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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.

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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 *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:*~~

~~D1193~~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~~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~~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

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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, Efficacy Data Requirements, "Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection" Standard Operating Procedure for Testing of Towelette Disinfectants against ~~*Salmonella choleraesuis , Staphylococcus aureus, Pseudomonas aeruginosa,* and *Mycobacterium bovis (BCG),* EPA/OPP Microbiology Laboratory, Ft. Meade, MD. SOP# MB09-01, Revised 11/08/00.~~Staphylococcus aureus and Pseudomonas aeruginosa, EPA/OPP Microbiology Laboratory, Ft. Meade, MD. SOP# MB09-02, Revised 12/31/06.

test organism after an aliquot of a test organism has been inoculated, evenly distributed, 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

This test method may be used to determine if a pre-saturated or impregnated towelette demonstrates antimicrobial effectiveness as a disinfectant on hard surfaces.

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 and fungi at 25 ± 2 °C).
6.2 *Sterilizer*—any suitable 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 × 75 × 2 mm slides, pre-cleaned (or other hard surfaces and sizes as appropriate).
6.9 *Glass Culture Tubes*—20 × 150 or 25 × 150 mm without lip or equivalent.
6.10 *Culture Tube Closures*—appropriate size nontoxic closures.
6.11 *Petri Dishes*—100 × 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.
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 *Tissue grinder*—sterile disposable or sterilizable glass.
6.29 *Orbital Shaker*—calibrated shaker.

7. Reagents

7.1 *Culture Media—Bacteria*
7.1.1 *Nutrient Broth—Pseudomonas aeruginosa*,
7.1.2 *Cystine Trypticase Agar—Pseudomonas aeruginosa*,
7.1.3 *Synthetic Broth—Salmonella ~~choleraesuis~~enterica and Staphylococcus aureus*.
7.1.4 Nutrient Agar.
7.1.5 Fluid Thioglycollate Broth.
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*
~~7.3.1Sabouraud Dextrose Agar plates.~~
7.3.1 Sabouraud Dextrose Agar plates/Potato Dextrose Agar.
7.3.2 Sabouraud Dextrose 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.

7.5 *Subculture Agar*

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

7.5.2 *Middlebrook 7H11 Agar*—Mycobacteria.

7.5.3 *Sabouraud Dextrose Agar*—Fungi.

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

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

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

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

8. Test Organisms

8.1 *Bacteria Test Organisms*:

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

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

8.2 *Mycobacteria Test Organisms* :

8.2.1 *Mycobacterium chelonae* (ATCC 35752).

8.2.2 *Mycobacterium bovis* (ATCC 35743)

8.2.3 Other mycobacteria strains may be tested using appropriate culture and subculture procedures.

8.3 *Fungi Test Organisms*:

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*—Maintain stock cultures of *S. aureus* and ~~*S. choleraesuis*~~ S. enterica on Nutrient Agar slants. Maintain stock cultures of *P. aeruginosa* on Cystine Trypticase ~~Agar slants.~~ Agar. Incubate freshly subcultured stock cultures for 48 ± 4 h at 35 ± 2 °C, then refrigerate cultures at ~~2-8°C~~ 2 to 8 °C for up to ~~30 days.~~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.

9.1.1 *Bacteria Inoculum Preparation*—From stock cultures, inoculate 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 ~~15~~30 consecutive daily transfers prior to use as inoculum for testing. Incubate the final transfer for 48 ± 4 h, and use these cultures in the test. Aseptically remove the pellicle from the *P. aeruginosa* culture before use in the test.

9.2 *Mycobacteria*—Maintain a stock culture of *Mycobacterium* organisms on Middlebrook 7H11 or 7H9 agar slants by monthly transfer and incubation for ~~15-30~~ 15 to 30 days at 35 ± 2 °C followed by storage at ~~2-8 °C.~~ 2 to 8°C.

9.2.1 *Mycobacteria Inoculum Preparation*—From stock culture, inoculate Modified Proskauer-Beck (MPB) Broth tubes and incubate ~~10-25~~ 10 to 25 days at 35 ± 2 °C. Add 1.0 mL of 0.1 % Tween 80 in saline to this ~~10-25-day~~ 10 to 25-day culture, transfer to a sterile tissue grinder and grind thoroughly. Finally transfer the appropriate volume of suspension from the grinder to a vessel appropriate for spectrophotometric determination. Calibrate a spectrophotometer to 650 nm using a MPB blank as the 100 % transmittance point. Measure transmittance of the culture suspension. Adjust culture suspension with MPB to a target of ~~15-20%~~8 to 12 % transmittance to achieve a $\geq 1.0 \times 10^4$ CFU/carrier concentration.

9.3 *Fungi*—Maintain a stock culture of *Trichophyton mentagrophytes* ~~on Sabouraud Dextrose agar slants by transferring at less than or equal to 3 month intervals and incubate 10 days at 25±2°C, followed by storage at 2-8°C.~~ on Sabouraud Dextrose or Potato Dextrose agar slants by transferring at less than or equal to 3 month intervals and incubate 10 days at 2 5± 2°C, followed by storage at 2 to 8°C. Alternatively, a water stock may be prepared and held at ambient temperature.

9.3.1 *Conidial Suspension Preparation*—From stock culture, inoculate Sabouraud Dextrose ~~Agar~~ or Potato Dextrose agar plates and incubate at 25 ± 2 °C for ~~10-15~~ 10 to 15 days. Transfer growth, using a sterile swab, to a glass screw-top vessel containing 5 sterile glass beads and 5 mL of 0.85-0.90 % sterile saline or other appropriate diluent per culture plate. Vortex mix the suspension for one min. Filter the conidial suspension through sterile cotton gauze or glass wool to remove the hyphal fragments. Estimate the conidial concentration by counting in a hemacytometer. Stock conidial suspension can be stored at 2-8 °C for one month. On the day of testing, standardize the conidial suspension using 0.85- to 0.9 % sterile saline to $\geq 1.0 \times 10^6$ conidia/mL. A 0.2 mL aliquot of Triton X-100 may be added to a 10 mL aliqout of conidial suspension to facilitate spreading.

9.4 *Inocula used for Testing Pre-Cleaned Surfaces* —Immediately prior to use, thoroughly vortex mix the prepared fungi suspension. Thoroughly vortex mix the prepared bacteria and mycobacteria suspension and allow to settle for ≥10 min prior to use.

9.5 *Inocula used for Testing Formulations as Disinfectants on Soiled Surfaces*—Immediately prior to use, thoroughly vortex mix the prepared fungi and mycobacterial suspension. Thoroughly vortex mix the prepared bacteria ~~and mycobacteria~~ suspension and allow to settle for ≥10 min prior to use. Transfer an aliquot of the suspension into a sterile tube and add an appropriate volume