge microorganisms. In these cases, autoclaving or irradiation of the fabric should reduce or eliminate contaminants.

## 11. Keywords

11.1 antimicrobial; bacteria; fabric; filter media; fungi; low solubility preservative; mold; polymer incorporated preservative; textile

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