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Standard Guide for Use of Standard Test Methods and Practices for Evaluating Antibacterial Activity on Textiles[1]

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1. Scope

1.1 This guide provides users with an index of procedures in the form of test methods, practices, and related international documents that are currently used in the textile industry for determining antibacterial properties of antimicrobial treated textile articles. This guide is not considered as all-inclusive for antimicrobial testing procedures related to textiles.

1.2 This guide identifies some existing ASTM and other industry standard test methods applicable for testing the antibacterial performance on textiles and discusses options within each method that have been used to address specific end-use performance expectations in addition to measuring wash durability of such activity.

1.3 This guide is intended to assist testing facilities in determining which test methods are appropriate for which treated articles based on type of antimicrobial active involved (diffusible versus non-diffusible), nature of test fabric, and expected end use.

1.4 The test methods indicated in this guide should be performed only by those trained in microbiological techniques, are familiar with textile antimicrobial agents and with the end use exposures of the antimicrobial treated textile material.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E2149 Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions

E2180 Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E3160 Test Method for Quantitative Evaluation of the Antibacterial Properties of Porous Antibacterial Treated Articles

E3162 Practice Measuring the Durability of Antibacterial Agents Applied to Textiles under Simulated Home Laundering Conditions

2.2 *AATCC Standards:*[3]

AATCC Test Method 61: Test Method for Colorfastness to Laundering: Accelerated. American Association of Textile Chemists and Colorists, RTP, NC

AATCC Test Method 90: Antibacterial Activity Assessment of Textile Materials: Agar Plate Method. American Association of Textile Chemists and Colorists, RTP, NC

AATCC Test Method 100: Antibacterial Finishes on Fabrics, Evaluation of. American Association of Textile Chemists and Colorists, RTP, NC

AATCC Test Method 147: Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method. American Association of Textile Chemists and Colorists, RTP, NC

[1] This guide is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Available from AATCC 1 Davis Dr Research Triangle Park, NC 27709-2215 USA. http://www.aatcc.org/

AATCC Test Method 211: Reduction of Bacterial Odor on Antibacterial Treated Textiles. American Association of Textile Chemists and Colorists, RTP, NC

2.3 *ISO Standards:*[4]

ISO 20743 Textiles – Determination of Antibacterial Activity of Antibacterial Finished Products

ISO 22196 Plastics – Measurement of Antibacterial Activity on Plastics Surfaces

2.4 *JIS Standards:*[5]

JIS L 1902 Testing for Antibacterial Activity and Efficacy on Textile Products

JIS Z 2801 Antimicrobial Products – Test for Antimicrobial Activity and Efficacy

2.5 *Other Standards:*

SNV 195920 Examination of the Antimicrobial Effect of Impregnated Textiles by the Agar Diffusion Test [4]

IBRG TEX13/005/1.0 Quantitative Method for Evaluating Bactericidal Activity of Textiles and Porous Materials and Articles[6]

IACM 0600 Standard Operating Procedure for Rapid Sample Rinse[7]

3. Terminology

3.1 For definitions of terms used in this guide see Terminology E2756.

4. Significance and Use

4.1 Antimicrobial agents are routinely used for treating textile materials for the reduction of biodeterioration and bacterial odor generation. Furthermore, textiles are treated to prevent or limit microbial cross-contamination in healthcare settings.

4.2 Antimicrobial agents used in textiles will vary with regard to their broad-spectrum effectiveness, biostatic/biocidal properties, and binding properties in or on particular substrates. When selecting antibacterial test methods as the sole means to predict end use behavior it is critical to understand the intended end use conditions of the treated articles.

4.3 Textile materials differ with regard to the knit/weave, fabric composition, and added functional feature (for example, water repellent, flame retardant, softener, whitener). Each of these factors may alter test results within a given method.

4.4 The test methods indicated below differ mainly in the procedure for inoculating samples, levels of nutrients in the bacterial challenge, organisms used, exposure times, and procedure for sterilization of test samples. Each of these parameters are often subject to industry modifications.

4.5 Some antimicrobial treated articles are not suitable for sterilization due to the sensitivity of these antimicrobial agents to high temperature and humidity. Furthermore, some antimicrobial agents may be unrealistically activated due to UV sterilization which could show false positive antimicrobial properties. Sterilization of test fabrics prior to testing should be avoided if possible. All modifications of the methods indicated below should be clearly indicated on associated test reports and should be appropriate to the antimicrobial technology used.

4.6 This guide is intended to review each commonly used industry test standard for its applicability with an understanding of each of the factors listed above. Further, it is the intention of this guide to indicate commonly used and generally accepted modifications of each standard when measuring specific end-use functionalities.

4.7 These test standards are not, in themselves, absolute indicators of real life performance. Such performance criteria are developed based on a series of antimicrobial and analytical test methods in addition to simulated real life use studies. All antimicrobial agents used for the treatment of textiles should be compliant with local regulatory agencies and should be deemed safe for the proposed end-use and claims.

5. Qualitative Antimicrobial Test Methods for Textiles

5.1 ***AATCC TM 147***—is a qualitative test to measure antibacterial activity of diffusible antimicrobial agents on treated textile material.

5.1.1 *Significance and Use*—The objective is to detect bacteriostatic activity on textile materials. The method is useful for obtaining a rough estimate of activity in that the growth of the inoculum organism decreases from one end of each streak to the other and from one streak to the next resulting in increasing degrees of sensitivity. The size of the zone of inhibition and the narrowing of the streaks caused by the presence of the antibacterial agent permit an estimate of the residual antibacterial activity after multiple washes.

5.1.2 Typical industry modifications include the use of multiple microbial organisms on a single plate. While the test method was developed to obtain a rough estimate of activity of a treated article by systematically decreasing the dose of organism across the surface of an agar plate, so too can this method be used as a fast determination of broad spectrum activity if multiple organisms are used. In many cases, four organisms are streaked lengthwise per agar plate (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans*) with a test fabric strip placed at 90° on the agar surface across streaks if multiple organisms are used. In many cases, four organisms are streaked lengthwise per agar plate (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans*) with a test fabric strip placed at 90° on the agar surface across streaks.

5.1.3 *Evaluation of the Test includes Determination of a Zone of Inhibition (ZOI)*—The width of the inhibition zone away from the treated substrate (in millimetres) or an evaluation of the level of growth underneath the test substrate. Care must be taken when evaluating growth directly underneath the sample. Some materials, including plastics and films, can make such intimate contact with the agar surface that no microbial growth is observed underneath the sample. It is recommended

[4] Available from International Organization for Standardization (ISO), 1, ch. de la Voie-Creuse, CP 56, CH-1211 Geneva 20, Switzerland, http://www.iso.org.

[5] Available from Japanese Industrial Standards Committee (JIS) 1-3-1 Kasumigaseki, Chiyoda-ku, Tokyo 100-8901, JAPAN. http://www.jisc.go.jp

[6] Available from the International Biodeterioration Research Group (IBRG), Pale Lane, Hartley Wintney, Hants, UK RG27 8DH. http://www.ibrg.org.

[7] Available from the International Antimicrobial Council (IAC), 2298 N. Eastman, Midland, MI 48642 USA. http://www.amcouncil.org.

to compare results of treated samples to an untreated sample composed of the same type of material to avoid false positives.

5.1.4 Lack of a ZOI does not necessarily indicate that the treated material does not contain an antimicrobial agent. In some cases, the nutrients available in agar medium or the agar matrix itself can deactivate the antimicrobial agent, leading to false-negative results. Alternative methods such as the Test Method E2149 or the Test Method E3160/AATCC 100 with low to no nutrient inoculum conditions can better define activity for those antimicrobials that are bound by nutrients or agar.

5.1.5 Growth directly under a test fabric does not necessarily indicate that the treated material does not contain an antimicrobial agent. If direct contact with the treated textile is required, bacteria may grow directly under the treated substrate without the needed intimate contact with the treated substrate. Alternative quantitative methods indicated below may be more appropriate for antimicrobial agents that are not diffusible into the surrounding medium.

5.1.6 Many test methods incorporate the agar based methodology for determining ZOI activity. AATCC TM 90, SNV 195920 and JIS L 1902 are examples of international standards that contain aspects of measuring zone of inhibition in an agar medium.

5.1.7 This test method can be a good bioassay method for detecting antimicrobial activity compared to untreated controls and is appropriate for use in quality control test programs.

6. Quantitative Antimicrobial Test Methods for Textiles

6.1 ***AATCC TM 100***—is designed to measure the antimicrobial activity of textiles after direct inoculation of the textile surface.

6.1.1 *Significance and Use*—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. After exposure, the bacterial challenge is eluted from the swatches and enumerated. The percent reduction of bacteria of the test fabric after 24 h versus the initial inoculum or a relative untreated control after identical contact time is calculated.

6.1.2 This method is written to provide a 24 h contact of the bacteria with the treated surfaces. Increased and decreased times may be used if accompanied by appropriate control fabrics to ensure the survival of the organism on the surface without treatment.

6.1.3 This original method indicated that the bacterial inocula should be prepared in full-strength nutrient broth. In the most recent update (2019), the levels of nutrients in this test have been changed to specify a dilute nutrient solution (1:20 full-strength Nutrient Broth). Diluted nutrient solutions have been shown to promote slight to moderate microbial growth over the 24 h contact time on a specified viability control fabric while not significantly affecting the antimicrobial additive on/in the fabric.

6.1.4 The method often requires modification for testing of hydrophobic samples. Options include the use of plastic films or cover slips to promote more intimate contact of the inoculum to the treated surface on non-absorbent or highly hydrophobic surfaces. This modification creates methodology similar to the ISO 22196 and JIS Z2801 test methods.

6.1.5 The use of neutralizers in the recovery broth is essential in order to deactivate any remaining antimicrobial agents which may carry over in the dilution tubes. The neutralizer should be selected based on its performance against the antimicrobial agent in question.

6.1.6 This test method is a good bioassay method for detecting biocidal activity compared to the initial inoculum (Time 0) or biostatic activity compared to an untreated control and is appropriate for use in quality control test programs.

6.1.7 This test method includes the use of both Gram positive bacteria (*Staphylococcus aureus*) and Gram negative bacteria. While the Gram negative *Klebsiella pneumoniae* is specified, many laboratories will substitute the Gram negative *Escherichia coli* as it has been shown to provide better repeatability. A standard industrial practice is to combine both Gram positive and Gram negative organisms into a single mixed inoculum. Retrieval on selective media has been shown to be effective at identifying both organisms while not affecting the overall antimicrobial reduction levels.

6.1.8 This test method requires the addition of an untreated Viability Control Fabric (VCF) to ensure that conditions are met that will provide microbial growth if possible. The VCF must demonstrate at least 1.5 log bacterial growth over the 24 h contact period under the parameters outlined in the method.

6.1.9 Sterilization of textile samples using steam autoclaving prior to microbiological testing can eliminate any inherent contamination on the sample, but also may alter the antimicrobial agent, resulting in either false positive or false negative results. If sterilization is performed, the method and reason for sterilization must be noted on the test report.

6.2 ***AATCC TM 211***—is designed to measure the antimicrobial and anti-odor activity of textiles after direct inoculation of the textile surface with specific surrogate organisms and nutrient solution and subsequently measuring the ammonia generated due to the bioconversion of urea to ammonia using a volatiles detection system.

6.2.1 *Significance and Use*—This test method provides a quantitative procedure for the comparison and evaluation of the degree of bacterial odor generation after a 20 ± 2 h exposure to the test bacteria on treated and untreated test fabric. Lower incubation times (6 to 8 h) have also been used and have been shown to effectively demonstrate anti-odor properties on antimicrobial treated textiles. This method is designed to measure the microbial generation of ammonia due to the bioconversion of urea using simple, inexpensive gas detection tubes.

6.2.2 This method is written to provide direct contact between specific ammonia generating bacteria and textiles treated and untreated with antimicrobial agents.

6.2.3 Specific ammonia generating organisms are employed as surrogate test organisms, taking advantage of the species' ability to produce ammonia while metabolizing urea or other protein residues. Ammonia is typically identified as a problem odor on textiles. Generation of ammonia on untreated textiles is a general indication of the presence of actively metabolizing bacteria. Likewise, lack of (or reduced) ammonia generation

compared directly to an identical untreated control fabric, indicate the control of bacterial metabolism on a treated textile surface.

6.2.4 The test method includes the use of the Gram positive bacteria *Staphylococcus saprophyticus* and the Gram negative bacteria *Proteus vulgaris*. These organisms were selected due to their ability to produce ammonia under these defined test conditions. Other bacteria that can produce a metabolic by-product which can be measured using a different Drager Tube may be used but these variations must be reported and recognized as a modification of this test method.

6.2.5 This test method does not directly measure biocidal activity on a treated textile but rather is predictive of biostatic properties provided by the addition of an antimicrobial agent.

6.2.6 This test method requires the addition of an untreated Viability Control Fabric (VCF) to ensure that conditions are met that will provide microbial growth if possible. The VCF should exceed the 1500 ppm ammonia limit on the Drager tubes within the 8 h incubation period.

6.2.7 Sterilization of textile samples using steam autoclaving prior to microbiological testing can eliminate any inherent contamination on the sample, but also may alter the antimicrobial agent, resulting in either false positive or false negative results. If sterilization is performed, the method and reason for sterilization must be noted on the test report.

6.3 ***Test Method E2149***—is designed to measure the antimicrobial activity of non-diffusible antimicrobial agents.

6.3.1 *Significance and Use*—Immobilized (cross-linked) antimicrobial agents are not free to diffuse into their environment under normal conditions of use. Textile methods, such as AATCC TM 147, that are directly dependent on the ready leachability of the antimicrobial agent from the treated fabric are inappropriate for evaluating immobilized antimicrobial agents. This test method ensures good contact between the bacteria and the treated fiber, fabric, or other substrate, by constant agitation of the test specimen in a challenge suspension during the designated 1-h contact test period.

6.3.2 Although this method is most appropriate for non-diffusible antimicrobial agents, such as the silane-quat technologies, it has been used to measure the efficacy of diffusible antimicrobial agents. However, the efficacy measured with diffusible antimicrobial agents will be a combination of efficacy from direct contact of microbes with the treated material and the efficacy of the agent in the buffer system after release from the treated material. It is recommended to use alternate test methods with antimicrobial agents that readily diffuse into the surrounding buffer system.

6.3.3 Typical industry modifications include the use of alternative bacterial or fungal species. Care must be used to ensure that all controls are used to indicate survival of the test organism on the recovery medium and in the untreated fabric control.

6.3.4 This method was originally written to provide 1-h contact between the microbial suspension and the treated article (Test Method E2149 – 10). In some cases, extended times (6 to 24 h) are required in order to demonstrate the full antimicrobial potential of a treated surface. However, great care must be taken to ensure survival of the organism over an extended time period. Untreated control samples are critical when measuring extended time periods in order to differentiate between the antimicrobial properties of the treated fabric and potential background activities resulting from other added functional features. If these or alternative contact times are used, this must be indicated as a Modification and listed appropriately in the test report.

6.3.5 This test method was originally designed to measure the activity of immobilized antimicrobial agents, therefore, the use of a neutralizer during the recovery step was not needed as no antimicrobial agent would be carried over into the dilution broth. The Test Method E2149 – 13 version includes the ability to measure diffusible or migrating types or unknown antimicrobial agents. If these agents are tested in this method, neutralizers must be added within the dilution step.

6.3.6 Sterilization of textile samples using steam autoclaving or UV prior to microbiological testing can eliminate any inherent contamination on the sample, but also may alter the antimicrobial agent, resulting in either false positive or false negative results. If sterilization is performed, the method and reason for sterilization must be noted on the test report.

6.3.7 This test method is a good bioassay method for detecting antimicrobial activity compared to untreated controls and is appropriate for use in quality control test programs.

6.4 ***Test Method E2180***—is specifically designed to measure the antimicrobial activity of highly hydrophobic surfaces.

6.4.1 *Significance and Use*—This method can be used to evaluate effectiveness of incorporated/bound antimicrobials in hydrophobic materials such as plastics, epoxy resins, as well as other hard surfaces. The aqueous based bacterial inoculum remains in close, uniform contact in a “pseudo-biofilm” state with the treated material. The percent reduction in the surviving populations of challenge bacterial cells at 24 h versus those recovered from a non-treated control is determined.

6.4.2 Test Method E2180 is designed to overcome hydrophobicity issues with treated material that could prevent contact between the treated substrate and the bacterial challenge. This method overcomes this contact issue by placing the bacterial challenge within an agar slurry which can then have direct contact with the treated surface.

6.4.3 This test method relies on the ability of the active antimicrobial agent to diffuse through the agar medium to reach the suspended bacterial challenge. Lack of antimicrobial activity in this method does not necessarily indicate that the treated material does not contain an antimicrobial agent.

6.4.4 Typical industry modifications include changes to the time points and challenge organisms examined during the test to those relevant for the intended use pattern of the textile.

6.4.5 Sterilization of textile samples using steam autoclaving or UV prior to microbiological testing can eliminate any inherent contamination on the sample, but also may alter the antimicrobial agent, resulting in either false positive or false negative results. If sterilization is performed, the method and reason for sterilization must be noted on the test report.

6.4.6 This test method is a good bioassay method for detecting antimicrobial activity compared to untreated controls and is appropriate for use in quality control test programs.