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Test Method for Antibacterial Finishes on Textile Materials: Assessment of

1. Purpose and Scope

1.1 This test method provides a quantitative procedure for the evaluation of the degree of antibacterial activity. Assessment of antibacterial finishes on textile materials is determined by the degree of antibacterial activity intended in the use of such materials. If only bacteriostatic activity (inhibition of multiplication) is intended, a qualitative procedure which clearly demonstrates antibacterial activity as contrasted with lack of such activity by an untreated specimen may be acceptable. However, if bactericidal activity is intended or implied, quantitative evaluation is necessary. Quantitative evaluation also provides a clearer picture for possible uses of such treated textile materials.

2. Principle

2.1 This test method 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 test fabric compared directly against an untreated control. After incubation, the bacterial challenge is eluted from the swatches and enumerated and a percent reduction by the fabric specimen is calculated.

3. Terminology

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

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

4. Safety Precautions

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

4.2 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.

4.3 Users **MUST** be consult manufacturers for specific details such as safety data sheets, equipment operating instructions, and other recommendations. Consult and follow all applicable health and safety regulations (e.g., OSHA standards and rules).

4.4 This test should be performed only

by appropriately trained personnel. The U.S. Department of Health and Human Services publication, *Biosafety in Microbiological and Biomedical Laboratories*, should be consulted (see 13.1).

4.5 **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.

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

5. Test Organisms

5.1 Test bacteria:

5.1.1 *Staphylococcus aureus*, ATCC No. 6538, Gram positive organism, CIP 4.83, DSM 799, NBRC 13276, NCIMB 9518 or equivalent strain (see 13.2 and 13.3).

5.1.2 *Klebsiella pneumoniae*, ATCC No. 4352, Gram negative organism, CIP 104216, DSM 789, NBRC 13277, NCIMB 10341 or equivalent strain (see 13.2 and 13.3).

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

6. Materials, Media and Reagents

6.1 Media and reagents. Suitable broth/agar media are:

6.1.1 Nutrient broth/agar.

6.1.2 Trypticase Soy broth/agar.

6.1.3 Sterile distilled or deionized water.

6.1.4 Triton X-100.

6.1.5 Neutralizing broth: Lethen broth, Dey Engley broth or other appropriate for the antimicrobial compound being tested.

6.1.6 Suspension Medium, 1:20 (5%) Nutrient broth or Trypticase Soy broth + 0.05% Triton X-100. Dilute the nutrient broth (TSB) 1:20 with distilled or deionized water containing 0.05% Triton X-100. Sterilize by autoclaving at $121 \pm 2^\circ\text{C}$. If not used immediately after preparation, store at $5-10^\circ\text{C}$.

6.2 Materials.

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

6.2.2 Inoculating loop.

6.2.3 Bunsen Burner or equivalent.

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

50°C ($113-122^\circ\text{F}$).

6.2.5 Pipettes and tips: 10-1000 μL pipettes and appropriate tips.

6.2.6 Culture tubes with non-screw caps; minimum 10 mL capacity.

6.2.7 Petri dishes, 100 mm diam. $\times$ 15 mm deep, sterile.

6.2.8 Forceps, sterile.

6.2.9 Stereomicroscope, minimum $40\times$ magnification.

6.2.10 Ruler.

6.2.11 Analytical balance, capable of measuring 10.0 ± 0.1 g.

6.2.12 Sterile specimen container with screw cap, 150-mL capacity or similar.

6.2.13 Exam gloves, nitrile or other.

6.2.14 Vortex mixer.

6.2.15 Steam sterilizer or autoclave.

6.2.16 Viability control fabric (see 13.4).

7. Preparation of Bacterial Inocula

7.1 Grow a fresh 18-h shake culture of each test specimen in sterile Tryptic Soy (or Nutrient) broth at $37 \pm 2^\circ\text{C}$ and 150-250 rpm prior to performing the test. These cultures should originate from a single colony selected from stock culture plates or growth on agar slants. Fresh stock plates should be generated weekly.

7.1.1 Each 18-h shake culture is then diluted with suspension medium (6.1.6), as appropriate for the estimated bacterial concentration, to obtain a bacterial concentration that is between 1.0×10^5 and 3.0×10^5 CFU/mL. These suspensions are used as the test inoculum. Prepared test inoculum is used within 2 h of preparation and bacterial concentration should be verified by appropriate enumeration method.

8. Test Specimens

8.1 Preparation. The following description will be in terms of fabric swatches. Textile materials not in fabric form can likewise be tested with the appropriate modification.

8.1.1 Size and shape of test swatches: Cut circular swatches 4.8 ± 0.1 cm (1.9 ± 0.03 in.) in diameter or square swatches $3.8 \times 3.8 \pm 0.1$ cm, from the test fabric. Use the number of swatches needed to equal 1.0 ± 0.1 g. Stack the swatches in a sterile specimen container with screw cap or other appropriate closed container. The number of swatches to be used is dependent on the fiber type and fabric construction. The number of swatches used per jar should be reported.

8.1.2 Untreated Controls. Swatches of the same fiber type and fabric construction