

Antibacterial Finishes on Textile Materials: Assessment of

Developed in 1961 by AATCC Committee RA31; revised 1965, 1981, 1988 (with title change), 1993, 1999; editorially revised 1969, 1971, 1974, 1985; reaffirmed 1977, 1981, 1989, 1998; editorially revised and reaffirmed 1986, 2004.

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 Swatches of test and control textile materials are tested qualitatively for antibacterial activity by AATCC Method 147. Those showing activity are evaluated quantitatively. Test and control swatches are inoculated with the test organisms. After incubation, the bacteria are eluted from the swatches by shaking in known amounts of neutralizing solution. The number of bacteria present in this liquid is determined, and the percentage reduction by the treated 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

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 Both the qualitative and quantitative tests should be carried out by persons with training and experience in the use of bacteriological techniques. The U.S. Department of Health and Human Services publication, *Biosafety in Microbiological and Biomedical Laboratories*, should be consulted (see 13.1).

4.2 CAUTION: Some of the bacteria used in this test are 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 and respiratory protection that prevents penetration by the bacteria.

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 13.2).

5. Limitations

5.1 For a qualitative, relatively quick and easily executed method to determine residual antibacterial activity of textile materials, refer to AATCC Method 147, Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method.

6. Test Organisms

6.1 Test bacteria.

6.1.1 *Staphylococcus aureus*, American Type Culture Collection No. 6538. Gram positive organism (see 13.10).

6.1.2 *Klebsiella pneumoniae*, Ameri-

can Type Culture Collection No. 4352. Gram negative organism (see 13.10).

6.1.3 Other suitable species can also be used.

7. Culture Medium

7.1 Suitable broth/agar media are Nutrient, Trypticase Soy and Brain-Heart Infusion.

Nutrient Broth:

Peptone (Bacto-peptone)

(see 13.3) 5 g

Beef extract (see 13.4) 3 g

Distilled water to 1000 mL

7.2 Heat to a boil to disperse ingredients. Adjust to pH 6.8 ± 0.1 with 1N sodium hydroxide (NaOH) solution. (This is not necessary if prepared, dehydrated medium is used.)

7.3 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.

7.4 Nutrient agar. Add 1.5% bacteriological agar to nutrient (or appropriate) broth (see 7.1). Heat to boiling. Check pH and adjust to 7.1 ± 0.1 using NaOH solution if necessary. Dispense in 15 ± 1 mL amounts in conventional bacteriological culture tubes. Plug and sterilize at 103 kPa (15 psi) for 15 min. (May be sterilized in 1000 mL borosilicate glass flasks and petri dishes poured from this.)

7.5 Slurry Inoculum Carrier (for hydrophobic fabrics) (see 7.2 and 7.3):

Sodium Chloride 8.5 g

Agar 3.0 g

Distilled Water 1000 mL

8. Maintenance of Culture of Test Organisms

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

8.2 Maintain stock cultures on nutrient or appropriate 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 13.5).

9. Qualitative Test (Screening or Presumptive Test)

9.1 For detection of bacteriostatic activity use AATCC Method 147 on a test specimen and control specimen using the organisms referred to above. For demonstration of bactericidal activity, proceed to the quantitative test described below.

10. Quantitative Test (Reference or Confirmatory Test)

10.1 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.

10.1.1 Size and shape of treated swatches: Cut circular swatches 4.8 ± 0.1 cm (1.9 ± 0.03 in.) in diameter, from the test fabric (preferably with a steel die). Stack the swatches in a 250 mL wide-mouth glass jar with screw cap. The number of swatches to be used is dependent on the fiber type and fabric construction. Use that amount of fabric which will absorb the 1.0 ± 0.1 mL of inoculum, and leave no free liquid in the jar. For example, 4 swatches of cotton print cloth will absorb 1 mL. The number of swatches used per jar should be reported.

10.1.2 Controls. Swatches of the same fiber type and fabric construction as test sample but containing no antibacterial finish (negative control).

10.1.3 Sterilization of samples. This is optional. The method to be used depends on the type of fiber and finish. Cotton, acetate and many manmade fibers can be sterilized in the autoclave. Wool can be sterilized by ethylene oxide or by intermittent (fractional) sterilization in flowing steam. The latter is also least damaging to certain finishes. Report method of sterilization, if used.

10.1.4 Size of inoculum per sample. Apply 1.0 ± 0.1 mL of an appropriate dilution of a 24 h broth culture of the test organism so that recovery from (1) untreated control fabric swatches or (2) treated test fabric swatches at "0" contact time (plated as soon as possible after inoculation) will show counts of $1-2 \times 10^5$ organisms. The dilution of the test organism should be made in nutrient (or appropriate) broth (see 7.1, 7.5 and 13.6).

10.2 Procedure.

10.2.1 Inoculation of fabrics. When using *Staphylococcus aureus*, shake a 24 h culture and let stand for 15-20 min before preparing the inoculum.* Place the swatches separately in sterile petri dishes and use a microliter pipette to inoculate them making sure that there is even distribution of the inoculum (see 13.7). Transfer these swatches aseptically to the jar. Screw the jar tops on tightly to prevent evaporation.

10.2.2 As soon as possible after inoculation ("0" contact time), add 100 ± 1 mL

of neutralizing solution to each of the jars containing the inoculated untreated control swatches, the inoculated treated test swatches and the uninoculated treated test swatches.

10.2.3 The neutralizing solution should include ingredients to neutralize the specific antibacterial fabric treatment and to take care of any pH requirements of the fabrics (from finishes, antibacterial agents, etc.). The neutralizing solution employed should be reported (see 13.8).

10.2.4 Shake the jars vigorously for one minute. Make serial dilutions with water and plate (in duplicate) on nutrient (or appropriate) agar. Dilutions of 10^0 , 10^1 , 10^2 are usually suitable.

10.2.5 Incubation over contact periods. Incubate additional jars containing inoculated untreated control swatches and jars containing inoculated treated test swatches at $37 \pm 2^\circ\text{C}$ ($99 \pm 3^\circ\text{F}$) for 18-24 h. Similar jars may be incubated over other periods (e.g., 1 or 6 h) to provide information about the bactericidal activity of the treatment over such periods.

10.2.6 Sampling of inoculated and incubated swatches. After incubation, add 100 ± 1 mL of neutralizing solution to jars containing untreated control swatches and to jars containing treated test swatches. Shake the jars vigorously for one minute. Make serial dilutions and plate (in duplicate) on nutrient (or appropriate) agar. Dilutions of 10^0 , 10^1 , 10^2 are usually suitable for treated test fabrics. Several different dilutions may be required for untreated control fabrics depending on the incubation period.

10.2.7 Incubate all plates for 48 h at $37 \pm 2^\circ\text{C}$ ($99 \pm 3^\circ\text{F}$) or other optimal temperature.

11. Evaluation

11.1 Report bacterial counts as the number of bacteria per sample (swatches in jar) not as the number of bacteria per mL of neutralizing solution. Report "0" counts at 10^0 dilution as "less than 100."

11.2 Calculate percent reduction of bacteria by the specimen treatments by one of the following formulas:

$$1) \quad 100 (B - A)/B = R$$

where:

R = % reduction

A = the number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated over the desired contact period

B = the number of bacteria recovered from the inoculated treated test specimen swatches in the jar immediately after inoculation (at "0" contact time)

$$2) \quad 100 (C - A)/C = R$$

where:

C = the number of bacteria recovered from the inoculated untreated control specimen swatches in the jar immediately after inoculation (at "0" contact time)

If " B and C " are not similar, the larger number should be used. If " B " and " C " are not significantly different, $(B + C)/2$ should be used as follows:

$$3) \quad 100(D - A/D) = R$$

where:

$$D = (B + C)/2$$

11.3 If an untreated control is not available, use the following calculation which allows for any background organisms that might interfere with the test.

$$Bg = 100 [(B - E) - (A - F)/B - E]$$

where:

A, B = (see 11.2)

E = the number of bacteria initially recovered from the uninoculated treated test sample (existing background organisms)

F = The number of bacteria recovered from the uninoculated, pre-wet treated test sample after incubation in the jar over the desired contact period (existing background organisms after contact period)

Bg = background organisms

11.4 For a valid test there should be: (1) "0" colonies of test organism recovered from the uninoculated treated test specimen swatches and (2) a significant increase in the numbers of bacteria recovered from the inoculated untreated control specimen swatches incubated for the specified contact time over the numbers of bacteria recovered from the inoculated untreated specimen swatches at "0" contact time (immediately after inoculation). This applies only if dilution was made in broth (see 10.1.4 and 13.6).

11.5 Report percent reduction of bacteria by the specimen treatment against each test organism.

11.6 The criterion for passing the test must be determined by the interested parties.

11.7 Report the dilution medium used.

12. Precision and Bias

12.1 Studies (see 13.9) indicate the following *within-laboratory* precision of the Standard Plate Count (SPC) Test: (a) *among-analyst* variation of 18% and (b) *within-analyst* variation of 8%.

13. Notes and References

13.1 Publication available from U.S. Department of Health and Human Services CDC/

*Using a 1 mL pipette, pad the inoculum carefully onto the fabric. If a strain of *Pseudomonas* that forms a pellicle is used, avoid including fragments of the pellicle in the inoculum.