Designation: E3058 – 16

Standard Test Method for Determining the Residual Kill Activity of Hand Antiseptic Formulations[1]

This standard is issued under the fixed designation E3058; 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 is designed to determine the residual killing activity of skin antiseptics against transient microbial skin flora on the hands .[2] It may be used to evaluate products that are used with the aid of water and rinsed off and those that are used without the aid of water and not rinsed off.

1.2 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects (see 21 CFR Parts 50 and 56).

1.3 This test method should be performed by persons with training in microbiology, in facilities designed and equipped for work with potentially infectious agents at biosafety level 2.

1.4 *Units*—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 determine the applicability of regulatory limitations prior to use.* For more specific precautionary statements see 8.1.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2752 Guide for Evaluation of Residual Effectiveness of Antibacterial Personal Cleansing Products

E1882 Test Method for Evaluation of Antimicrobial Formulations by the Agar Patch Technique

E2197 Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Federal Standards:*[4]

21 CFR Part 50 Protection of Human Subjects

21 CFR Part 56 Institutional Review Boards

3. Terminology

3.1 *Definitions*—For definitions of terms used in this document, see Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *healthcare personnel hand rub, n*—an antimicrobial gel, foam, liquid, spray, or wipe, applied by rubbing to reduce the transient microbial skin flora on hands that are not visibly soiled, and which does not require a post-treatment water rinse. Such agents may also be referred to as hand rubs, hygienic hand rubs, hand sanitizers, or hand antiseptics.

3.2.2 *healthcare personnel hand wash, n*—a cleanser requiring rinsing or a non-rinse agent intended to reduce transient microbial skin flora on the hands.

3.2.3 *room temperature, n*—temperature in the range of 20 to 25°C (68 to 77°F).

3.2.4 *test bacteria, n*—an applied suspension of bacteria having characteristics that permit ready identification of colonies. Test bacteria are used to simulate a transient topical microbial contaminant. These may also be referred to as test organisms, marker organisms, simulants, or contaminants.

3.2.5 *test material, n*—a product or formulation that incorporates an antimicrobial ingredient(s).

4. Summary of Test Method

4.1 This test method uses adult subjects who have provided a written informed consent and whose hands have been determined to be free from any clinical evidence of skin disorders, dermatosis, cuts, lesions, or hangnails at the time of

[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.

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[2] Rutter J.D., Angiulo K., Macinga D.R., Measuring residual activity of topical antimicrobials: is the residual activity of chlorhexidine an artefact of laboratory methods?*J. Hosp. Infect*. 88:113-115, 2014

[3] 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.

[4] Available from U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St., NW, Washington, DC 20401-0001, http://www.access.gpo.gov.

participation in the study. Subjects are to refrain from use of any antimicrobials for at least 7 days prior to the initiation of the test procedure (see 12.3).

4.2 Subjects' hands are pre-treated with the test material to load the antimicrobial onto the skin. Test material remains on the hands for a pre-determined time (the time selected to demonstrate the test product's residual kill activity) prior to contamination.

4.3 Subjects press each fingerpad onto a stainless steel disc contaminated with approximately 7 $\log_{10}$ CFU of test organism (using one disc per fingerpad), which transfers approximately 6 $\log_{10}$ CFU of test organism to each fingerpad (that is, approximately 10% transfer). The test organism, *Staphylococcus aureus* (ATCC 6538), remains viable upon drying and is stable on both the stainless steel discs and on the fingerpads over the course of the experiment. The fingerpads are exposed to the challenge organism for pre-determined times.

4.4 The test bacterium is then recovered from the fingerpads by rubbing each fingerpad for 30 s in a Petri dish containing 10 ml neutralizer (one Petri dish per fingerpad).

4.5 Residual killing by the test material is measured by comparing the number of test bacteria recovered from contaminated fingerpads at specific time intervals after contamination to the number recovered at time zero (treated fingerpad with zero time to allow for reduction in microorganism after application).

5. Significance and Use

5.1 Many marketed hand antiseptics make claims of "long-lasting protection" or "extended kill" (for example 6 hours), which are typically based on results of testing as described in Test Method E1882 or Guide E2752, or both. At this time there are no standard methods for evaluating a hand antiseptic formulation for its ability to kill microorganisms on hands when a "dry" contamination event occurs at some time after product use. This test method provides a method to substantiate residual kill claims for hand antiseptics.

6. Apparatus

6.1 *Aluminum bars*—Discs are attached to these to avoid movement and / or sticking of the discs to the fingerpads during contamination (see Fig. 1). Any of several types may be used, for example, multipurpose 6061 aluminum rectangular, ⅜ in. × ½ in. × 6 in.[5].

6.2 *Colony Counter*—Any of several types may be used; for example, Quebec darkfield colony counters and similar devices. Automated, computerized plater/counter systems may also be used.

6.3 *Discs*—1 cm diam. and 0.7 mm thick made from sheets of brushed stainless steel, AISI Type 430 (E2197).

6.4 *Handwashing Sink*—Sufficient in size to permit handwashing without the touching of hands to sink surface or other subjects.

6.5 *Humidity Chamber*—Capable of maintaining 50-60% relative humidity in the chamber for 24 h at room temperature.

6.6 *Humidity Monitor (Hygrometer)*—Calibrated and capable of displaying relative humidity in 1% increments

6.7 *Water Faucet(s)*—Located above the sink at a height to permit hands to be held higher than the elbow during the washing procedure.

6.8 *Tap Water Temperature Regulator and Temperature Monitor*—To set and maintain the tap water temperature at 40 ± 2°C

6.9 *Incubator*—Capable of maintaining a temperature of 35 ± 2°C.

6.10 *Biological Safety Cabinet.*

6.11 *Miscellaneous Labware*—Continuously adjustable pipetters (1-mL and 0.2-mL capacity) and sterile pipette tips, sterile serological pipettes (5.0-mL capacity), sterile culture tubes, sterile disposable Petri dishes, sterile syringes, Erlenmeyer flasks, sterile loops and beakers.

6.12 *Sampling Petri dishes*—Sterile dishes measuring 100 mm × 15 mm, and able to hold 10 mL sampling solution (see 7.7).

6.13 *Absorbance Meter*—Capable of reading at 625 nm with a 1 cm path length.

6.14 *Sterilizer*—Any steam sterilizer capable of processing culture media and reagents.

[5] The sole source of supply of the apparatus known to the committee at this time is available from McMaster Carr, part number 8975K614. http://www.mcmaster.com/#. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

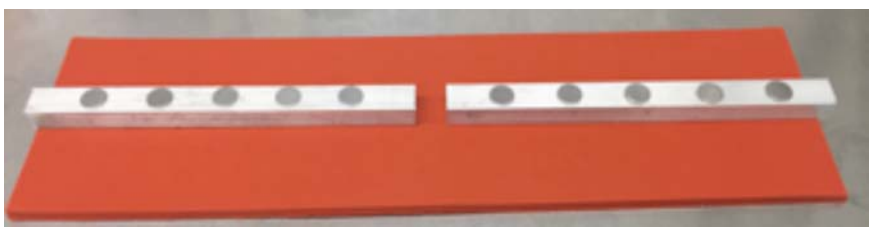

FIG. 1 Aluminum Bar

6.15 *Timer (Stop-Clock)*—Type that can be read for minutes and seconds.

6.16 *Vortex Mixer*—Any vortex that will ensure proper mixing of culture.

7. Reagents and Materials

7.1 *Antibiotic Ointment*—A topical, triple-antibiotic ointment for application to the hands after the final decontamination.

7.2 *Cleansing Wash*—A mild, proven non-antimicrobial liquid soap. May be purchased commercially or prepared according to the instructions: Soft Soap, 200 g/L: Linseed oil 50 parts by weight Potassium hydroxide 9.5 parts Ethanol 7 parts Distilled or high purity water as needed Add linseed oil to a solution of potassium hydroxide in 15 parts water and heat up to approximately 70°C while constantly stirring. Add the ethanol and continue heating while stirring until the saponification process is completed and a sample dissolves clearly in water and almost clearly in alcohol. The weight of the soft soap is then brought up to 100 parts by addition of hot water. Take 200 g of the soft soap in 1 L of water. Dispense in to appropriate containers and sterilize in an autoclave.

7.3 *Chlorhexidine Skin Cleanser*—Antiseptic skin cleanser containing 4 % chlorhexidine gluconate (w/v) for hand decontamination.

7.4 *Culture Media:*

7.4.1 *Broth*—Soybean-casein digest broth (tryptic soy broth) is recommended.

7.4.2 *Agar Plating Media:*

7.4.2.1 *Plating Medium*—Soybean-casein digest agar (tryptic soy agar [TSA]) containing an effective inactivator for the test material, if necessary is recommended.

NOTE 1—Ensure that stock culture of *S. aureus* and any subsequent subculture used produces golden-colored colonies on soybean-casein digest agar.

7.4.2.2 *S. aureus Plating Medium*—HardyCHROM [6], containing an effective inactivator for the test material, if necessary, may be used as an alternative to the standard plating media. Other indicator media for *S. aureus* may be appropriate but should be validated prior to use.

NOTE 2—*S. aureus* forms smooth, deep pink to fuchsia-colored colonies when grown on HardyCHROM.[6] The growth of most other organisms, including *Staphylococcus epidermidis* are partially to completely inhibited.

7.5 *Dilution and Sampling Fluid*—Dissolve 0.4 g KH2PO4, 10.1 g Na2HPO4, 1.0 g isooctylphenoxypolyethoxyethanol (for example, Triton X-100), and appropriately validated neutralizers, if necessary (see Note 3), in distilled water. Adjust pH to 7.8 ± 0.1 with 0.1 N HCl or 0.1 N NaOH and bring volume to 1 L with distilled water.

NOTE 3—A neutralizer validation should be conducted according to Test Methods prior to the study. Test Methods E1054 provides a list of neutralizers appropriate for commonly used antimicrobial agents. In some cases (for example, some alcohol-based hand rubs) neutralization is achieved by dilution alone, therefore, inclusion of an inactivator is only required if neutralization of the test material cannot be achieved upon dilution (see 7.5).

7.6 *Ethanol Solution*—70 % ethanol in water (v/v) for hand decontamination.

7.7 *Test Material*—Use directions provided with the test material. If directions are not provided, use the directions given in this method.

7.8 *Negative Control*—70 % ethanol in water (v/v).

8. Hazards

8.1 Application of microorganisms to the skin may involve a health risk. Determine the antibiotic sensitivity profile of the test bacteria prior to applying to the skin. After the test has been completed, decontaminate the subject's hands and follow proper procedures to reduce infection risk (13.11). If an infection occurs, provide the antibiotic susceptibility profile to the attending clinician.

9. Test Bacteria

9.1 *Staphylococcus aureus* ATCC 6538 (methicillin-sensitive) is the recommended test bacterial species. *S. aureus* is differentiated from resident microbial skin flora (including *Staphylococcus epidermidis*) colonies by colony morphology and pigmentation on standard plating media (see 7.4.2.1) or with chromogenic indicator medium (see 7.4.2.2).

10. Preparation of Test Bacteria Suspension

10.1 *Preparation of S. aureus:*

10.1.1 Prepare a stock culture of *S. aureus* ATCC 6538 (no more than 5 transfers from original ATCC vial) by inoculating approximately 5 mL of soybean-casein digest broth (see 7.4.1) from a frozen stock or lyophilized vial and incubate for 18-24 h at 35 ± 2°C.

10.1.2 Using a sterile bacteriological loop inoculate a sufficient number of soybean-casein digest agar plates (see 7.4.2.1) from the overnight culture for colony isolation and incubate for 18-24 h at 35 ± 2°C.

10.1.3 Using a sterile bacteriological loop, gently scrape or rub surface of agar to remove golden-colored colonies and suspend in fresh soybean-casein digest broth to an absorbance of 0.2-0.25 at 625 nm at a path length of 1 cm (or other appropriate measurement based on your absorbance meter specifications) to approximate a titer of 8.5-9.0 $\log_{10}$ CFU/ml. Remove an aliquot from the suspension, dilute and plate for counting. An isolation streak plate should be made to confirm purity.

11. Inoculation of Stainless Steel Discs

11.1 Mix the test bacterial suspension using a vortex for 30 s to homogenize it.

11.2 Use a calibrated pipette to transfer 10 µL of the bacterial inoculum to the center of each sterile disc (6.3). Do not spread the inoculum to avoid operator variability and also to maintain uniform thickness of the inoculum on all discs. For consistency, the same pipette tip should be used when inoculating a given batch of discs. If possible, the inoculation should

[6] Trademarked HardyCHROM*Staph aureus*, available from Hardy Diagnostics