

Test Method for the Reduction of Bacterial Odor on Antibacterial-Treated Textiles

1. Purpose and Scope

1.1 This test method is for the evaluation of the reduction of odor generated from textiles treated with antibacterial finishes.

1.2 This procedure is meant to evaluate single layer of a textile. For multiple layered textiles, such as carpet, the test is to be used only on the layer of the material that has been treated.

1.3 The reduction of bacterial odor is to be determined by testing a comparable fabric that has not been treated with an antibacterial finish.

1.4 For evaluation of the antibacterial efficacy of a textile, existing antibacterial test methods for the bactericidal/static effect of the product should be used. See Note 13.6.

2. Principle

2.1 This procedure is intended to be used to supplement results from industry standard antibacterial efficacy tests that do not measure the issue of odor reduction.

2.2 Ammonia-generating organisms are employed as the surrogate test organisms of which their ability to produce ammonia while they metabolize urea or protein residue is utilized. Ammonia is a component of bacterial odor that can be smelled in many problem areas including sink drains, diaper pails, incontinence products, and pet bedding.

2.3 *Proteus vulgaris* is the surrogate gram-negative organism while *Staphylococcus saprophyticus* is a gram-positive surrogate organism.

2.4 The results obtained from this test may be used as a relative comparison of the effects of antibacterial treatments on odor generation over time when compared to each other and to untreated fabrics. The lower limit of detection for ammonia by this method is 2 - 3 ppm, which is at or near the mean concentration for olfactory detection according to at least one study (see 13.1) although another source has reported it at 17 ppm (see 13.2).

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 that kills bacteria (bactericide) or interferes with the multiplication, growth or activity of bacteria

(bacteriostatic).

3.3 **odor**, n.—the sensation that results when olfactory receptors in the nose are stimulated by particular chemicals in gaseous form. (Source Princeton Word Net)

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 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 trained personnel. Consult the U.S. Department of Health and Human Services publication *Biosafety in Microbiological and Biomedical Laboratories* (see 13.13).

4.5 CAUTION: The bacteria used in this test may be 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 and respiratory protection that prevents penetration by the bacteria.

5. Limitations

5.1 This method, as written, is limited to bacterial organisms capable of producing ammonia as a metabolic by-product. Other bacteria that can produce a metabolic by-product which can be measured by different gas detector tubes may be used, but these variations must be reported. It is not known whether results from different gas detector tubes are comparable.

6. Apparatus, Reagents, Materials (see 13.4)

6.1 Volumetric flasks, 5 mL, glass, with a #8 stopper.

6.2 Gas detector color diffusion tubes for ammonia, calibrated 2.5 to 1500 ppm-h

(Draeger ammonia columns is an example of a suitable gas detector tube).

6.3 Sealing film, thermoplastic, with two-way stretch.

6.4 Fabric scissors.

6.5 Inoculating loop.

6.6 Incubator, 37 ± 2°C, 75% RH equipped with an orbital shaker used to agitate overnight cultures.

6.7 Serological pipettes, 1.0 mL, sterile.

6.8 Brain-Heart Infusion Broth (BHIB)

6.9 Urea, Molecular Biology Certified, >99.9%.

6.10 Filter sterilization apparatus.

6.11 Test Organisms, *Proteus vulgaris* or *Staphylococcus saprophyticus*.

6.11.1 *Proteus vulgaris* ATCC 29905 (or 8427), CIP 104989, DSM 13387, NCIMB 13426, CCUG 35382T, (see 13.5).

6.11.2 *Staphylococcus saprophyticus* ATCC 15305, CIP 76.125, DSM 20229, NCIMB 8711.

6.12 Bacterial agar for organism maintenance slants (BHIA, TSA, or NA).

7. Specimens

7.1 Cut three-1.0 g specimens from each sample fabric of each test. Each specimen is then cut into sufficiently small pieces that can be inserted through the neck of the 5-mL volumetric flasks.

7.2 Prepare one specimen from a control sample as in 7.1.

7.2.1 The control sample should be from a fabric with the same fiber content and fabric construction (weave/knit, fabric count, yield) as the test sample, but with no antibacterial finish.

7.2.2 If a true control sample cannot be generated due to manufacturing constraints, use a textile fabric of similar construction.

7.2.3 A suitable viability control fabric will generate ammonia during the testing period with the specified testing conditions.

8. Preparation of Reagents

8.1 Two media preparations are required for the test.

8.1.1 Growth media: BHIB.

8.1.2 Test media: 5% BHIB with 2% urea.

8.2 Heat BHIB as needed to dissolve completely; dispense into 10 mL volumes in conventional bacteriological culture tubes. Plug and sterilize at 103 kPa (15 psi) for 15 min.

8.3 Just prior to testing, and after cooling BHIB to room temperature, determine the amount of BHIB needed to conduct the testing described below. Dilute BHIB with sterile deionized water to achieve 5% BHIB. Add 2% w/v of urea to the predetermined amount of BHIB and mix well. Sterilize using a 0.2-μm filter.

8.4 Using a 4-mm inoculating loop, transfer the culture in BHIB from stock culture. Incubate cultures at $37 \pm 2^\circ\text{C}$.

8.5 Maintain stock cultures on Tryptic Soy Agar (TSA) or appropriate agar slants. Store at $5 \pm 1^\circ\text{C}$ and transfer once a week to fresh agar.

9. Procedure

9.1 Sterilization is not necessary for the test. But if fabrics are sterilized, include method of sterilization in the final test report.

9.2 Place 1.0 g of the cut pieces of each specimen in a separate volumetric flask so that they rest in a pile within the bulb of the flask.

9.3 Prepare test organism *P. vulgaris* or *S. saprophyticus* using a broth culture incubated for 18 - 24 hour at $37 \pm 2^\circ\text{C}$ and shaking at 110-250 rpm.

9.4 Adjust the suspension with 5% BHIB containing 2% w/v urea to obtain the test inoculum containing $1-5 \times 10^5$ cfu per mL of the test organism. Store this test inoculum at $2-8^\circ\text{C}$ until ready for testing.

9.5 If the evaluation of antibacterial finish(es) is to be undertaken after home laundering, interest parties should agree on the laundering conditions from AATCC LP1 and the number of launderings. The laundering conditions should be stated with the test results.

NOTE: Inoculum must be used within 2 hours following adjustment.

NOTE: Other concentrations of nutrient may be used in testing as long as they support ammonia generation on the untreated control fabric. These changes must be outlined in the report.

9.5 Apply 1.0 mL of the inoculum with 2% urea directly onto each specimen, taking care to ensure total uptake of the inoculum by the fabric.

NOTE: If reduction of the test volume is necessary to prevent oversaturation of a test specimen, do not use less than 0.5 mL of test inoculum. When the volume of test inoculum is decreased, the concentration of the bacterial cells in the inoculum shall be increased to provide the same number of bacterial cells as when the normal volume of test inoculum is applied unless otherwise specified.

9.6 Seal the flask opening with film or a similar substrate.

9.7 Place all the flasks in an incubator operating at $37 \pm 2^\circ\text{C}$ and incubate for 20 ± 2 h.

9.8 For each flask, open a gas detector tube by snapping off the neck as directed in the detector's instructions, and insert the open end into the neck of each volumetric flask, sealing off the neck from the atmosphere.

9.9 Secure the gas detector tubes in the necks using strips of sealing film as required to ensure the seal.

9.10 Place all the flasks in an incubator operating at $37 \pm 2^\circ\text{C}$ and record the test start time.

9.11 Monitor the flasks once each hour for signs of color change from yellow to blue within the packing of each gas detector tube. For each flask, record the reading associated with the color change on the tube scale and the time elapsed from the start.

NOTE: The gas detector tubes are only certified for an 8-hour exposure time-frame. The exposure time with the column must be stopped after eight hours.

9.12 It is recommended that, once a color change is detected, the flasks are to

be read and recorded every 30 minutes thereafter.

9.13 The test is complete for each specimen when it reaches the top of the scale or the 8-h time frame has elapsed (whichever is first). The end point time will be utilized for ppm and % reduction calculations.

10. Calculations

10.1 For each test and control specimen, divide the endpoint reading by the corresponding elapsed time to obtain the ppm of ammonia generated (Eq. 1).

$$\text{ppm} = \frac{(\text{ppm} \cdot \text{h reading})}{\text{h exposure}} \quad (\text{Eq. 1})$$

10.2 If replicates are used, calculate the mean ppm for all test specimens in the sample. Calculate the mean ppm for all control specimens.

10.3 Using the ppm from Eq. 1, calculate the % reduction of ammonia by the treated fabric using Eq. 2.

$$R = 100 \frac{(B - A)}{B} \quad (\text{Eq. 2})$$

where:

R = % reduction

A = mean ppm of ammonia from the treated sample

B = mean ppm of ammonia from the untreated sample (control)

11. Report

11.1 Describe or identify the sample tested.

11.2 Report that the sample was tested using AATCC TM211-2021

11.3 Report the testing conditions:

11.3.1 Control sample used.

11.3.2 Test organism used, including supply source and strain number.

11.4 Report the test results:

11.4.1 Ammonia concentration (ppm) for each test and control specimen.

11.4.2 End point (hours) for each test and control specimen.

11.4.3 Reduction of ammonia (%) by test sample.

12. Precision and Bias

12.1 *Precision.* In 2020, 3 laboratories completed a study with 2 treated fabrics and an untreated control fabric. Fabric A was the control. Fabric B and C had the same antibacterial treatment, but Fabric C was tested after 25 home laundering cycles. Each lab used 3 specimens and 1 operator. The test organism was *Proteus vulgaris* (two different strains).

12.1.1 Table I gives the repeatability and reproducibility for this test method.

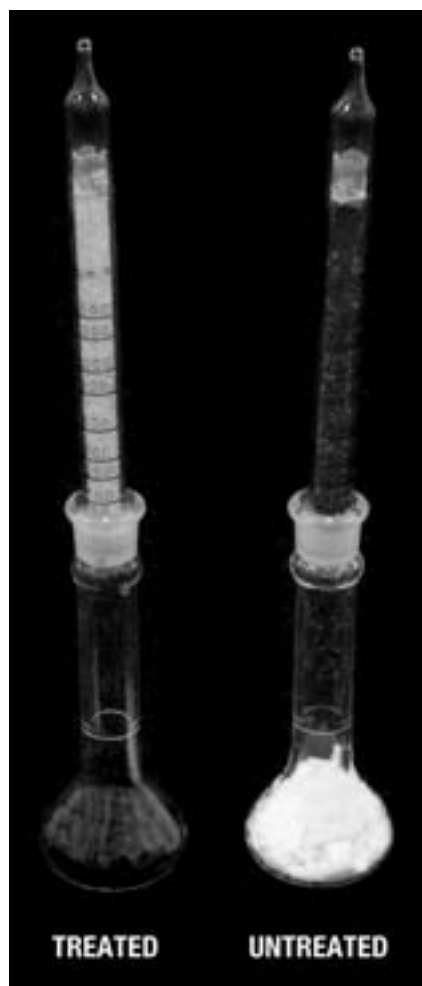


Fig. 1—Test vessels containing adequately treated and untreated fabrics after 6 h of incubation at $37 \pm 2^\circ\text{C}$.