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

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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.

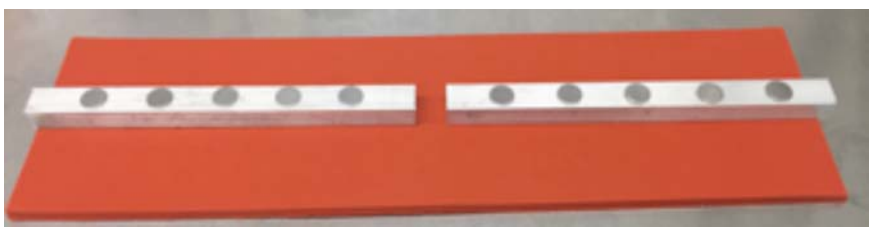

FIG. 1 Aluminum Bar

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

be done in the laminar flow hood to avoid disturbance of the inoculum during transport to the hood.

11.3 Transfer the inoculated discs to a biological safety cabinet for approximately 1 hour to dry the inocula.

11.4 Transfer the dried inoculated discs to a 50-60% relative humidity chamber and maintain at room temperature for 17 to 24 h.

NOTE 4—Variation in the relative humidity within the test facility can affect the rate of transfer of the test organism to the fingerpads. Storage of the discs within a specified humidity range prior reduces this variability.

12. Subjects

12.1 Recruit a sufficient number of healthy adult human subjects who have no clinical evidence of dermatosis, cuts, lesions, hangnails, or other skin disorders on the hands or forearms. A minimum of eight subjects should be used to test for each test material. The total number of subjects used will depend on the number of test materials, the purpose of the study, and the regulatory requirements governing the study.

12.2 It is the responsibility of the user of this test method to obtain the necessary approval from an Institutional Review Board (IRB) or Independent Ethics Commission (IEC) for the use of adult human subjects for testing and to obtain informed and written consent from those selected for the study before starting the tests.

12.3 Instruct subjects to avoid contact with antimicrobial products for the duration of the test and for at least 7 days prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions, soaps, and antimicrobial cleaning and disinfectant products. Bathing in biocide-treated pools, hot tubs, or spas must be avoided. Harsh chemicals such as acids, bases, and solvents must also be avoided. Subjects may not have or apply nail polish, artificial nails, or nail polish remover, or have undergone nail treatment during the 7-day pre-test conditioning period or on the single test day. Subjects may not use topical or systemic antimicrobials, antibiotics, or steroids other than for contraception or post-menopausal indications, and must agree to abstain from these materials until the completion of the study. Provide subjects with a kit of non-antimicrobial personal care products for exclusive use during the test and include rubber gloves to be worn when contact with antimicrobial or harsh chemicals cannot be avoided.

13. Procedure

13.1 *Admission to Testing*—Instruct each subject to return to the laboratory for testing after they having refrained from using antimicrobials for at least seven days. Question the subject to confirm adherence to the study requirements (see 12.3). Inspect the subject's hands and forearms to confirm the absence of clinical signs of skin disorders as described in 12.1. Admit the subject into the test if each of the above criteria is met. Instruct the subject to remove all jewelry from their hands and arms and to clip their fingernails to a uniform length (free edge ≤1 mm). Subjects should demonstrate an ability to rub their fingerpads in Petri dish to check for dexterity in all fingers prior to being admitted into testing.

13.2 *Cleansing Wash*—Instruct the subject to perform a 30-s cleansing wash (see 7.2). This procedure removes oil and dirt from the hands.

13.2.1 Have subject thoroughly wet their hands and forearms under tap water.

13.2.2 Dispense 5 mL of the cleansing wash (see 7.2) into the subject's cupped hands and instruct subject to spread over hands up to the wrists.

13.2.3 Instruct subject to wash all surfaces of the hands in a vigorous manner for 30 ± 2 s. If the lather becomes too dry, add a small amount of water to maintain lather.

13.2.4 Instruct subject to rinse thoroughly from wrists to fingertips under tap water for 30 ± 2 s. Have the subject exercise caution to avoid contact with the sink and fixtures, eliminating the chance of recontamination from the sink surfaces.

13.2.5 Hand subject a clean, dry paper towel and instruct them to lightly pat their hands dry.

13.2.6 After completing the cleansing wash, have each test subject wait at least five minutes prior to the next phase of the study. Instruct the test subject to avoid touching anything with their hands to avoid contamination.

13.3 *Test Material Application*—Apply the test material in accordance with its use directions. If test material directions are not available, use the appropriate test material application procedure described in 13.3.1 or 13.3.3.

13.3.1 For demonstration of residual activity, more than one test material application may be required. If the test material is applied more than one time, the negative control must be applied an equal number of times. The number of test material applications is reported in the results.

13.3.2 *Hand Wash Formulations:*

13.3.2.1 Have subject thoroughly wet their hands under tap water.

13.3.2.2 Dispense 1.5 mL of the test material into the subject's cupped hands.

13.3.2.3 Instruct subject to wash all surfaces of the hands and fingers, including the nails, in a vigorous manner for 30 ± 2 s. If the lather becomes too dry, add a small amount of water to maintain lather.

13.3.2.4 Instruct subject to rinse thoroughly from wrists to fingertips under tap water for 30 ± 2 s. Have the subject exercise caution to avoid contact with the sink and fixtures, eliminating the chance of recontamination from the sink surfaces.

13.3.2.5 Hand subject a clean, dry paper towel and instruct them to lightly pat their hands dry.

13.3.3 *Hand Rub Formulations:*

13.3.3.1 Dispense 1.5 mL of test material into the subject's cupped hands from an appropriate dispenser or syringe.

13.3.3.2 Within 5 s, instruct the subject to distribute test material over all surfaces of the hands and fingers, paying attention to the nails, and to continue rubbing until the product is dry. Subject should exercise caution to retain the test material in the hands.

NOTE 5—The volume output from a foaming dispenser can be calculated by measuring the mass dispensed (g) and dividing by the density of the test material (g/ml). If the density of the test material is unknown, a

mass of 1.3 g is approximately equal to 1.5 ml for formulations containing between 60% (v/v) and 90% (v/v) ethanol.

13.4 *Measurement of Residual Activity*—Residual activity is assessed at any selected time after the last treatment application (for example, 30 minutes, 1 hour, 4 hours, and so forth) by measuring the survival of the test bacteria applied to the fingerpads by dry contact contamination after pre-selected exposure times.

13.4.1 The following is an example of a procedure for assessing residual activity 30 minutes after the last treatment using exposure times of 1 minute, 5 minutes, 20 minutes, and 1 hour.

13.4.2 All ten fingerpads are contaminated by firmly pressing onto the prepared stainless steel discs. The two thumbs are to be used as time-zero controls. The remaining fingerpads are randomized such that one fingerpad on each hand is used for each exposure time, and the numbers of bacteria recovered from the two fingerpads representing each exposure time are averaged.

13.5 *Contamination of the Fingerpads:*

13.6 Within 5 min. prior to the contamination of fingerpads, remove contaminated discs from the humidity chamber (see 11.4). Fingerpads should be contaminated as soon as possible after removing discs from chamber, and no more than 5 min. should pass from the time discs are removed until fingerpads are contaminated. Contaminated fingerpads must not touch each other or any surfaces.

13.7 The subject will first contaminate thumbs by pressing each thumbpad firmly onto the contaminated disc for 2 s. The contaminated thumbpads will be used for the time-zero (baseline) recovery.

13.8 For determination of residual kill, the subjects will contaminate each randomly chosen fingerpad from each hand by pressing each firmly onto a contaminated disc (one per fingerpad) for 2 seconds. Repeat until all remaining fingerpads have been contaminated.

NOTE 6—It may be necessary to contaminate fingerpads one at a time to allow sufficient time for sampling. For example, thumbs may be contaminated first and sampled immediately. The other fingerpads may then be contaminated in a pre-determined order allowing for timely sampling.

13.9 After the appropriate exposure time, whether for immediate time-zero recovery or for the remaining four time points, contaminated fingerpads will be sampled individually by firmly rubbing the fingerpads in circular motion with firm pressure for 30 s. on the bottom of sampling Petri dishes (see 6.12) containing 10 mL of dilution and sampling fluid (see 7.5). One Petri dish is to be used for each fingerpad sample. After recovery of bacteria from the fingerpads, the sample will be processed for enumeration (see Section 14).

NOTE 7—It may be useful to place Petri dishes on rubber or other non-skid surface to ensure Petri dish movement is minimized during sampling.

13.10 Four time points will be evaluated, one per finger per hand with the thumbs acting as the time-zero baseline sample. Evaluations will be made at pre-determined intervals (see 13.4.1). After each sampling procedure is completed the subject will dry their two fingerpads by pressing onto a dry paper towel, ensuring other fingerpads do not touch any surface.

13.11 Subjects will be instructed not to leave the laboratory for any reason once testing begins except in the case of an emergency. Additionally, subjects will be required to wear protective garments and instructed not to touch their clothing, faces, or any other body parts with their hands during the test period. On completion of testing, subjects will be required to perform a 1-min. rinse with 70% (v/v) ethanol or 70% (v/v) isopropyl alcohol and air-dry, followed by a supervised 4-min. wash with a 4% chlorhexidine gluconate solution. A topical antibiotic ointment will be applied to the hands following the decontamination procedure.

14. Enumeration of Bacteria

14.1 Enumerate the *S. aureus* in the recovered sampling solution (see 13.9) using standard microbiological techniques, such as spread plating or spiral plating. The pour plate technique is not recommended.

14.1.1 1 Prepare dilutions of the recovered sampling solution (see 13.9) in dilution and sampling fluid (see 7.5). Use an appropriate indicator medium (see 7.4.2.1) with suitable neutralizer, if necessary, as the recovery medium.

14.1.2 Incubate prepared plates 24 ± 4 h at 35 ± 2°C.

14.1.3 Count *S. aureus* colonies using an appropriate colony counter (see 6.2) based on manufacturer's instructions for the indicator medium (see 7.4.2.1).

15. Calculation or Interpretation of Results

15.1 To determine surviving organisms, record raw data as CFU/plate. Average duplicate plates (2 plates from each replicate dilution) and multiply by the dilution factor to arrive at CFU/mL finger.

15.2 Convert the surviving organisms (CFU/finger) to $\log_{10}$ and average the left and right finger values for each sampling interval.

15.3 Determine $\log_{10}$ reductions at each recovery interval by subtracting $\log_{10}$ recovery values at each exposure time from $\log_{10}$ recovery values at time-zero.

15.4 It may be desirable to compare the test material with other test formulations. If this is the case, an equivalent number of subjects should be assigned to each formulation on a random basis. All test parameters will be equivalent for products, although the wash procedure for an established product may be different. All test products should be run concurrently.

16. Precision and Bias

16.1 A precision and bias statement cannot be made for this method at this time.

17. Keywords

17.1 alcohol-based hand rub; alcohol foam; antimicrobial; antiseptic wipe; contaminant; efficacy; hand antiseptic; hand sanitizer; healthcare personnel handrub; healthcare personnel handwash; *Staphylococcus aureus*; residual kill; residual activity