Designation: E3363 – 23

Standard Test Method for Quantitative Performance Evaluation of Antimicrobial Towelettes[1]

This standard is issued under the fixed designation E3363; 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 quantitatively determines the effectiveness of various sizes of antimicrobial towelettes in treating hard, non-porous surfaces against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

1.2 This test method may be used to evaluate towelettes for antimicrobial efficacy against additional microorganisms (with necessary modifications).

1.2.1 This test method does not differentiate between chemical inactivation of the test microbe and mechanical removal of inoculum from a surface; rather, product efficacy is considered a combination of both attributes of a towelette-based formulation.

1.3 This test method involves the use of hazardous materials, chemicals, and infectious microorganisms and therefore should be performed only by those trained in microbiological techniques in facilities designed and equipped for work with infectious agents at the appropriate biosafety level, a BSL-2 or higher laboratory; specifications provided in the "Biosafety for Biomedical and Microbiological Laboratories" (BMBL), 6th edition (BMBL).

1.4 It is the responsibility of the investigator to determine whether Good Laboratory Practices (GLP Standards—For example, 40 CFR, Part 160 of FIFRA) are required and to follow them when appropriate.

1.5 Strict adherence to the protocol is necessary for the validity of the test results.

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

1.7 *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.*

1.8 *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]

D5465 Practices for Determining Microbial Colony Counts from Waters Analyzed by Plating Methods

E1054 Practices for Evaluation of Inactivators of Antimicrobial Agents

E2362 Practice for Evaluation of Pre-saturated or Impregnated Towelettes for Hard Surface Disinfection

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *AOAC Standards:*[3]

Official Method 955.15 Use-Dilution Method for Testing Disinfectants against Staphylococcus aureus. Revised 2013

Official Method 964.02 Use-Dilution Method for Testing Disinfectants against Pseudomonas aeruginosa. Revised 2013

2.3 *Centers for Disease Control:*[4]

Biosafety in Microbiological and Biomedical Laboratories 6th Edition, U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health, HHS Publication No. (CDC) 21- 1112, Revised June 2020.

2.4 *U.S. Government Regulations:*[5]

40 CFR Part 160 Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards

[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 Sept. 1, 2023. Published September 2023. DOI: 10.1520/E3363–23.

[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] For referenced AOAC standards, visit the AOAC website, www.aoacofficialmethod.org/index.

[4] Available from https://www.cdc.gov/labs/BMBL.html.

[5] Available from U.S. Government Publishing Office (GPO), 732 N. Capitol St., NW, Washington, DC 20401, http://www.gpo.gov.

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard refer to Terminology E2756.

3.1.2 *antimicrobial towelette (wipe), n*—a piece of porous material pre-saturated or impregnated with an antimicrobial liquid that is meant for decontamination of environmental surfaces by wiping.

3.1.3 *carriers, n*—sterile plastic (for example, polystyrene) Petri plates (150 mm by 15 mm).

3.1.4 *colony forming unit (CFU), n—in microbiology*, a visible mass of cells (algae, bacteria, or fungi) originating from either an individual cell or cluster of cells that have been placed onto or dispersed into a solid or semi-solid nutrient medium and subsequently incubated under prescribed conditions.

3.1.5 *diluted test suspension, n*—microbial suspension with no soil.

3.1.6 *dilution blank, n*—tubes of phosphate buffered saline (PBS), phosphate buffered dilution water (PBDW), or similar inert phosphate buffer solution.

3.1.7 *final test suspension, n*—microbial suspension with the 3-part soil load (25 µL BSA stock, 35 µL yeast extract stock, 100 µL mucin stock, and 340 µL microbial test suspension).

3.1.8 *quality control (QC), n*—the procedures, products or services that meet a laboratory's specified standards of quality.

3.1.9 *soiled carrier, n*—Petri plates (see 3.1.3) spotted with sterile 5 % non-heat inactivated fetal bovine serum.

3.1.10 *soil suspension, n*—3-part soil load, comprised of 25 µL BSA stock, 35 µL yeast extract stock, 100 µL mucin stock.

NOTE 1—Fetal bovine serum or other animal serum as desired may be added to the bacterial suspension to achieve the desired level of soil depending on the target regulatory agency and claim desired.

3.1.11 *treated carrier, n*—Petri plates (see 3.1.3) inoculated with final test suspension.

3.1.12 *two carrier (2-carrier) set, n*—one dried soiled carrier and one dried treated carrier.

4. Summary of Test Method

4.1 This test method provides detailed instructions for performing a quantitative evaluation of antimicrobial efficacy of a towelette when challenged against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The test method may be evaluated with additional microorganisms, though modification might be necessary to accommodate recovery of surviving organism.

4.2 Petri plates, spotted with a suspension of sterile 5 % non-heat-inactivated fetal bovine serum, are used as the soiled carriers. Each soiled carrier is spotted with five spots (10 µL each) of sterile 5 % non-heat-inactivated fetal bovine serum and allowed to dry.

4.3 Petri plates, inoculated with a suspension of vegetative bacteria and 3-part soil, are used as the treated carriers. Each treated carrier is inoculated with five spots (10 µL each) of the final test suspension and allowed to dry.

4.4 One dried soiled carrier and one dried treated carrier are together referred to as a 2-carrier set.

4.5 The dried carriers are exposed to the test substance by applying a pre-saturated towelette to the inner bottom surface of the carriers using a prescribed pattern of wiping, and the treated carrier is allowed to stand for a specific contact time. At the end of the contact time, two aliquots of an appropriate neutralizer are added to the treated carrier, one prior to the initial scraping of the carrier surface and the second aliquot prior to the secondary scraping. Both done with a sterile cell scraper at each instance to dislodge and suspend inoculum remaining on the surface. The inoculum-neutralizer suspension is collected, diluted, and enumerated using membrane filtration and plated onto recovery media (Tryptic Soy Agar).

4.6 Control and treated carrier CFUs are enumerated using membrane filtration (Practice D5465).

4.7 The mean $\log_{10}$ density (LD) recovered from treated carriers is compared to the mean $\log_{10}$ density recovered from the control carriers. This calculation is used to determine the efficacy of the product based on the mean $\log_{10}$ reduction (LR) value.

5. Significance and Use

5.1 The plastic Petri plate (carrier) provides a closed system for enumeration and easy application of a pre-saturated or impregnated antimicrobial towelette by an analyst.

5.2 Aliquoting of sterile 5 % non-heat-inactivated fetal bovine serum (five 10 µL spots) onto soiled carriers and inoculation of final test suspension onto treated carriers (five 10 µL spots) is conducted using a template and a positive displacement pipette, thereby ensuring a precise inoculum level and uniform distribution of soil and final test suspension.

5.3 A single towelette is tested per 2-carrier set, eliminating the likelihood of cross contamination between carriers.

5.4 The corkscrew-patterned circular motion of the product application (wipe outside to inside, wipe inside to outside using the wiping template; see Annex A3 – Annex A6) ensures uniform coverage and contact of disinfectant with the inoculated surface.

5.5 The addition of neutralizer to the treated carriers at the end of the contact time results in neutralization of the test substance. This standard test method provides a procedure for performing neutralization verification to confirm that the microbicidal, microbistatic, or both types of activity of a test substance has been reduced by 50 % at the end of the contact time (see Annex A1 for neutralization verification procedure).

5.6 The design of this standard test method minimizes any loss of viable organisms through carrier wash-off.

5.7 It is optional to adjust (dilution in PBS) the inoculum to achieve desired control counts of 5.0 $\log_{10}$ CFU/carrier to 6.5 $\log_{10}$ CFU/carrier.

5.8 Include, where applicable, comparisons of the test to other similar procedures such as Practices E1054 and E2362.