Designation: ~~E2149 – 10~~ E2149 – 13

Standard Test 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 ~~100-2004),~~100), flexibility of retrieval at different contact times, use of inappropriately applied static conditions (as in AATCC ~~147-2004),~~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 ~~a leaching antimicrobial is determined both pre- and~~ 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 ~~147-2004~~147 Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method

AATCC ~~100-2004~~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.

---

[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.

Current edition approved ~~May 1, 2010~~July 1, 2013. Published ~~May 2010~~December 2013. Originally approved in 2001. Last previous edition approved in ~~2001~~2010 as ~~E2149 – 01~~E2149 – 10.~~, which was withdrawn in 2010 and reinstated in May 2010. DOI: 10.1520/E2149-10.~~ DOI: 10.1520/E2149-13.

[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. Significance and Use

4.1 ~~Immobilized, as chemically~~ Chemically bonded, antimicrobial agents are not free to diffuse into their environment under normal conditions of use. ~~Textile test methods, such as AATCC 147-2004, 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 test period.

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 ~~Liquid analysis~~ Leaching of ~~antimicrobial activity of non-leaching agents provides the ability to completely wet-out a test substrate. With the~~ 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~~use of wetting-agent surfactants, false negatives observed when comparing hydrophobic and hydrophilic substrates can be reduced.~~ 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 ~~is not intended for directly comparing the activities of leaching and non-leaching antimicrobial agents. In liquid environments, leaching biocides may release the active ingredient at differential rates. Furthermore, residual antimicrobial activity of leaching biocides may be present in serial dilution and may exert additional activity after desired contact time, unless adequately sequestered at end of test. Controls for both of these factors are not included in this test method; therefore, screening protocols are introduced to identify the presence~~ 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~~of leaching biocides.~~ 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

~~5.1 *Agar bore,* 8-mm diameter.~~

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 making streak plates periodically, observing for colonies characteristic of *Escherichia coli*, and Gram-staining.

~~NOTE 2—Original method, ASTM E2149-01, specified *Klebsiella pneumoniae* as test organism. *Escherichia coli* is used in this test method as it is easier to handle and is a more universally accepted test type organism.~~

8.1.2 Alternative organisms can be substituted depending on the end use of the product. However, the precision and bias statement has been developed using *Escherichia coli* ATCC 25922. There is are no data to support a precision and bias statement for other organisms at this point. Use of alternate organisms shall be included in the report, in addition to any other modification of media, buffer, bacterial concentration, etc.

9. Parameters

9.1 Surface preparation or conditioning must be specified. Prior manipulation of the specimen may be required in order to demonstrate maximum activity in a desired time frame and must be reported and compared to identically handled controls.

9.2 The weight, size, and material of construction of specimen must be specified.

9.3 Specimens should be prepared such that they can maximize agitation and are reflective of a recordable ratio of surface area to test titer.

10. Preparation of Bacterial Inoculum

10.1 Grow a fresh 18 h shake culture of *Escherichia coli* in sterile Tryptic Soy Broth at 35 ± 2°C prior to performing the test.

10.2 Dilute the culture with the sterile buffer solution until the solution has an absorbance of 0.28 ± 0.02 at 475 nm, as measured spectrophotometrically. This has a concentration of 1.5-3.0 × 10^8 CFU/mL. Dilute appropriately into sterile buffer solution to obtain a final concentration of 1.5-3.0 × 10^5 CFU/mL. This solution will be the working bacterial dilution.

11. Test Specimen

11.1 *Preparation of Test Specimen:*

11.1.1 *Fabric and Paper*—Samples are selected on weight basis and weighed to 1.0 ± 0.1 g.

11.1.2 *Powder and Granular Material*—Weigh to 1.0 ± 0.1 g. The material must settle after shaking so that no specimen interferes with the retrieval and counting techniques.

11.1.3 *Other Solids (Surface Treatment)*—Reduce the solid in size to fit into the flask or use a sterile wide-mouth bottle. Use a specimen that gives 4 in.^{2} (25.8 cm^2) of treated surface area. Specimen may also be selected on weight basis, ±0.1 g, at the discretion of the investigator. Care must be exercised during shaking not to break the flask or bottle. The untreated specimen of the solid must not absorb the solution. If appropriate to the nature of the test specimen, it can be mounted as a seal for the test container so that only the treated surface is in direct contact with the inoculum.

NOTE 2—Solids anticipated in this part of the method are plastics, glass beads or chips, ceramics, metal chips, or similar hard surfaces. Sample mass

[4] The sole ~~source of supply of the apparatus~~ 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.