Designation: E2362 – 22

Standard Practice for Evaluation of Pre-saturated or Impregnated Towelettes for Hard Surface Disinfection[1]

This standard is issued under the fixed designation E2362; 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 practice is designed to evaluate the antimicrobial activity of pre-saturated or impregnated towelettes when used as a hard surface disinfectant.

1.2 It is the responsibility of the investigator to determine whether Good Laboratory Practices (GLP's) are required and to follow them when appropriate.

1.3 This practice should be performed only by those trained in microbiological techniques.

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

1.5 Appropriate modifications to the practice may be required when testing organisms not specified herein.

1.6 *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.*

1.7 *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]

D1193 Specification for Reagent Water

E1054 Practices for Evaluation of Inactivators of Antimicrobial Agents

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Federal Standard*

40 CFR, Part 160 Good Laboratory Practice Standards[3]

3. Terminology

3.1 For definitions of terms used in this practice, refer to Terminology E2756.

3.2 *Definitions:*

3.2.1 *carrier, n*—a transportable surface onto which a test organism will be inoculated and dried.

3.2.1.1 *Discussion*—The carrier will be treated with the test substance and subcultured for survivors.

3.2.2 *CFU, n*—colony forming units

3.2.3 *disinfectant, n*—a physical or chemical agent or process that destroys pathogenic or potentially pathogenic microorganisms in/on surfaces or objects.

3.2.4 *impregnated, adj*—saturated with test substance.

3.2.5 *neutralizer, n*—a component used to render an active agent incapable of destroying organisms by chemical or physical means.

3.2.6 *pre-saturated, adj*—to be filled or impregnated with test substance prior to the time of its intended use.

3.2.7 *towelette, n*—A paper, cloth or non-woven blend material used as a transporter for a cleaning and/or disinfection agent.

4. Summary of Practice[4]

4.1 A towelette impregnated or pre-saturated with a test substance is used to treat a carrier which has been inoculated with a test organism after an aliquot of a test organism has been inoculated, evenly distributed to an inoculation area of approximately one square inch (approximately 625 mm), and

[1] This practice 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 the Superintendent of Documents, U.S. Government Printing Office, Washington D.C. 20402

[4] United States Environmental Protection Agency, Standard Operating Procedure for Disinfectant Towelette Test Against *Staphylococcus aureus, Pseudomonas aeruginosa, and Salmonella enterica*, EPA/OPP Microbiology Laboratory, Ft. Meade, MD. SOP# MB09-05, Revised 1/30/13.

***A Summary of Changes section appears at the end of this standard**

dried onto the carrier. The carrier is wiped using the pre-saturated or impregnated towelette simulating the application of the test substance and then held for a pre-determined contact time. After the specified contact time, the test substance remaining on the carrier is neutralized and the carrier is subcultured to recover surviving test organism.

5. Significance and Use

5.1 This practice may be used to determine if a pre-saturated or impregnated towelette demonstrates antimicrobial effectiveness as a disinfectant on hard surfaces. This practice provides survivor results in the form of a qualitative endpoint (growth positive versus growth negative). The results generated by following this practice do not provide for specific quantitative reductions.

6. Apparatus

6.1 *Incubator*—any calibrated incubator that maintains a temperature specific for propagation of organisms. (for example, bacteria and mycobacteria at 36 °C ± 1 °C and fungi at 27.5 °C ± 2.5 °C).

6.2 *Sterilizer*—any suitable, calibrated steam sterilizer that produces the conditions of sterilization is acceptable.

6.3 *Test Towelettes*—with instructions for use.

6.4 *Timer (Stop-clock)*—a calibrated timer that displays min and s.

6.5 *Spectrophotometer*—calibrated to 650 nm.

6.6 *Mixer*—a vortex mixer is recommended.

6.7 *pH meter*—a calibrated pH meter to determine the pH of media.

6.8 *Nonporous Test Carriers*—borosilicate glass slides, 25 mm × 75 mm slides, pre-cleaned (or other hard surfaces and sizes as appropriate).

6.9 *Glass Culture Tubes*—20 mm × 150 mm, 25 mm × 150 mm, and 38 mm × 100 mm or 38 mm × 200 mm without lip, or equivalent, sterile.

6.10 *Culture Tube Closures*—appropriate size nontoxic closures.

6.11 *Petri Dishes*—100 mm × 15 mm, glass and plastic, sterile.

6.12 *Balance*—a calibrated balance sensitive to 0.1 g.

6.13 *Micropipettor*—calibrated for dispensing 10 µL.

6.14 *Forceps*—sterilizable or pre-sterilized.

6.15 *Sterilizer Apparatus*—a bunsen burner or other appropriate heat sterilizer.

6.16 *Bacteriological Culture Loop*— 4 mm inside diameter loop of platinum or platinum alloy wire or sterile disposable plastic loops of appropriate size.

6.17 *Colony Counter*—any one of several types may be used, for example Quebec.

6.18 *Gloves*—sterile gloves not possessing antimicrobial properties.

6.19 *Pipette*—sterile volumetric pipettes.

6.20 *Glass Jars*—100 mL or other appropriate vessel.

6.21 *Filter Paper*—9 cm (Whatman No. 2, or equivalent) sterilized prior to use.

6.22 *Thermometer*—calibrated thermometer.

6.23 *Glass Beads*—3 –5 mm sterile beads.

6.24 *Gauze*—sterile cotton gauze.

6.25 *Hemacytometer*—calibrated hemacytometer.

6.26 *Glass Wool*—sterile grease free glass wool.

6.27 *Hot air oven*—ability to maintain ≥180°C.

6.28 *Refrigerator*—calibrated to maintain 5 °C ± 3°C.

6.29 *Ultra-Cold Freezer,* Calibrated to maintain ≤ -70 °C

6.30 *Glass Tissue Grinder or Macerator,* sterile.

6.31 *Sterile cryovials,* (for example, 1.5 mL with screw cap)

6.32 *Centrifuge,* calibrated.

7. Reagents

7.1 *Culture Media—Bacteria*

7.1.1 Nutrient Broth or Synthetic Broth—*Pseudomonas aeruginosa*,

7.1.2 Cystine Trypticase Agar—*Pseudomonas aeruginosa*,

7.1.3 Synthetic Broth—*Salmonella enterica and Staphylococcus aureus*.

7.1.4 Fluid Thioglycollate Broth.

7.1.5 Tryptic Soy Broth (TSB)

7.1.6 Tryptic Soy Broth with 15% v/v glycerol (Cyroprotectant solution)

7.2 *Culture Media—Mycobacteria*

7.2.1 Middlebrook 7H11 or 7H9 Agar Slants.

7.2.2 Modified Proskauer-Beck Broth.

7.3 *Culture Media—Fungi*

7.3.1 Sabouraud Dextrose Agar plates/Glucose Agar plates.

7.3.2 Sabouraud Dextrose Agar slants/Glucose Agar slants.

7.4 *Neutralizing Subculture Media*—A neutralizing growth medium capable of supporting the growth of the test organism following exposure to the test material in accordance with Practices E1054. For Mycobacterium, horse serum (which may be supplemented with additional neutralizers) is recommended.

7.5 *Subculture Agar*

7.5.1 Tryptic Soy Agar with or without sheep blood—Bacteria.

7.5.2 Middlebrook 7H11 Agar—Mycobacteria.

7.5.3 Sabouraud Dextrose Agar or Glucose Agar—Fungi.

7.6 *Subculture Media*—Mycobacteria

7.6.1 Modified Proskauer-Beck Broth[5]

7.6.2 Kirchner's Medium[5]

7.6.3 Middlebrook 7H9 Broth or TB broth

7.7 Other subculture agars, broths and neutralizers may be used where appropriate.

[5] AOAC Official Method 965.12 Tubcerculocidal Activity of Disinfectants. AOAC International, Chapter 6.