

Test Method for Antimicrobial Activity of New Carpets

1. Purpose and Scope

1.1 This test method is designed to determine the antimicrobial activity of new carpet materials and consists of three procedures: a qualitative antibacterial assessment, a quantitative antibacterial assessment and qualitative antifungal assessment. It may also be used to evaluate the effect of a cleaning process (agreed upon by the interested parties) on the antimicrobial resistance of carpets.

2. Principle

2.1 This test method consists of three procedures, Test I, II and III, which can be used singly or in combination.

2.1.1 Test I is a qualitative assessment of antibacterial activity. Specimens of the test material, including corresponding untreated controls of the same material (if available but not required), are placed in intimate contact with the agar surface which has been previously streaked with a bacterial culture. After incubation, a clear area of interrupted growth underneath and along the sides of the test material indicates antibacterial activity of the specimen. Standard strains of bacteria are used with *Staphylococcus aureus* (gram-positive) and *Klebsiella pneumoniae* (gram-negative), the representative organisms.

2.1.2 Test II provides a quantitative procedure for the comparison and evaluation of the degree of antibacterial activity after a 24 h exposure to the test bacteria on the carpet swatches. Test carpets are inoculated with the test organisms. After incubation, the bacteria are eluted from the swatches by shaking in known amounts of neutralizing liquid. The number of bacteria present in this liquid is determined, and the percent reduction by the specimen is calculated.

2.1.3 Test III is a qualitative assessment of the antifungal activity of carpet and resistance to a common fungal organism on a sabouraud dextrose agar medium.

3. Referenced Documents

NOTE: Use current versions of all publications unless otherwise specified.

3.1 AATCC TM138, Test Method for Cleaning: Washing of Textile Floor Coverings (see 31.1).

3.2 AATCC TM171, Test Method for Carpets: Cleaning of; Hot Water Extraction (see 31.1).

3.3 ASTM E2471, Standard Test

Method for Using Seeded-Agar for the Screening Assessment of Antimicrobial Activity In Carpets (see 31.2).

3.4 ASTM E1402, Standard Guide for Sampling Design (see 31.2).

3.5 Centers for Disease Control. Biosafety in Microbiological and Biomedical Laboratories (see 31.3).

4. Terminology

4.1 **activity**, n.—*of an antimicrobial agent*, a measure of effectiveness of the agent.

4.2 **antibacterial agent**, n.—any chemical which kills bacteria (bactericide) or interferes with the multiplication, growth or activity of bacteria (bacteriostat).

4.3 **antifungal agent**, n.—any chemical which kills or inhibits the growth of fungi.

4.4 **antimicrobial agent**, n.—any chemical which kills or inhibits the growth of microorganisms.

4.5 **bacterial resistance**, n.—*in textiles*, resistance to the development of visible bacterial growth and accompanying odors, resulting from bacterial degradation of fibers or soil on them, as distinguished from musty fungal odors.

4.6 **mildew resistance**, n.—*in textiles*, resistance to the development of unsightly fungal growths and accompanying unpleasant, musty odors on carpet materials exposed to conditions favoring such growths.

4.7 **rot resistance**, n.—*in textiles*, resistance to deterioration of a carpet material as a result of fungal growth in or on it.

4.8 **zone of inhibition**, n.—clear area of no visible growth of a microorganism, cultured onto the surface of agar growth medium, in proximity to the borders of a specimen placed in direct contact with this agar surface.

NOTE: A zone of inhibition occurs as result of the diffusion of an antimicrobial agent from the specimen.

5. Safety Precautions

5.1 The safety precautions specified in the method/procedure are ancillary to the testing procedures and are not intended to be all inclusive.

5.2 It is the user's responsibility to reference applicable safety data sheets, use safe and proper techniques, and wear appropriate personal protective equipment in handling materials in this standard.

5.3 Users MUST consult manufactur-

ers for specific details such as equipment operating instructions and other recommendations. Consult and follow all applicable health and safety regulations (e.g. OSHA standards and rules).

5.4 See 3.5, 8.1, 11.1 for additional safety precautions and personnel training related to handling bacteria and fungi.

6. Uses and Limitations

6.1 This test method is designed for use only for new carpets and must not be used for carpets that have been laid down and used.

I. Qualitative Assessment of Antibacterial Activity on Carpets: Single Streak Method

7. Apparatus (see 31.4)

7.1 Incubator maintained at $37 \pm 2^\circ\text{C}$ ($99 \pm 4^\circ\text{F}$).

7.2 Water bath maintained at $45\text{--}50^\circ\text{C}$ ($113\text{--}122^\circ\text{F}$).

7.3 Bunsen Burner or equivalent.

7.4 Stereomicroscope, minimum 40 $\times$ magnification.

7.5 Autoclave.

7.6 Vortex or equivalent.

8. Materials (see 31.4)

8.1 Bacteria species suitable for the intended end-use of the test sample. Some bacteria are pathogenic (i.e., capable of infecting humans and producing disease). Take every necessary and reasonable precaution to eliminate risk to laboratory personnel and others in the associated environment. Sterilize all contaminated samples and test materials prior to disposal. Any or all the following bacteria may be used:

8.1.1 Gram-positive: *Staphylococcus aureus*, ATCC 6538, CIP 4.83, DSM 799, NBRC 13276, NCIMB 9518 or equivalent strain (see 31.5 and 31.6).

8.1.2 Gram-negative: *Klebsiella pneumoniae*, ATCC 4352, CIP 104216, DSM 789, NBRC 13277, NCIMB 10341 or equivalent strain (see 31.5 and 31.6)

8.1.3 Other species agreed upon by the interested parties.

8.2 Broths/agars; Nutrient broth/agar, Trypticase Soy broth/agar, Brain-Heart Infusion broth/agar, Müller-Hinton broth/agar or other capable of growing and maintaining the chosen bacterial test organism.

8.3 Distilled or deionized water, sterile.

8.4 Inoculating loop, 4-mm.

- 8.5 Pipets, 1 mL, sterile or equivalent.
- 8.6 Culture Tubes with caps; minimum 10 mL capacity, sterile
- 8.7 Petri dishes, 100 mm diam. × 15 mm deep, flat-bottom, sterile.
- 8.8 Forceps, sterile.
- 8.9 Ruler, marked in millimeters.

9. Sampling

9.1 Test results are valid only when the samples are statistically representative (see ASTM E1402).

9.2 Sampling must be random. Every unit of product must have a mathematically equal chance of becoming a sample; and every portion of each sample must be equally likely to become a test specimen.

9.3 All specimens must be alike within the variations due to pure chance. There must be no differences within samples assignable to known causes.

10. Specimens

10.1 Test specimens (non-sterile) are cut by hand or with a die. They may be any convenient size although oblong specimens cut 25 × 50 mm are recommended.

10.2 If possible, test a specimen of the same material treated in exactly the same way with whatever other finishing agents were used, but without the antibacterial agent. However, this is not essential for the validity of the test. Many standard finishing chemicals will give strong antibacterial activity even after many cleanings.

10.3 Test 1 test specimen. Additional test specimens may be used to increase the precision of the average.

11. Procedure

11.1 This test should be performed only by trained personnel. Consult the U.S. Department of Health and Human Services publication *Biosafety in Microbiological and Biomedical Laboratories* (see 3.5).

11.2 If durability data is desired, carpet specimens can be tested before and after being cleaned by a test method agreed upon by the interested parties i.e. AATCC TM138 (see 3.1) or AATCC TM 171 (see 3.2)

11.3 Dispense sterilized (appropriate medium) agar cooled to 45 ± 2°C (117 ± 4°F) by pouring 15.0 ± 2.0 mL into each standard (15 × 100 mm) flat bottomed Petri dish. Allow the agar to gel firmly before inoculating.

11.4 Prepare the test organism by taking a sterilized inoculation loop and inoculating separate culture tubes of the appropriate broth with the organism to be used. Incubate the cultures at 37 ± 2°C (99 ± 4°F) for 18-24 h.

11.5 Prepare inoculum by transferring

1.0 ± 0.1 mL of a 18-24 h broth culture into 9.0 ± 0.1 mL of sterile distilled water contained in a test tube or small flask. Mix well using appropriate agitation or by using a vortex.

11.6 Using a 4-mm inoculating loop, load one loop full of the diluted inoculum and transfer it to the surface of the sterile agar plate by making one long streak of approximately 75 mm in length across the center of the plate. Do not break the surface of the agar while making the streak.

11.7 Gently press the test specimen transversely across the inoculum streak to ensure intimate contact with the agar surface. An easy technique is to press the specimen to the agar surface with a biological section lifter or with a spatula which has been sterilized in a flame and then air-cooled immediately before use. Test both the pile (face fibers) and the backing of the carpet on separate agar plates.

11.8 Incubate the plates at 37 ± 2°C (99 ± 3°F) for 18-24 h.

12. Evaluation

12.1 Examine the incubated plates for interruption of growth along the streak of inoculum beneath the specimen and for a clear zone of inhibition beyond the specimen edge.

12.2 Calculation of zone of inhibition size is optional. The width of the zone of inhibition around the test specimen may be calculated using Eq 1:

$$W = (T - D)/2 \quad (\text{Eq. 1})$$

where:

W = width of clear zone of inhibition in mm

T = total width of test specimen and clear zone in mm

D = width of the test specimen in mm

12.3 The criterion for passing the test must be agreed upon by the interested parties.

12.4 The size of the zone cannot be construed as a quantitative evaluation of antibacterial activity. The report of results will include an observation of zones of inhibition and growth under the specimen if present.

13. Report

13.1 Describe or identify the sample tested.

13.2 Report that the sample was tested using AATCC TM174-2022 Test I.

13.3 Report the testing conditions:

13.3.1 Size of test specimen used.

13.3.2 Number of test replicates used.

13.3.3 Number of carpet cleanings (if any) and the method used.

13.3.4 If the test specimen was sterilized prior to testing and the method of

sterilization.

13.3.5 Agar media used.

13.3.6 Organism used.

13.3.7 incubation time.

13.4 Report the test results:

13.4.1 Any observed growth underneath the sample.

13.4.2 Any observed zone of inhibition.

13.5 Describe any modification(s) of the published standard.

14. Precision and Bias

14.1 *Precision.* Precision for this test method has not been established. Until a precision statement is generated for this test method, use standard statistical techniques in making any comparisons of test results for either *within-laboratory* or *between-laboratory* averages.

14.2. *Bias.* The antimicrobial activity can be defined on in terms of a test method. There is no independent method for determining the true value. As a means of evaluating this property, the method has no known bias.

II. Quantitative Assessment of Antibacterial Activity on Carpets

15. Apparatus (see 31.4)

15.1 Incubator maintained at 37 ± 2°C (99 ± 4°F).

15.2 Water bath maintained at 45-50°C (113-122°F).

15.3 Bunsen Burner or equivalent.

15.4 Autoclave.

15.5 Vortex or equivalent.

16. Materials (see 31.4)

16.1 Bacteria species suitable for the intended end-use of the test sample. Some bacteria are pathogenic (i.e., capable of infecting humans and producing disease). Take every necessary and reasonable precaution to eliminate risk to laboratory personnel and others in the associated environment. Sterilize all contaminated samples and test materials prior to disposal. Any or all the following bacteria may be used:

16.1.1 Gram-positive: *Staphylococcus aureus*, ATCC 6538, CIP 4.83, DSM 799, NBRC 13276, NCIMB 9518 or equivalent strain (see 31.5 and 31.6).

16.1.2 Gram-negative: *Klebsiella pneumoniae*, ATCC 4352, CIP 104216, DSM 789, NBRC 13277, NCIMB 10341 or equivalent strain (see 31.5 and 31.6).

16.1.3 Other species agreed upon by the interested parties.

16.2 Broths/agar; Nutrient broth/agar, Trypticase Soy broth/agar, Brain-Heart Infusion broth/agar, Müller-Hinton broth/agar or other capable of growing and maintaining the chosen bacterial test organism.