



Designation: E2362 – 22

# Standard Practice for Evaluation of Pre-saturated or Impregnated Towelettes for Hard Surface Disinfection<sup>1</sup>

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 ( $\epsilon$ ) 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:*<sup>2</sup>

[D1193 Specification for Reagent Water](#)

[E1054 Practices for Evaluation of Inactivators of Antimicrobial Agents](#)

<sup>1</sup> 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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<sup>2</sup> For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[E2756 Terminology Relating to Antimicrobial and Antiviral Agents](#)

2.2 *Federal Standard*

[40 CFR, Part 160 Good Laboratory Practice Standards](#)<sup>3</sup>

## 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<sup>4</sup>

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

<sup>3</sup> Available from the Superintendent of Documents, U.S. Government Printing Office, Washington D.C. 20402

<sup>4</sup> 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 *Hemocytometer*—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<sup>5</sup>

7.6.2 Kirchner's Medium<sup>5</sup>

7.6.3 Middlebrook 7H9 Broth or TB broth

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

<sup>5</sup> AOAC Official Method 965.12 Tuberculocidal Activity of Disinfectants. AOAC International, Chapter 6.

7.8 *Soil*—Blood Serum, such as heat inactivated fetal bovine serum or other appropriate alternative soil.

7.9 *Dilution Fluid*—sterile phosphate buffered water (PBDW), sterile saline or Butterfield's Buffer. (See Specification **D1193**.)

7.10 Sterile saline + 0.05 % v/v Triton X-100

7.11 Sterile 0.1 % v/v Polysorbate (Tween) 80

7.12 *Carrier Preparation Solutions*—70 % to 95 % isopropyl alcohol, deionized or distilled water.

## 8. Test Organisms

### 8.1 Bacterial Test Organisms:

8.1.1 *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 10708), and *Pseudomonas aeruginosa* (ATCC 15442)-received lyophilized.

8.1.2 Other bacterial organisms may be tested using appropriate culture and subculture procedures.

### 8.2 Mycobacterial Test Organism:

8.2.1 *Mycobacterium bovis*—(BCG) (Organon teknika or ATCC 35743)

8.2.2 Other mycobacterial strains may be tested using appropriate culture and subculture procedures.

### 8.3 Fungal Test Organisms:

8.3.1 *Trichophyton mentagrophytes* (ATCC 9533)

8.3.2 Other fungi may be tested using appropriate culture and subculture procedures.

## 9. Preparation of Organism

9.1 *Bacteria*<sup>6</sup>—Preparation of frozen stock cultures for *S. enterica*, *S. aureus*, and *P. aeruginosa*.—Using a tube containing 5 mL–6 mL TSB, aseptically withdraw 0.5 mL to 1.0 mL and rehydrate the lyophilized culture. Aseptically transfer the entire rehydrated pellet back into the original tube of broth. Mix well. Incubate for 24 h ± 2 h at 36 °C ± 1 °C. Using a sterile spreader, inoculate a sufficient number of TSA plates (for example, 5 to 10 plates per organism) with 100 µL each of the culture. Incubate plates at 36 °C ± 1 °C for 24 h ± 2 h. Following incubation, add 5 mL cryoprotectant solution (TSB with 15% v/v glycerol) to the surface of each agar plate. Resuspend the cells in this solution using a sterile spreader or a sterile swab and aspirate the cell suspension from the surface of the agar. Transfer suspension into a sterile vessel. Repeat by adding another 5 mL cryoprotectant to the agar plates, resuspend the cells, aspirate suspension and pool with the initial cell suspension. Alternately, 10 mL cryoprotectant solution may be added per plate for resuspending with subsequent aspiration. Mix the pooled contents of the vessel thoroughly. Immediately after mixing, pipet approximately 1.0 mL quantities of the diluted suspension into cryovials. Place and store cryovials in –70°C or below freezer; these are the frozen stock cultures. Each cryovial is considered as single use only. Store stock cultures up to 18 months. Reinitiate stocks using a new lyophilized culture.

9.1.1 *Bacteria Inoculum Preparation*—For *S. aureus* and *S. enterica*, defrost a single cryovial at room temperature and briefly vortex to mix. Add 10 µL of the thawed frozen stock to a tube containing 10 mL synthetic broth and then vortex to mix. Incubate at 36 °C ± 1 °C for 24 h ± 2 h. Briefly vortex the 24 h culture prior to transfer. For this final subculture step, inoculate a sufficient number of 20 mm × 150 mm tubes containing 10 mL synthetic broth with 10 µL per tube of the 24 h synthetic broth culture; incubate 48 h to 54 h at 36 °C ± 1 °C. Using a Vortex-style mixer, mix synthetic broth test cultures 3 s to 4 s and let stand 10 min at room temperature before continuing. Remove the upper portion of each culture, leaving behind any debris or clumps, and transfer to a sterile flask or tube; pool cultures in the flask and swirl to mix. Aliquot a sufficient volume of culture into a sterile test tube.

9.1.1.1 For each bacterium, one daily transfer is required prior to the inoculation of a final test culture. Daily cultures may be subcultured for up to 5 d; each daily transfer may be used to generate a test culture. For the purpose of achieving the carrier count range, final cultures may be adjusted by dilution in growth medium or by concentration using centrifugation (for example, 5000 g for 20 min) resuspending the pellet in the appropriate volume of sterile test culture medium.

9.1.2 For *P. aeruginosa*, defrost a single cryovial at room temperature and briefly vortex to mix. Each cryovial should be single use only. Add 10 µL of the thawed frozen stock to a tube containing 10 mL broth (synthetic or nutrient broth) and then vortex to mix. Incubate at 36 °C ± 1 °C for 24 h ± 2 h. Do not vortex the 24 h culture prior to transfer. For this final subculture step, inoculate a sufficient number of 20 × 150 mm tubes containing 10 mL broth (synthetic or nutrient) with 10 µL per tube of the 24 h broth culture; incubate 48 to 54 h at 36 °C ± 1 °C. Do not shake 48 h to 54 h test culture. The pellicle from the 48 h to 54 h cultures must be removed from the broth either by decanting the liquid aseptically into a sterile tube, by gently aspirating the broth away from the pellicle using a pipet, or by removal with a vacuum. Avoid harvesting pellicle from the bottom of the tube.

9.1.2.1 Any disruption of the pellicle resulting in dropping, or breaking up of the pellicle in culture before or during its removal renders that culture unusable in the test. This is extremely critical because any pellicle fragment remaining will result in uneven clumping and layering of organism, allowing for biased exposure to disinfectant and causing false-positive results. Pool the test culture from each tube and visually inspect culture for pellicle fragments. Presence of pellicle in the final culture makes it unusable for test. Using a Vortex-style mixer, mix test cultures 3 s to 4 s and let stand 10 min at room temperature before continuing. Remove the upper portion of each culture, leaving behind any debris or clumps, and transfer to a sterile flask or tube; pool cultures from tubes in the flask and swirl to mix. Aliquot a sufficient volume of culture into a sterile test tube.

9.1.2.2 One daily transfer is required prior to the inoculation of a final test culture. Daily cultures may be subcultured for up to 5 days; each daily transfer may be used to generate a test culture. For the purpose of achieving the carrier count range, final cultures may be adjusted by dilution in growth medium or

<sup>6</sup> AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants. AOAC International, Chapter 6.

by concentration using centrifugation (for example, 5000 g for 20 min) resuspending the pellet in the appropriate volume of sterile test culture medium.

**9.2 *Mycobacteria***—Maintain a stock culture of *Mycobacterium* organisms on Middlebrook 7H11 or 7H9 agar slants by monthly transfer and incubation for 15 days to 20 days at  $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ . Slants may be stored at  $5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$  for up to six weeks.<sup>5</sup>

**9.2.1 *Mycobacteria Inoculum Preparation***—From stock culture, inoculate Modified Proskauer-Beck (MPB) Broth tubes and incubate 21 to 2 days at  $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ . Using a sterile transfer loop, transfer culture to a sterile glass tissue grinder. Add 1.0 mL of 0.1 % polysorbate (Tween) 80. Grind to break up large clumps or aggregates. Dilute the culture with 9 mL of Modified Proskauer-Beck Broth. Transfer the suspension to a sterile test tube and allow to settle for 10 min to 15 min. Remove the upper portion to a sterile tube or flask, leaving behind any debris or clumps. Pool cultures, as applicable, and swirl to mix. Dilute the culture to achieve  $20\text{ } \pm 1\text{ } \% \text{ T}$  at 650 nm using Modified Proskauer-Beck Broth.<sup>5</sup>

**9.3 *Fungi***—Maintain a stock culture of *Trichophyton mentagrophytes* on Sabouraud Dextrose Agar (SDA) or Glucose agar slants by transferring at less than or equal to 3 month intervals and incubate 10 d at  $27.5\text{ }^{\circ}\text{C} \pm 2.5\text{ }^{\circ}\text{C}$ , followed by storage at  $5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$ .

**9.3.1 *Conidial Suspension Preparation***—For *T. mentagrophytes*, prepare Petri dish cultures ( $\geq 5$  plates) by planting inoculum from a stock culture at the center of the glucose agar or SDA plate and incubating culture at  $27.5\text{ }^{\circ}\text{C} \pm 2.5\text{ }^{\circ}\text{C}$  for 10 d to 15 d. Remove mycelial mats from surface of the agar plate cultures, using a sterile spatula or similar device. Transfer growth to a heat-sterilized glass tissue grinder and macerate with 25 mL sterile physiological saline solution (0.85 % NaCl) or 0.85 % saline with 0.05 % Triton X-100, or to sterile Erlenmeyer flask containing 25 mL sterile saline solution with glass beads and shake thoroughly. (Maintain the ratio of 25 mL solution per 5 plates harvested.) Filter the suspension through sterile absorbent cotton or equivalent to remove hyphal elements. Estimate the density of the conidial suspension by counting in a hemacytometer or by direct plate count using glucose agar or SDA. Store suspension at  $5\text{ }^{\circ}\text{C} \pm 3\text{ }^{\circ}\text{C}$ . This represents the stock spore suspension; it should contain approximately  $10^7$  conidia/mL -  $10^8$  conidia/mL. Use for up to 4 weeks for preparing test suspensions of conidia. Standardize test conidial suspension as needed by diluting (using sterile saline solution) or concentrating the stock spore suspension so that it contains a minimum of  $5 \times 10^6$  conidia/mL. Add 0.02 mL Triton X-100/10 mL suspension to facilitate spreading, if previously not incorporated.

**9.4 *Inocula used for Testing Pre-Cleaned Surfaces***—No organic soil load is added.

**9.5 *Inocula used for Testing Formulations as Disinfectants on Soiled Surfaces***—Transfer an aliquot of the suspension into a sterile tube and add an appropriate volume of blood serum (soil) to yield a 5 % organic soil load (for example, 19 mL of the test organism suspension plus 1 mL fetal bovine serum). Perform a sterility control of the blood serum by adding 1.0 mL

of serum to a tube of appropriate recovery broth, (for example, Fluid thioglycollate medium) and incubate with the test.

**9.6 *Organism Purity***—Subculture each test organism to the appropriate agar, incubate with the test, and examine for purity.

## 10. Procedure

### 10.1 *Preparation of Carriers:*

**10.1.1** Test carriers should be submerged in 70 % to 95 % ethyl or isopropyl alcohol, then rinsed with deionized or distilled water.

**10.1.2** Place the test carriers into a large glass dish and sterilize in a hot air oven for  $\geq 2$  h at  $\geq 180\text{ }^{\circ}\text{C}$ .

**10.1.3** After sterilization, place each carrier horizontally into separate glass or plastic Petri dishes containing 2 pieces of sterile filter paper. Transfer at a minimum the required number of carriers for testing including a minimum of 6 carriers for the population control, 1 to 3 carriers for each viability control, and 1 carrier for the carrier sterility control.

### 10.2 *Inoculation of Carriers:*

**10.2.1** Using a pipette or 4.0 mm inside diameter (i.d.) loop, transfer 0.01 mL (10  $\mu\text{L}$ ) of the test organism to the nonporous carrier which has been placed horizontally inside the above mentioned Petri dish.

**10.2.2** Spread the inoculum suspension evenly over the designated test area (an approximate 1 in. by 1 in. area, approximately 25 mm by 25 mm on the end of the slide) and within 3 mm of the edge using the sterile pipette tip or 4.0 mm id loop used for inoculation and recover with the Petri dish lid.

### 10.3 *Carrier Drying:*

**10.3.1** Place all Petri dishes containing inoculated carriers into an incubator equilibrated at  $36\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ , for 30 min to 40 min., until dry.

### 10.4 *Carrier Treatment/Application of Product:*

**10.4.1** Wipe the inoculated test area according to label instructions or the procedure under test. The wiping procedure should closely simulate the direction for intended use.

**NOTE 1**—The carrier treatment phase is most easily performed with more than one technician. The technician performing the wiping (treatment) procedure must wear sterile gloves prior to handling the towelette under test and must not touch anything except the towelette under test and inoculated carriers. Aseptic technique is critical in minimizing potential environmental contamination of the carrier subcultures.

**10.4.2** The area of the towelette used for wiping should be rotated so as to expose a new surface of the towelette surface in the course of each carrier treatment. One towelette may be used to treat multiple carriers (typically 10 carriers/wipe).

**10.4.3** The wiping procedure should be performed at staggered intervals so as to allow for the prescribed exposure time before subculture.

**NOTE 2**—The technician should allow enough time between each carrier wiping (for example, 30 s) to allow for the exact exposure time prior to subculturing. The exposure time starts immediately following the completion of treatment.

**10.4.4 *Optional***—Following the treatment of the last carrier in the set, the towelette or liquid expressed from the towelette