

Antimicrobial Activity Assessment of Carpets

Developed in 1991 by Committee RA31; revised 1992; editorially revised and reaffirmed 1993; reaffirmed 1998; editorially revised 2004.

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:

1.1.1 A qualitative antibacterial assessment.

1.1.2 A quantitative antibacterial assessment.

1.1.3 A qualitative antifungal assessment.

1.2 This test method 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, the principles of which are given in Sections 6, 14 and 22.

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 degradation 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 27.1).

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. NOTE: 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 of January 1, 1989). 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 27.2).

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 worn.

1. 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 nutrient agar 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*, American Type Culture Collection No. 6538 (see 27.3).

7.2 *Klebsiella pneumoniae*, American Type Culture Collection No. 4352 (see 27.3).

8. Culture Medium

8.1 Suitable broth/agar media are Nutrient, Trypticase Soy and Brain-Heart Infusion (BHI).

8.2 Nutrient Broth

Beef Extract	3 g
Peptone	5 g
Distilled water	to 1000 mL

8.3 Heat to a boil to disperse ingredients. Adjust to pH 6.8 with 1N sodium hydroxide (NaOH) solution. (This is not necessary if prepared, dehydrated medium is used.)

8.4 Dispense in 10 mL amounts in conventional bacteriological culture tubes (i.e., 125 × 17 mm) plug and sterilize at 103 kPa (15 psi) for 15 min.

8.5 Nutrient Agar (see 27.4). Add 1.5% bacteriological agar to nutrient broth. Heat to boiling. Check pH and adjust to 7.0-7.2 using NaOH solution if necessary. Dispense in 15 mL amounts in conventional bacteriological culture tubes, plug and sterilize at 103 kPa (15 psi) for 15 min.

9. Maintenance of Culture of Test Organisms

9.1 Using a 4 mm inoculating loop, transfer the culture daily in nutrient broth for not more than two weeks. At the conclusion of two weeks, make a fresh transplant from stock culture. Incubate cultures at $37 \pm 2^\circ\text{C}$ ($99 \pm 3^\circ\text{F}$).

9.2 Maintain stock cultures on nutrient agar slants. Store at $5 \pm 1^\circ\text{C}$ ($41 \pm 2^\circ\text{F}$) and transfer once a month to fresh agar (see 27.5).

10. Test 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.

11. Procedure

11.1 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.

11.2 Dispense sterilized nutrient agar cooled to $45 \pm 2^\circ\text{C}$ ($113 \pm 4^\circ\text{F}$) by pouring 15.0 ± 2.0 mL into each 100.0 mm diameter flat bottomed Petri dish. Allow agar to gel firmly before inoculating.

11.3 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.

11.4 Using a 4 mm inoculating loop, load one loopful 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.

11.5 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.

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

12. Evaluation and Report

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 speci-

men 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 diameter of test specimen and clear zone in mm

D = diameter of the test specimen in mm

12.2 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.

12.3 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.

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. Precision and Bias

13.1 A precision and bias statement is not applicable because data are not generated by this method.

II. Quantitative Assessment of Antibacterial Activity on Carpets

14. Principle

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

14.2 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 27.7).

15. Test Organisms

15.1 See Section 7.

16. Culture Medium

16.1 See Section 8.

17. Maintenance of Culture of Test Organisms

17.1 See Section 9.

18. Test Specimens

18.1 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.

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

18.1.2 Do not sterilize carpet samples prior to testing. Sections 18.1.1 and 20.3 address any problems that might arise due to the presence of background organisms on the carpet.

19. Procedure

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

19.2 Apply 0.1-0.5 mL of an 18-24 h broth bacterial inoculum adjusted to $1-2 \times 10^5$ 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 27.6) and then briefly blotting it on filter paper.

19.3 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.

19.4 As soon as possible after inoculation (0 contact time), add 100 ± 0.1 mL of neutralizer solution to the jar (see 27.9).

19.5 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.

19.6 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.

19.7 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.

19.8 Sampling of inoculated and incubated swatches. After incubation, add 100 ± 0.1 mL of neutralizer solution to jars containing treated carpet discs. Shake the jars 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 for treated test specimens. Several different dilutions may be required for untreated control carpets depending on the incubation period.

19.9 Incubate all plates for 24 h at $37 \pm 2^\circ\text{C}$ ($99 \pm 3^\circ\text{F}$).