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Standard Guide for Evaluation of Residual Effectiveness of Antibacterial Personal Cleansing Products[1]

This standard is issued under the fixed designation E2752; 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 guide is designed to demonstrate the effectiveness of an antibacterial personal cleansing product in reducing the numbers of a marker organism (representing transients) both immediately and after prolonged exposure to (cleansing) washing when used as recommended under simulated use conditions. The method demonstrates the effect of residual antibacterial activity by means of inhibition of proliferation of bacteria on the skin after the contact period. Antimicrobial activity is compared with a vehicle or to a baseline organism count.

1.2 A knowledge of microbiological techniques is required for these procedures.

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

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 may involve hazardous materials, operations, and equipment. 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.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E1874 Test Method for Recovery of Microorganisms From Skin using the Cup Scrub Technique

2.2 *Federal Document:*[3]

21 CFR Parts 50 and 56 Code of Federal Regulations: Protection of Human Subjects; Institutional Review Boards

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *marker organism, n*—an applied inoculum of an organism that has characteristics that allow it to be readily identified or differentiated. Marker organisms are used to simulate transient microorganisms.

3.1.2 *occlusion, n*—covered and sealed from the outside environment.

3.1.3 *occlusive chamber, n*—a covering that protects the sampling surface without contacting the sampling surface.

3.1.4 *personal cleansing products, n*—products used for personal hygiene such as soaps, hand sanitizers, towelettes, body washes, and so forth.

3.1.5 *transient organisms, n*—organisms from the environment that contaminate but do not normally permanently colonize skin.

3.1.6 *vehicle, n*—a formulation for delivery of the antimicrobial agent.

4. Summary of Test Method

4.1 This guide is conducted on a group of volunteer subjects who refrained from using oral and topical antimicrobials for at least one week.

4.2 The test sites are washed multiple times with the cleansing product or vehicle. After washing, the sites are inoculated with a marker organism and occluded for a specified period of time after which the sites are sampled and enumerated for the marker organism. Activity of the cleansing product is measured by comparing microbial counts from treated sites to those derived from the sites treated with vehicle or to an untreated baseline organism count.

[1] This guide 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] 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] DLA Document Services Building 4/D 700 Robbins Avenue Philadelphia, PA 19111-5094 http://quicksearch.dla.mil/

5. Significance and Use

5.1 The procedure is used to evaluate personal cleansing products containing antibacterial ingredients that are intended to reduce the number of organisms on intact skin. It also may be used to demonstrate the effect of residual antibacterial activity by means of inhibition of the proliferation of bacteria on the skin after contact.

6. Apparatus

6.1 *Colony Counter*—Use any of several types.

6.2 *Incubator*—Any incubator capable of maintaining a suitable temperature ±2°C.

6.3 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization.

6.4 *Timer (Stop Clock)*—One that displays hours and minutes.

6.5 *Table*—Any elevated surface, such as a 1 by 2-m table with mattress or similar padding to allow the subject to recline, when applicable.

6.6 *Handwashing Sink*—A sink of sufficient size to permit panelist to wash without touching hands to the sink surface or other panelist.

6.7 *Water Faucet(s)*—To be located above the sink at a height that permits the hands to be held higher than the elbows during the washing procedure.

6.8 *Tap Water Temperature Regulator and Temperature Monitor*—To monitor and regulate water temperature.

7. Reagents and Materials

7.1 *Bacteriological Pipettes*—Sterile, of appropriate capacity.

NOTE 1—Presterilized/disposable bacteriological pipettes are available from most laboratory supply houses.

7.2 *Water Dilution Bottles*—Any sterilizable container having a 100 to 200-mL capacity and tight closure.

NOTE 2—Milk dilution bottles of 160-mL capacity have a screw-capped closure and are available from most local laboratory supply houses.

7.3 *Scrub Cups*—Sterile cylinders, height approximately 2.5 cm, inside diameter of convenient size. Useful sizes range from approximately 1.5 to 4.0 cm.

7.4 *Teflon Policeman or Rubber Policeman*—Can be fashioned in the laboratory or purchased.

7.5 *Pipettor*—With disposable tips capable of delivering 10 µL.

7.6 *Graduated Cylinders*—Sterile, of appropriate capacity.

7.7 *Beakers*—Sterile, of appropriate capacity.

7.8 *Occlusive Chamber*—For covering inoculated test sites.

NOTE 3—Occlusive chambers or plastic weigh boats of appropriate size available from laboratory supply houses

7.9 *Adhesive Dressing*—For securing the occlusive chamber.

NOTE 4—Adhesive dressings such as adhesive tape, surgical tape, or others secural devices.

7.10 *Bacterial Cultures*—Such as *Staphylococcus aureus* ATCC 27217 or *Escherichia coli* ATCC 11229, or others as appropriate.

7.11 *Test Formulations*—With directions for use.

7.12 *Sampling and Dilution Fluid*—Sterile Butterfield's phosphate buffered water, containing an antimicrobial inactivator specific for the test formulation as determined by Test Method E1054.

7.13 *Plating Medium*—Soybean-casein digest agar or equivalent as appropriate with neutralizers, as determined by Test Method E1054.

7.14 *Sterile Culture Tubes,* or equivalent with closures of appropriate capacity.

8. Test Control and Baseline Skin Sites

8.1 Select skin sites appropriate for target flora and the test material's intended use. Where possible, a contralateral site is recommended for application of the vehicle or for the microorganism count control.

NOTE 5—Forearms are a convenient site for most applications.

9. Subjects

9.1 *Number of Subjects*—The number of subjects required depends on the statistical confidence for the expected test results, the variability encountered in the study, and the relative efficacy of the antibacterial agent evaluated.

9.1.1 Recruit a sufficient number of healthy adult volunteers who have no clinical evidence of dermatoses, open wounds, or other skin disorders that affect the integrity of the test.

9.2 Instruct the subjects to avoid contact with antimicrobials for at least one week prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions, and soaps, also such material as acids bases and solvents. Bathing in biocide-treated pools, hot tubs, spas, and so forth, should be avoided. Provide volunteers with a kit of non-antimicrobial personal care products for exclusive use during the test. Volunteers must wear rubber gloves when contact with antimicrobial agents cannot be avoided.

10. Procedure

10.1 *Treatment(s) Application Procedure:*

10.1.1 Application of test material is assigned by a predetermined randomized application schedule. Each subject will have an active and a vehicle or no vehicle treatment site.

10.1.2 Application of test and vehicle material consists of an equal number of washes. For demonstration of residual activity, more than one test material application may be required. Schedule applications at least one hour apart.

10.1.3 Perform all washes under supervision of a technician trained in the methodology. The following are suggested treatment procedures for evaluating two rinse-off products, a bar soap product, and a liquid soap product; and two no-rinse products, a liquid gel product and a pre-wetted towelette.

10.1.4 *Bar Soap Products and Vehicle*—Check, record, and maintain the temperature of the water at 35 to 38°C before each wash. Water flow should be 4L/min. Remove all jewelry from hands and wrists prior to start of wash procedure. Subjects can

wash their own arms. The start time of each wash should be recorded. The following wash procedure is recommended.

10.1.4.1 Subjects wash their *left* arm first with the assigned test product or vehicle formulation:

(1) Wet the volar surface of the forearm under the running water.

(2) Wet the appropriate bar of soap under the running water.

(3) The subjects rub the bar on their forearm, using an up-and-down motion from the wrist to the elbow, for 15 s.

(4) Then set the bar down and continue to rub the lather on their forearm using the same up-and-down motion from the wrist to the elbow, for an additional 45 s.

(5) Rinse the forearm for 15 s. Do not rub!

(6) Pat the forearm dry using a paper towel. Do not rub!

(7) Repeat steps 1 to 6 on their *right* forearm with the other formulation.

10.1.4.2 The above wash procedure is conducted a total of nine (9) times. There will be three (3) washes each day on the first, second, and third days of the study. The washes are to be at least one (1) hour apart.

10.1.5 *Liquid Soap Products and Vehicle*—Check, record, and maintain the temperature of the water at 35 to 38°C before each wash. Water flow should be 4L/min. Remove all jewelry from hands and wrists prior to start of wash procedure. Subjects can wash their own arms. The start time of each wash should be recorded. The following wash procedure is recommended.

10.1.5.1 Subjects wash their *left* arm first with the assigned test product or vehicle formulation:

(1) Wet the volar surface of the forearm under the running water.

(2) 1.8 mL of liquid soap product is dispensed from a syringe onto the volar surface of the forearm.

(3) The subjects rub the liquid soap on their forearm, using an up-and-down motion from the wrist to the elbow, for 45 s.

(4) They rinse their forearm for 15 s. Do not rub!

(5) Pat the forearm dry using a paper towel. Do not rub!

(6) Repeat steps 1 to 5 on the *right* forearm with the other formulation.

10.1.5.2 The above wash procedure is conducted a total of nine (9) times. There are three (3) washes each day on the first, second, and third days of the test period. The washes are to be at least one (1) hour apart.

10.1.6 *No-Rinse, Liquid Gel, and Placebo*—Remove all jewelry from hands and wrists prior to start of procedure. The time of treatment should be recorded at the start of application. Subjects can treat their own arms. The start time of each treatment should be recorded. The following procedure is recommended.

10.1.6.1 Subject's *left* forearm is treated first with the assigned test product or vehicle formulation:

(1) 1.8 mL of product is dispensed from a syringe onto the volar surface of the forearm.

(2) The subjects rub the formulation on their forearm, using an up-and-down motion from the wrist to the elbow, for 15 s.

(3) Allow subject's forearm to air dry.

(4) Repeat steps 1 to 3 on the *right* forearm with the other formulation.

10.1.6.2 The above procedure is conducted a total of three (3) times at least one (1) hour apart.

10.1.7 *Pre-Wetted Towelette or Wipe Product and Vehicle*—Remove all jewelry from hands and wrists prior to start of procedure. The time of treatment should be recorded at the start of wiping. A technician will wipe each subject's arm. The technician will wear gloves for this procedure. The start time of each treatment should be recorded. The following procedure is recommended.

10.1.7.1 Subject's *left* forearm is treated first with the assigned test product or vehicle formulation:

(1) The technician will unfold the test towelette and wipe the forearm in an up-and-down motion for 15s.

(2) The technician will flip the towelette and wipe the forearm with the opposite side of the towelette in an up-and-down motion for another 15 s.

(3) Allow subject's forearm to air dry.

(4) Repeat steps 1 to 3 on the *right* forearm with the other formulation.

10.1.7.2 The above wipe procedure is conducted a total of three (3) times at least one (1) hour apart.

10.2 *Evaluation Procedure*—Residual activity is assessed at any selected time after the last treatment application, for example, 1 hour, 6 hours, 24 hours, and so forth, by measuring the survival of marker bacteria applied under occlusion to the treated skin sites after pre-selected exposure times, for example, 30 minutes, two hours, or five hours depending on study objectives. The following is an example of a procedure for assessing residual activity 24 h after the last treatment using exposure times of 30 min, 2 h and 5 h.

10.2.1 Approximately 24 h after the final treatment application, three (3) evenly spaced circular test sites are marked along the midsection of the volar aspect of each forearm, avoiding the area on the wrist and elbow crease. The lower site is near the wrist and the upper site is near the elbow. Mark the sites by pressing a 3.0 cm inside diameter glass cylinder, inked with a stamp pad, against the skin.

10.2.2 Using a micro pipettor (section 7.5), inoculate the center of each circular site with 10 µL of the bacterial culture to obtain 10^6 to 10^7 colony forming units (CFUs) of *S. aureus*. A sterile, disposable, inoculating loop is used to evenly spread the inoculum within the center of the test site while remaining 4 to 5 mm from the marked edge. The culture is prepared as described in section 10.5.

10.2.3 Each inoculated site is immediately occluded by covering it with an occlusive chamber (section 7.8) and secured to the skin with an adhesive dressing (section 7.9).

10.3 *Harvesting of the Surviving Organisms*—All inoculated sites are sampled for surviving organisms. One (1) test site on each arm is harvested at each of the three (3) time periods 30 ± 5 min, 2 h ± 5 min, and 5 h ± 5 min after inoculation. The randomization will indicate the time period for each site. See Test Method E1874. The following procedure is used for harvesting:

10.3.1 Aseptically remove occlusive covering from site to be sampled.

10.3.2 A hollow glass cylinder 2.2 cm inside diameter is positioned in the middle area of the test site avoiding contact with the ink-stamped edge.

10.3.3 Pipet one (1.0) mL sterile sampling fluid (section 7.12) with suitable neutralizers into the cylinder.

10.3.4 Massage the skin inside the cylinder for 60 s with a sterile policeman (section 7.4).

10.3.5 Remove the fluid by pipetting it into an empty sterile culture tube.

10.3.6 Add another 1.0 mL of sampling fluid for a 30 s scrub.

10.3.7 Remove the fluid from the second scrub and pool with the fluid from the first scrub.

10.3.8 Care must be taken during this process to prevent the sampling fluid from spilling into an adjacent site that has not been sampled.

10.3.9 After samples are collected, paper toweling is used to blot the site dry.

10.3.10 After each test site is harvested, disinfect with 70 % ethanol. When the harvesting of the last test site is completed, both forearms will be washed for approximately 60 s with chlorhexidine topical (4 % chlorhexidine gluconate). After the arms have been washed with chlorhexidine topical, a small amount of bacitracin/polymyxin antibiotic ointment will be applied to each test site.

10.4 *Microbial Counts*—Each pooled sample is mixed thoroughly. Tenfold serial dilutions of each sample are prepared in dilution fluid (section 7.12). Duplicate quantitative pour or spread plates are prepared in plating medium (section 7.13). Incubate prepared plates at suitable growth temperature, ±2°C for 24 to 72 h, or until colonies are visible.

10.5 *Preparation of Test Organism(s)*—Transfer culture(s) no more than five (5) times from ATCC stock (once every 18 to 24 h) into appropriate culture broth (section 7.15). Make the last transfer into a volume of media large enough to provide enough organism(s) for the test. Adjust culture concentration by dilution in dilution fluid without neutralizer (section 7.12) to between 1×10^{8} and 1×10^{9} CFU/mL.

11. Interpretation

11.1 Determine the residual effectiveness at each evaluation interval. At each interval compare CFU counts using a Wilcoxon paired signed-rank test to determine which of the test articles has the greatest antibacterial activity. In addition, a binomial (sign) test may be performed on these data. Perform a within treatment analysis using the Wilcoxon paired signed ranks test to compare antibacterial activity. P-values ≤0.05 are considered statistically significant.

12. Precision and Bias

12.1 A precision and bias statement cannot be made for this guide at this time.

13. Keywords

13.1 antibacterial wash; cup scrub; residual activity