Designation: E2149 – 13

StandardTest Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents Under Dynamic Contact Conditions[1]

This standard is issued under the fixed designation E2149; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method is designed to evaluate the antimicrobial activity of non-leaching, antimicrobial-treated specimens under dynamic contact conditions. This dynamic shake flask test was developed for routine quality control and screening tests in order to overcome difficulties in using classical antimicrobial test methods to evaluate substrate-bound antimicrobials. These difficulties include ensuring contact of inoculum to treated surface (as in AATCC 100), flexibility of retrieval at different contact times, use of inappropriately applied static conditions (as in AATCC 147), sensitivity, and reproducibility.

1.2 This test method allows for the ability to evaluate many different types of treated substrates and a wide range of microorganisms. Treated substrates used in this test method can be subjected to a wide variety of physical/chemical stresses or manipulations and allows for the versatility of testing the effect of contamination due to such things as hard water, proteins, blood, serum, various chemicals, and other contaminants.

1.3 Surface antimicrobial activity is determined by comparing results from the test sample to controls run simultaneously.

1.4 The presence of an antimicrobial that requires neutralization is determined by the post-test.

1.5 Proper neutralization of all antimicrobials must be confirmed using Test Methods E1054.

1.6 This test method should be performed only by those trained in microbiological techniques.

1.7 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.8 *This standard may involve hazardous materials, operations, and equipment. 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]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

2.2 *AATCC Documents:*[3]

AATCC 147 Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method

AATCC 100 Antibacterial Finishes on Fabrics

3. Summary of Test Method

3.1 The antimicrobial activity of a substrate-bound, non-leaching antimicrobial agent is dependent upon direct contact of microbes with the active chemical agent. This test determines the antimicrobial activity of a treated specimen by shaking samples of surface-bound materials in a concentrated bacterial suspension for a one hour contact time. The suspension is serially diluted both before and after contact and cultured. The number of viable organisms from the suspension is determined and the percent reduction (or $\log_{10}$ reduction) is calculated by comparing retrievals from appropriate controls.

4. Significance and Use

4.1 Chemically bonded, antimicrobial agents are not free to diffuse into their environment under normal conditions of use. 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 test period.

[1] This test method 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 American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709, http://www.aatcc.org.

4.2 The metabolic state of the challenge species can directly affect measurements of the effectiveness of particular antimicrobial agents or concentrations of agents. The susceptibility of the species to particular biocides could be altered depending on its life stage (cycle). One-hour contact time in a buffer solution allows for metabolic stasis in the population. This test method standardizes both the growth conditions of the challenge species and substrate contact times to reduce the variability associated with growth phase of the microorganism.

4.3 Leaching of an antimicrobial is dependent upon the test conditions being utilized and the ultimate end use of the product. For example, water soluble antimicrobials will be prone to removal from the test surface using the method described in Section 13 but insoluble compounds will not. It is for this reason that the use of the term leaching throughout this document is limited to only the testing conditions described herein. To determine if a compound is immobilized in all conditions or during the end use of the product additional testing may be required.

4.4 This test method cannot determine if a compound is leaching into solution or is immobilized on the substrate. This test method is only intended to determine efficacy as described in 4.5 and subsequent portions of the method.

4.5 This test method is intended to evaluate antimicrobial agents that are not removed from the surface by the aqueous testing conditions, as evaluated by Section 13. If an antimicrobial agent that is shown to be removed from the surface by Section 13 is utilized in this test methodology, controls must be included such that appropriate neutralization steps are including during recovery and enumeration.

4.6 The test is suitable for evaluating stressed or modified specimens, when accompanied by adequate controls.

NOTE 1—Stresses may include laundry, wear and abrasion, radiation and steam sterilization, UV exposure, solvent manipulation, temperature susceptibility, or similar physical or chemical manipulation.

5. Definitions

5.1 *Immobilized:* The antimicrobial remains on the surface of the article throughout the test as determined by the absence of bactericidal activity in Section 13. A neutralizer does not need to be included for this type of antimicrobial

5.2 *Leaching:* Removal of the antimicrobial from the surface by the test conditions being utilized, resulting in a concentration high enough to cause bactericidal activity as defined in Section 13. A valid neutralizer must be utilized for this type of antimicrobial

6. Apparatus

6.1 *Air displacement pipettes,* Eppendorf or equivalent, 100 to 1000 µL with disposable tips.

6.2 *Analytical balance,* to weigh chemicals and substrates and to standardize inoculum delivery volumes by pipettes.

6.3 *Glassware:*

6.3.1 *Contact Flask,* 250 mL Erlenmeyer flask, capped, autoclavable.

6.3.2 *Test tubes,* 18 × 150 mm rimless bacteriological test tubes used for growing test organisms and for serial dilution.

6.4 *Incubator,* capable of maintaining a temperature of 35 ± 2°C.

6.5 *Shaker, wrist action,* capable of aggressive agitation of bacteria and substrate solutions.

6.6 *Spectrophotometer,* capable of measuring an absorbance of 475 nm.

6.7 *Sterile serological pipettes,* capable of 50 and 10 mL capacity.

6.8 *Sterilizer,* any suitable steam sterilizer producing the conditions of sterility.

6.9 *Vortex mixer,* to vortex dilution tubes during serial dilutions.

6.10 *Water bath,* for short term storage of liquefied agar media, capable of maintaining 45 to 50°C.

7. Reagents

7.1 *Buffer Solution*—The following solution is prepared from reagent-grade chemicals. For buffer stock solution (0.25M KH_2PO_4): Prepare a fresh stock solution at least once every 6 months as follows: Weigh 34 ± 0.1 g of potassium dihydrogen phosphate into a 1000 mL beaker. Add 500 mL of distilled water. Adjust pH to 7.2 ± 0.1 with a dilute solution of NaOH. Dilute to 1000 mL; transfer to a flask and store at 4°C. For working buffer solution (0.3mM KH_2PO_4): Prepare a fresh solution at least once every 2 months as follows: Transfer 1 ± 0.01 mL of stock buffer solution with a sterile pipette to flask containing 800 mL of distilled water. Cap, sterilize and store at room temperature.

7.2 *Media:*

7.2.1 *Tryptic Soy Broth,* prepared according to manufacturer's directions.

7.2.2 *Plate Count Agar,* prepared according to manufacturer's directions.

7.3 *Wetting Agent Surfactant*—Agents must be shown by prior testing at the intended use concentration not to cause a reduction or increase in bacterial numbers. DC Q2-5211[4] at 0.01 % final dilution of working buffer solution has been shown to be effective.

8. Test Organism

8.1 *Escherichia coli,* American Type Culture Collection No. 25922.

8.1.1 Cultures of the test organism should be maintained according to good microbiological practice and checked for purity on a routine basis. Consistent and accurate testing requires maintenance of a pure, uncontaminated test culture. Avoid contamination by use of good sterile technique in plating and transferring. Avoid mutation or reversion by strict adherence to monthly stock transfers. Check culture purity by

[4] The sole supplier of DC Q2-5211 known to the committee at this time is Dow Corning, Midland, MI. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.