Designation: ~~E2362 – 09~~ E2362 – 15

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

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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 and health practices and to determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]
D1193 Specification for Reagent Water
E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents
2.2 *Federal Standard*
40 CFR, Part 160 Good Laboratory Practice Standards[3]

3. Terminology

3.1 *carrier, n*—a transportable surface onto which a test organism will be inoculated and dried. The carrier will be treated with the test substance and subcultured for survivors.

3.2 *CFU, n*—colony forming units

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

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

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

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

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

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[1] This practice is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of ~~Subcommitee~~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. 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,~~ distributed to an inoculation area of approximately one square inch (approximately 625 mm), and 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. ~~The used towelette, after the contact time, is also cultured for surviving test organism.~~

5. Significance and Use

5.1 This ~~test method~~ 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 ~~35 ± 2 °C~~36 ± 1 °C and fungi at ~~25 ± 2 °C).~~27.5 ± 2.5 °C).

6.2 *Sterilizer*—any ~~suitable~~ 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 ~~× 2~~ mm slides, pre-cleaned (or other hard surfaces and sizes as appropriate).

6.9 *Glass Culture Tubes*—20 mm × 150 ~~or~~mm, 25 mm × 150 ~~mm without lip or equivalent.~~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 ~~forceps.~~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.

[4] United States Environmental Protection Agency, ~~Efficacy Data Requirements, "Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection"~~ Standard Operating Procedure for ~~Testing of Towelette Disinfectants against~~Disinfectant Towelette Test Against *Staphylococcus ~~aureus and~~ aureus, Pseudomonas aeruginosa, and Salmonella enterica,* EPA/OPP Microbiology Laboratory, Ft. Meade, MD. SOP# ~~MB09-02,~~MB09-05, Revised ~~12/31/06.~~1/30/13.

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

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

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

6.30 ~~*Tissue grinder*—~~*Glass Tissue Grinder or Macerator,* ~~sterile disposable or sterilizable glass.~~sterile.

6.31 ~~*Orbital Shaker*—~~*Sterile cryovials,* ~~calibrated shaker.~~ (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 Nutrient Agar.~~

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/Potato 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 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.

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 ~~*Bacteria*~~*Bacterial Test Organisms:*

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

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

8.2 ~~*Mycobacteria*~~*Mycobacterial Test ~~Organisms:~~Organism:*

8.2.1 *Mycobacterium bovis*—~~Mycobacterium chelonae~~(BCG) (Organon ~~(ATCC 35752).~~ teknika or ATCC 35743)

~~8.2.2 *Mycobacterium bovis* (ATCC 35743)~~

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

8.3 ~~*Fungi*~~*Fungal Test Organisms:*

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

8.3.1 *Trichophyton mentagrophytes* (ATCC 9533)

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

9. Preparation of Organism

9.1 ~~*Bacteria—Bacteria*~~[6] ~~–~~Preparation of frozen stock cultures for S. enterica, S. aureus, and P. aeruginosa.~~—Maintain stock cultures of~~Using a tube containing ~~S. aureus~~5–6 mL TSB, aseptically withdraw 0.5 to 1.0 mL and ~~S. enterica~~rehydrate the ~~on Nutrient Agar slants. Maintain stock cultures~~ lyophilized culture. Aseptically transfer the entire rehydrated pellet back into the original tube of ~~P. aeruginosa~~broth. Mix ~~on Cystine Trypticase Agar. Incubate freshly subcultured stock cultures for 48 ± 4 h at 35 ± 2 °C, then refrigerate cultures at 2 to 8 °C for up to one month. Stock cultures used for inoculation of broth cultures should not undergo more than 5 passages from the first subculture from the ATCC frozen stock.~~well. Incubate for 24 ± 2 h at 36 ± 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 ± 1ºC for 24 ± 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*—~~From~~For *S. aureus* and *S. enterica,*~~stock cultures, inoculate~~ 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 ± 1°C for 24 ± 2 h. Briefly 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 ~~of the appropriate fresh culture broth and incubate for 24 ± 4 h at 35 ± 2 °C. Using a 4 mm inside diameter transfer loop, transfer one loopful of the culture into fresh culture broth. Make at least 3 but less than 30 consecutive daily transfers prior to use as inoculum for testing. Incubate the final transfer for 48 ± 4 h, and use these~~ synthetic broth with 10 µL per tube of the 24 h synthetic broth culture; incubate 48 to 54 h at 36 ± 1°C. Using a Vortex-style mixer, mix synthetic broth test cultures 3 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 ~~test. Aseptically remove the pellicle from the~~flask and swirl to mix. Aliquot a ~~P. aeruginosa~~sufficient volume ~~culture before use in the test.~~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 ± 1ºC for 24 ± 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 ± 1°C. Do not shake 48 to 54 h test culture. The pellicle from the 48 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 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 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 to ~~30~~20 days at ~~35 ± 2 °C followed by storage at 2 to 8°C.~~ 36 ± 1 °C. Slants may be stored at 5 ± 3 °C for up to six weeks.[5]

[6] AOAC Official Method 961.02 Germicidal Spray Products as Disinfectants. AOAC International, Chapter 6.