

Test Method for Antimicrobial Activity Assessment of New Carpets

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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, depending on the extent of proof of antimicrobial activity the end-user is interested in acquiring. Test I is a qualitative assessment of antibacterial activity using both a Gram-positive and Gram-negative bacteria that can produce results in 24 h. 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. Then the bacterial challenge is eluted from the swatches and enumerated and a percent reduction by the carpet specimen is calculated. Test III is for assessment of the antifungal activity of carpet and resistance to a common fungal organism on nutrient rich medium.

3. Terminology

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

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

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

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

3.5 **bacterial resistance**, n.—*in textiles*, resistance to the development of visible bacterial growth and accompanying odors, resulting from bacterial degra-

dation of fibers or soil on them, as distinguished from musty fungal odors.

3.6 **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.

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

3.8 **zone of inhibition**, n.—clear area of no 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.

4. Safety Precautions

NOTE: These safety precautions are for information purposes only. The precautions are ancillary to the testing procedures and are not intended to be all inclusive. It is the user's responsibility to use safe and proper techniques in handling materials in this test method. Manufacturers MUST be consulted for specific details such as material safety data sheets and other manufacturer's recommendations. All OSHA standards and rules must also be consulted and followed.

4.1 This test should be performed only by trained personnel. The U.S. Department of Health and Human Services publication *Biosafety in Microbiological and Biomedical Laboratories* should be consulted (see 25.1).

4.2 CAUTION: Some of the microorganisms used in these tests are allergenic and pathogenic; i.e., capable of infecting humans and producing disease. Therefore, every necessary and reasonable precaution must be taken to eliminate this risk to the laboratory personnel and to personnel in the associated environment. Wear protective clothing, respiratory protection, and impervious gloves when working with the organisms. Choose respiratory protection that prevents penetration by the spores.

4.3 Good laboratory practices should be followed. Wear safety glasses in all laboratory areas.

4.4 All chemicals should be handled with care.

4.5 An eyewash/safety shower should be located nearby for emergency use.

4.6 Sterilize all contaminated samples and test materials prior to disposal.

4.7 Exposure to chemicals used in this procedure must be controlled at or below levels set by government authorities (e.g., Occupational Safety and Health Administration's [OSHA] permissible exposure limits [PEL] as found in 29 CFR 1910.1000 (see web site: www.osha.gov for latest version). In addition, the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values (TLVs) comprised of time weighted averages (TLV-TWA), short term exposure limits (TLV-STEL) and ceiling limits (TLV-C) are recommended as a general guide for air contaminant exposure which should be met (see 25.2).

5. Uses and Limitations

5.1 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

6. Principle

6.1 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.

7. Test Organisms

7.1 *Staphylococcus aureus*, ATCC 6538, CIP 4.83, DSM 799, NBRC 13276, NCIMB 9518 or equivalent strain (see 25.3 and 25.4).

7.2 *Klebsiella pneumoniae*, ATCC 4352, CIP 104216, DSM 789, NBRC 13277, NCIMB 10341 or equivalent strain (see 25.3 and 25.4).

7.3 Other suitable species can also be used depending on the intended end-use of the test sample.

8. Materials, Media and Reagents

8.1 Media and Reagents. Suitable

broth/agar media are:

- 8.1.1 Nutrient broth/agar.
- 8.1.2 Trypticase Soy broth/agar.
- 8.1.3 Brain-Heart Infusion broth/agar.
- 8.1.4 Müller-Hinton broth/agar.
- 8.2 Equipment and Supplies:
 - 8.2.1 Incubator maintained at $37 \pm 2^\circ\text{C}$.
 - 8.2.2 Inoculating Loop.
 - 8.2.3 Bunsen Burner or equivalent.
 - 8.2.4 Water bath, maintained at $45\text{--}50^\circ\text{C}$.
 - 8.2.5 Pipettes, 1 mL, sterile or equivalent.
 - 8.2.6 Culture Tubes with caps, minimum 10 mL capacity.
 - 8.2.7 Petri dishes, 100 mm diameter $\times$ 15 mm deep, sterile.
 - 8.2.8 Forceps, sterile.
 - 8.2.9 Stereomicroscope, minimum 40 $\times$ magnification.
 - 8.2.10 Ruler.

9. Test Specimens

9.1 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.

9.2 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. Procedure

10.1 If durability data are desired, carpet specimens must be tested before and after being cleaned by a test method agreed upon by the interested parties.

10.2 Dispense sterilized (appropriate medium) agar cooled to $45 \pm 2^\circ\text{C}$ ($117 \pm 4^\circ\text{F}$) 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.

10.3 Prepare inoculum by transferring 1.0 ± 0.1 mL of a 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.

10.4 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.

10.5 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.

10.6 Incubate the plates at $37 \pm 2^\circ\text{C}$ ($99 \pm 3^\circ\text{F}$) for 18-24 h.

11. Evaluation and Report

11.1 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. The width of the zone of inhibition around the test specimen may be calculated using the following equation:

$$W = (T - D)/2$$

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

11.2 The criterion for passing the test must be agreed upon by the interested parties. To constitute acceptable antibacterial activity, there must be no bacterial colonies directly under the sample in the contact area.

11.3 Report the results of testing the carpet both before and after cleaning. The number of cleanings is to be agreed upon by the interested parties.

11.4 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.

12. Precision and Bias

12.1 Precision and bias 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.

II. Quantitative Assessment of Antibacterial Activity on Carpets

13. Principle

13.1 This test method provides a quantitative procedure for the evaluation of the degree of antibacterial activity.

13.2 Test carpets are inoculated with the test organisms. After incubation, the bacteria are eluted from the swatches by shaking in known amounts of liquid. The number of bacteria present in this liquid is determined, and the percent reduction by the specimen is calculated (see 25.5).

14. Test Organisms

14.1 See Section 7.

15. Materials, Media and Reagents

15.1 See Section 8.

16. Test Specimens

16.1 Cut a circular disc of approximately 48 mm in diameter from the test carpet (preferably with a steel die). Place the disc in a 250 mL wide-mouth glass jar with screw cap. The carpet disc should lie flat at the bottom of the jar.

16.1.1 An uninoculated treated carpet may be used to determine the level of background organisms present on the carpet.

16.1.2 Do not sterilize carpet samples prior to testing.

17. Procedure

17.1 If durability data are required, test carpet specimens before and after being cleaned in accordance with a method agreed upon by the interested parties.

17.2 Apply 0.1-0.5 mL of an 18-24 h broth bacterial inoculum adjusted to $1\text{--}2 \times 10^5$ CFU on the prewetted carpet fibers. The dilution of the test organism may be prepared in sterile 0.85% saline solution or suitable buffer if a steady-state culture is needed during the contact period. If, however, this test is to be performed under in-use conditions, use nutrient broth as the dilution medium. The carpet disc may be prewetted by dipping it in sterile deionized water or in water containing 0.05% of a non-bacteriocidal wetting agent (see 25.6) and then briefly blotting it on filter paper.

17.3 Inoculate the carpet fibers evenly using a sterile pipette and place the specimen in a glass jar. Screw the jar top on tightly to prevent evaporation.

17.4 As soon as possible after inoculation (0 contact time), add 100 ± 0.1 mL of neutralizer solution to the jar (see 25.7).

17.5 Shake the jar, either mechanically or by hand, vigorously for 1 min. Make serial dilutions and plate (in duplicate) on nutrient (or appropriate) agar. Dilutions of 10^0 , 10^1 and 10^2 are usually suitable.

17.6 The neutralizer solution should include ingredients to neutralize the specific antibacterial carpet treatment and to adjust the pH to 6-8. Report the neutralizer used.

17.7 Incubation over contact periods. Incubate additional jars containing inoculated carpet discs at $37 \pm 2^\circ\text{C}$ ($99 \pm 3^\circ\text{F}$) for 6-24 h. Similar jars may be incubated over other periods (i.e., 1 or 6 h) to provide information about the bacteriocidal activity of the treatment over such periods.

17.8 Sampling of inoculated and incu-