Designation: E2471 – 05 (Reapproved 2016)

Standard Test Method for Using Seeded-Agar for the Screening Assessment of Antimicrobial Activity In Carpets[1]

This standard is issued under the fixed designation E2471; 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

Today's modern commercial carpets (especially modular carpet tile) often incorporate antimicrobial agents either in or on the face fibers or incorporated into the primary backing (attachment point of carpet fiber to the backing structure). The American Association of Textile Chemists and Colorists (AATCC) Method 174 permits both qualitative and quantitative antibacterial assessment and antifungal assessment (qualitative only) of antimicrobial treatments in or on carpet. However, the method is not suited for rapid screening of antimicrobials low in water solubility or that have slow diffusion rates when incorporated into the carpet's primary backing layer. The test method described here provides a rapid screen of antimicrobial activity in or on carpets and allows for the simultaneous assessment of multiple components of the carpet (not just the fibers).

1. Scope

1.1 This test method is designed to evaluate (qualitatively) the presence of antimicrobial activity in or on carpets. Use this test method to qualitatively evaluate both antibacterial and antifungal activity.

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 simultaneously evaluates (both visual and stereo-microscopic) antimicrobial activity both at the fiber layer and at the primary backing layer of carpet.

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

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

1.6 *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 *American Association of Textile Chemists and Colorists (AATCC) Standard:*

Method 174-2007, Antimicrobial Activity Assessment of Carpets[2]

3. Terminology

3.1 *Definitions:*

3.1.1 *face fiber, n*—the wear layer of the carpet; can be composed of nylon, polypropylene, wool, or other natural or synthetic polymers. Typically, face fiber is tufted into a woven or non-woven scrim and then coated with latex to bond the face fiber securely to the backing; this latex coated scrim forms the primary backing.

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

3.1.3 *primary backing, n*—the uppermost layer of carpet backing where carpet fiber bundles are physically attached at the base to the backing structure. This layer is typically constructed of synthetic latex (ethylene vinyl acetate, styrene butadiene, or a thermo-polymer; that is, ethylene vinyl acetate hot-melt adhesive).

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

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

4. Summary of Test Method

4.1 Cut carpet samples into small rectangular pieces either via a carpet knife or mechanical die and press. Shave half of the face fiber on each sample using electric hair clippers and arrange in sterile Petri dishes (typically with the shaven half of the sample facing the center of the dish. Cool molten agars (full or partial complement) to 45 ± 2°C and inoculate with the challenge bacteria or mold conidia. Following wrist action mixing, immerse samples into the seeded-molten agar, place into a Petri dish and pour additional seeded agar into the dish to surround but not cover the test sample. Incubate the Petri dish for 24 to 72 h at 30 ± 2°C. Visually and microscopically examine both at the face fiber and shaven (primary backing) layer for inhibition of the challenge microorganisms. Report the presence of carpet surface inhibition (for low water soluble or slow migrating antimicrobials) or zone of inhibition for water soluble antimicrobials.[3]

5. Significance and Use

5.1 This test method provides for rapid screening of antimicrobial treatments located in or on the carpet face fiber or incorporated into the backing structure of the carpet (or both).

5.2 This test method simulates actual use conditions that may occur on carpets (for example, food and beverage spills, soiling from foot traffic, 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 carpet components for antimicrobial activity.

5.5 Carpets may be cleaned prior to testing with this test 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 *Sterile Petri Dishes,* 150 mm.

6.4 *Incubators,* set at required temperatures (30 ± 2°C and 37 ± 2°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 Carpet.*

6.12 *Electric Hair Clippers* (Oster Golden A5 or equivalent #30 Blade).

6.13 *Canned Air* (compressed air for surface dusting).

6.14 *Sterile Petri Dishes,* 100 mm.

6.15 *Carpet Knife* (razor knife).

6.16 *Mechanical Die (Optional),* 2.5 by 3.8 cm.

6.17 *Hydraulic Press (Optional).*

6.18 *Sterile Funnel,* with a glass wool plug.

6.19 *Counting Chamber* (hemocytometer).

6.20 *Light Microscope,* 10 and 40× objectives.

6.21 *Disposable Examination Gloves.*

7. Reagents

7.1 *Media:*

7.1.1 *Tryptic Soy Broth.*

7.1.2 *Tryptic Soy Agar.*

7.1.3 *Potato Dextrose Agar.*

7.1.4 *Sterile 0.85 % Saline,* with 0.1 % polysorbate 80.

7.2 *Test Organisms*—Specific organisms 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 carpet is expected to perform.

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

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

7.2.3 Fungus *Aspergillus niger* ATCC 9642.

NOTE 1—Originally deposited as *Aspergillus niger*, the current ATCC designation is *Aspergillus brasiliensis*.

8. Procedure

8.1 Grow 18 hour tryptic soy broth cultures of *Staphylococcus aureus* at 37 ± 2°C and *Serratia marcescens* at 30 ± 2°C. These cultures should originate from 18 to 24 h growth stock culture plates or agar slants. Origination from glycerol stocks with two transfers is also permissible.

8.2 Prepare a suspension of fungal conidia by harvesting mature conidia from a 1 week old stock culture plate or slant grown at 30 ± 2°C. Pour sterile 0.85 % saline with 0.1 % polysorbate 80 over the growth, agitate the liquid with a sterile glass rod, and filter the hyphal fragments by pouring the suspension through a sterile funnel plugged with glass wool.

8.3 Prepare 200-mL lots of ½ strength tryptic soy and ½ strength potato dextrose agars in 250-mL Erlenmeyer flasks and autoclave. Cool the molten agars to 45 ± 2°C in a water bath.

8.4 Cut carpet samples (minimum of duplicate carpet samples for each challenge organism) 2.5 by 3.8 cm.

8.5 With gloved hands, aseptically shear half of the fiber from the 3.8-cm length of carpet using electric clippers. The sheared half of the carpet sample should have fibers no longer than 2 ± 1 mm. Use canned air to remove the loose fibers from each carpet sample after shaving.

8.6 Place carpet samples into 150-mm Petri dishes with the shaven half of each sample facing the center of the dish.

[3] *Technical Manual of the American Association of Textile Chemists and Colorists*, 2000, Volume 75, American Association of Textile Chemists and Colorists.

Physically separate samples in the dish so they do not touch one another. Each dish should contain replicates of the same sample versus a control (if available).

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

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

8.9 Inoculate 200-mL lots of cooled (45 ± 2°C) tryptic soy agar with 2.0 mL of standardized bacterial inoculum (final cell density 1-2×10^5 CFU/mL). Wrist-action mix the agar for 30 seconds.

8.10 Inoculate 200-mL lots of cooled (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 seconds.

8.11 Immerse each carpet sample into the seeded agar using flame sterilized forceps. Then place each sample into the Petri dish as described in 8.6. Pour a sufficient amount of the seeded agar into the Petri dish to cover the bottom of the dish and to surround but not cover the sample (20- to 25-mL molten agar volume is typical).

8.12 Allow the seeded agar to gel around the carpet samples (10 min).

8.13 Incubate all samples at 30 ± 2°C for 24 to 72 hours.

9. Report

9.1 The report shall contain the following elements:

9.1.1 Report gross examination of the fiber layer and shaven primary backing layer for direct surface inhibition or a zone of inhibition surrounding the carpet sample at 24, 48, and 72 hours, or both.

9.1.2 Report the results of a stereo-microscopic (10 to 30× magnification) inspection. Examine both the unshaven fiber layer and the shaven primary backing layer of the carpet samples for evidence of bacterial or fungal inhibition. Compare the observations to a non-treated control carpet or growth in distal areas of the Petri dish away from the carpet samples (that is, the center of dish).

9.1.3 Key for reporting degree of microbial inhibition on the carpet 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 hours 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

9.1.3.4 CIZ = complete inhibition of the challenge microorganism with the presence of a zone of inhibition (average size reported in mm).

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 Carpet with plush fibers (>10-mm pile height) or those constructed of wool and hemp may absorb an excess volume of the seeded agar making them less likely to demonstrate meaningful fiber layer inhibition.

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

11. Keywords

11.1 antimicrobial; bacteria; carpet; fungi; low solubility preservative; mold