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Standard Guide for The 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.

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 and health practices and determine the applicability of regulatory limitations prior to use.*

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

E2722 Test Method for Using Seeded-Agar for the Screening Assessment of Antimicrobial Activity in Fabric and Air Filter Media

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *AATCC Standards:*[3]

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

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

[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/

[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

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, and exposure times.

4.5 This guide is intended to review each commonly used industry standard test method 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 method when measuring specific end-use functionalities.

4.6 These test methods 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 degrees 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 degrees 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 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 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 L1902 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.

5.2 ***Test Method E2722***—is designed to evaluate qualitatively the presence of antibacterial and antifungal activity in or on fabrics or air filter media.

5.2.1 *Significance and Use*—This test method provides for rapid screening of antimicrobial treatments located in or on fabrics and air filter media. The method simulates actual use conditions that may occur on fabrics and provides a means to screen for activity and durability of an antimicrobial treatment under conditions of organic loading.

5.2.2 Typical industry modifications include the use of multiple microbial organisms.

5.2.3 This test method provides for a simultaneous assessment of multiple fabric components for antimicrobial activity, for example, fabric, component fibers with polymer incorporated treatments, and back coating if present.

5.2.4 This test method may not be suited for covalently bonded (non-soluble or non-leaching) antimicrobials such as silane-modified quaternary ammonium compounds or antimicrobials with limited migration through nutrient agar.

5.2.5 Growth next to a test fabric does not necessarily indicate that the treated material does not contain an antimicrobial agent. If direct contact with the treated textile or some migration of the antimicrobial agent into the nutrient agar is required, bacteria may grow next to the test material without the needed intimate contact with the treated substrate. Alternative quantitative methods indicated below may be more appropriate for antimicrobial agents that are not readily diffusible into the surrounding medium.

5.2.6 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 (that is, recovery at Time 0) is calculated.

6.1.2 This method was originally 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 Although the current method appears to indicate that the bacteria inocula should be prepared in full-strength nutrient broth, the levels of nutrients in this test often are modified to a dilute nutrient solution (for example, 1:20 or 1:500 dilution of full-strength Nutrient Broth) or a non-nutrient solution such as water, saline or phosphate buffer. Diluted nutrient solutions have been shown to promote slight to moderate microbial growth over the 24 h contact time. It is recommended to include the inoculum carrier solution that was utilized in the test in the test report to allow readers to compare results among different test labs.

6.1.4 The method often requires modification for testing of hydrophobic samples. Options include suspending the bacteria in a dilute agar slurry or 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 test method.

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.

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 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. It is recommended to avoid steam sterilization of samples unless absolutely necessary.

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

6.2.1 *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.

6.2.2 Although this method is most appropriate for non-diffusible antimicrobial agents, it can be 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.

6.2.3 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.

6.2.4 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 (up 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.

6.2.5 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. 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.

6.2.6 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. It is recommended to avoid steam sterilization of samples unless absolutely necessary.

6.2.7 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.

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

6.3.1 *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.

6.3.2 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.

6.3.3 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.

6.3.4 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.

6.3.5 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. It is recommended to avoid steam sterilization of samples unless absolutely necessary.

6.3.6 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.

6.4 ***ISO 20743/JIS L1902***—is designed for the determination of antibacterial activity of antibacterial finished products on absorbent or porous textiles. The methods also can be applied to other materials such as polyurethane foams. Both methods are very similar with the exception of alternative bacterial enumeration options within the JIS methods.

6.4.1 *Significance and Use*—This International Standard specifies quantitative test methods to determine the antibacterial activity of antibacterial finished textile products including nonwovens.

6.4.2 This method is composed of three different inoculation methods that are used based on the intended application and on the environment in which the textile product is used. The user can select the most suitable of the three methods which include an absorption method, transfer method and printing method.

6.4.3 Bacterial inocula are prepared in a 1:20 dilution of Nutrient Broth in "purified water". However modifications of the nutrient loadings and substitution of the diluent (saline or buffer instead of de-ionized or distilled water) are often made similar to the AATCC 100 test method above and IBRG TEX13 mentioned below (6.1.4 and 6.6.2 respectively). As with all of the methods described in this guide, it is recommended that the test lab include the details of the inoculum preparation and dilution in the test report.

6.4.4 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. It is recommended to avoid steam sterilization of samples unless absolutely necessary.

6.4.5 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.

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.

6.5 ***ISO 22196/JIS Z 2801***—is designed for the determination of antibacterial activity on plastic surfaces but has been modified to include many other non-porous surfaces including textiles and coated substrates. Both methods are very similar with the exception of alternative bacterial enumeration options within the JIS methods.

6.5.1 *Significance and Use*—This International Standard specifies a method of evaluating the antibacterial activity of antibacterial-treated plastic products (including intermediate products). It may also be suitable for other non-porous materials such as textiles.

6.5.2 The method uses a slightly lower level of nutrients in the test inoculum (1:500 nutrient dilution) and is intended to be used on non-absorbent materials. In these scenarios, a liquid inoculum is placed directly onto the treated article. A sanitized plastic cover slip/film is placed directly on top of the inoculum in order to evenly distribute the inoculum across the treatment surface. After a specified time (usually 24 h and a relative humidity of not less than 90%), the sample is removed and the remaining viable cells are quantified.

6.5.3 With appropriate modifications such as increasing viscosity of the inoculum by the addition of agar (See Test Method E2180 or by decreasing the inoculum volume, this method can be used to measure the antimicrobial activity of highly hydrophobic surfaces.

6.5.4 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. It is recommended to avoid steam sterilization of samples unless absolutely necessary.

6.5.5 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.

6.5.6 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.

6.6 ***IBRG TEX13/005/1.0***—is a method that determines the basic antibacterial properties of textiles and porous materials and articles treated with a biocide with the intention of introducing disinfectant/hygienic properties.

6.6.1 *Significance and Use*—This method was developed from two ISO standards (ISO 22196 and the "germ count" section of ISO 20743) by harmonizing the testing parameters described in them. These modifications were validated by two,

statistically-designed international ring tests conducted by the International Biodeterioration Research Group (IBRG).

6.6.2 A liquid suspension of bacteria is applied to materials both treated and untreated with antimicrobial finishes. Replicate samples of each material are inoculated with a specified number of bacteria suspended in a solution containing a low concentration of nutrients (1:500 dilution).

6.6.3 The samples are incubated under conditions of controlled temperature and humidity for 24 h.

6.6.4 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.

6.6.5 International ring tests specifically used both *E. coli* and *Staphylococcus aureus* as test organism. Alternative organisms may be used as long as recovery media and cultivation techniques are accommodated accordingly.

6.6.6 Changes in the sizes of populations of the test organisms are calculated in relation to the numbers present on the untreated variants or in relation to the total number of organisms applied to the sample, or both, and are expressed as log reductions.

6.6.7 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. It is recommended to avoid steam sterilization of samples unless absolutely necessary.

6.6.8 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.

7. Keywords

7.1 antimicrobial; antibacterial; neutralizer; inoculum bioload; leaching; migrating; immobilized; cross-linked; nonleaching; textiles and textile surfaces