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Standard Test Method for Evaluation of Surgical Hand Scrub Formulations[1]

This standard is issued under the fixed designation E1115; 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 measure the reduction of microbial flora on the skin. It is intended for determining both immediate and persistent (continuing antimicrobial effect) microbial reductions, after single or repetitive treatments, or both. It may also be used to measure cumulative antimicrobial activity after repetitive treatments.

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.

1.4.1 In this test method, SI units are used for all applications, except for distance, in which case inches are used and SI units follow in parentheses.

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.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2180 Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials

2.2 *Other Documents:*

21 CFR Parts 50 and 56[3]

AATCC 147–2004 Antibacterial Assessment of Textile Materials: Parallel Streak Method[4]

JIS Z 2801 :2000, Antimicrobial Products—Test for Antimicrobial Activity and Efficacy[5]

USP 32 United States Pharmacopeia, Chapter 61 "Microbial Limits Test", 2009[6]

3. Terminology

3.1 *Definitions:*

3.1.1 *active ingredient*—a substance added to a formulation specifically for the inhibition or inactivation of microorganisms.

3.1.2 *cleansing wash*—a non-antimicrobial wash intended to remove gross soil or residues from the hands.

3.1.3 *cleansing wash formulation*—a liquid castile soap or other liquid soap with neutral pH which does not contain an antimicrobial.

3.1.4 *cumulative effect*—a progressive decrease in the number of microorganisms recovered following repeated applications.

3.1.5 *internal reference formulation*—a formulation with demonstrated performance characteristics within the laboratory.

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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 U.S. Government Printing Office, 732 N. Capitol St., Washington, DC 20401, U.S. Government Bookstore, http://bookstore.gpo.gov/baskets/cfr-listing.jsp.

[4] Technical Manual of the American Association of Textile Chemists and Colorists (AATCC), 2009, Vol 82, P.O. Box 12215, Research Triangle Park, NC 27709, http://www.aatcc.org.

[5] Available from Japanese Industrial Standards Committee, Divisional Council on Consumer Life, Japanese Standards Association (JSA), 4-1-24 Akasaka Minato-Ku, Tokyo, 107-8440, Japan, http://www.jsa.or.jp.

[6] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

3.1.6 *neutralization*—a process that results in quenching or inactivation of the antimicrobial activity of a formulation. This may be achieved through dilution of the formulation or through the use of chemical agents called neutralizers.

3.1.7 *persistence*—prolonged or extended antimicrobial activity that prevents or inhibits the proliferation or survival of microorganisms after treatment.

3.1.8 *sampling fluid*—a buffered solution that aids in recovery of microorganisms from the skin and neutralization of the active ingredient in test and internal reference formulations.

3.1.9 *test formulation*—a formulation containing an active ingredient(s).

4. Summary of Test Method

4.1 This test method is conducted on individuals selected from a group of subjects who have refrained from using any antimicrobials for at least one week prior to initiation of the test. Subjects are selected from this group on the basis of high initial bacterial count, $\geq 1 \times 10^5$ CFU/per hand as determined by baseline measurements of the bacteria on their hands using the recovery techniques in this method.

4.2 The selected subjects perform a simulated surgical scrub under the supervision of an individual competent in aseptic technique. One hand of each subject is sampled immediately after the scrub (within 1 min), and the other hand, 6 h after scrubbing. Only one hand of a subject is sampled at a specified time. Optionally, another sampling time, 3 h for example, can be added between the immediate and 6 h sampling times. If this is desired, the panel size must be increased by 50 % to obtain the same number of data points at each designated sampling interval. Also, a sampling time randomization must be generated such that one-third of the hands are sampled at each sampling interval with only one hand of a subject being sampled at a sampling time interval.

NOTE 1—Data for submission to some regulatory bodies may require the addition of a positive and negative control in addition to the test product. For the negative control, 0.9 % saline can be used when testing alcohol products and the product vehicle can be used as the negative control when testing non-alcoholic products.

4.3 If demonstration of cumulative activity is desired, eleven additional scrubs are performed over a 5-day period, one additional time on Day 1, three times on Days 2, 3, and 4 and once on Day 5. The hands are sampled again after the last scheduled scrub.

5. Significance and Use

5.1 The procedure in this test method should be used to evaluate the activity of the test formulation in reducing the bacterial population of the hands immediately after a single use and to determine persistent activity (inhibition of growth) after 6 h. Optionally, measurements of persistent activity after a 3 h period and measurements of cumulative activity may be made after repetitive uses over a five day period.

6. Apparatus

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

6.2 *Incubator*—Any incubator capable of maintaining a temperature of 30 ± 2°C.

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

6.4 *Timer (stop-clock)*—one that displays minutes and seconds.

6.5 *Hand Washing Sink*—A sink of sufficient size to permit subjects to wash without touching hands to sink surface or other subjects.

6.5.1 *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. (It is desirable for the height of the faucet(s) to be adjustable.)

6.6 *Tap Water Temperature Regulator and Temperature Monitor*—To monitor and regulate water temperature to 40 ± 2°C.

7. Reagents and Materials

7.1 *Petri Dishes*—100 by 15 mm. Required for performing Standard Plate Count.

NOTE 2—Pre-sterilized/disposable plastic petri dishes are available from most local laboratory supply houses.

7.2 *Bacteriological Pipets*—10.0 and 2.2 or 1.1-mL capacity.

NOTE 3—Pre-sterilized/disposable bacteriological pipets are available from most local laboratory supply houses.

7.3 *Water-Dilution Bottles*—Any sterilizable container having a 150 to 200-mL capacity and tight closures may be used.

NOTE 4—Dilution bottles of 160-mL capacity having a screw-cap closure are available from most local laboratory supply houses.

7.4 *Cleansing Wash Formulation*—A mild, non-antimicrobial soft soap such as the following or any other liquid soap with neutral pH which does not contain an antimicrobial:

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

7.4.1 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.

7.5 *Gloves for Sampling*—Loose-fitting, unlined, powder-free latex gloves which possess no antimicrobial properties,[7] or equivalent.

NOTE 5—A zone of inhibition test such as AATCC 147–2004, Test Method E2180, or Japanese Standard JIS Z 2801 may be used to evaluate antimicrobial properties of gloves.

7.6 *Test Formulation*—Directions for use of active test formulation should be utilized if available. If not available, use directions provided in this test method (see ~~11.3~~11.1.3).

7.7 *Water*—Sterile deionized water or equivalent (Specification D1193, Type III).

7.8 *Sampling Fluid*[8]—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, 1.0 g isooctylphenoxypolyethoxyethanol (for example, Triton X-100), and appropriately validated neutralizers in 1 L distilled water. Adjust pH to 7.8 ± 1 with 0.1 *N* HCl or 01. *N* NaOH. Dispense to achieve a final volume of 75 ± 1 mL and sterilize.

7.9 *Dilution Fluid*—Sterile Butterfield's buffer[9] or other suitable diluent adjusted to pH 7.2 ± 0.1 with effective neutralizer for the test material. Adjust pH with 0.1 *N* HCl or 0.1 *N* NaOH. See Test Methods E1054.

7.10 *Agar*—Soybean-casein Digest agar (USP 32) or other solid media appropriately validated to support growth of the test organism with appropriate neutralizers if needed.

NOTE 6—Inadequate neutralization may result in false interpretation of the test data. The use of excess chemical neutralizers may exert a toxic effect on the recovery of bacterial cells. The goal, therefore, is to stop antimicrobial activity as early as possible in the sampling/plating process. If it can be demonstrated that antimicrobial activity is quenched or inactivated in the sampling fluid then, to reduce the chance of possible toxic effects, inactivators should not be added to the dilution fluid or plating media.

7.11 *Scrub Sponge and Nail Cleaner Stick*—Such as E-Z Scrub 160[10] or any equivalent may be used.

8. Subjects

8.1 Recruit a sufficient number (see X1.1) of healthy subjects who have no clinical evidence of dermatoses, open wounds, or other skin disorders. Exclude any individual receiving antibiotic therapy and any individual sensitive to natural rubber or latex or to a component of the formulation(s) being tested.

8.2 Instruct the subjects to avoid contact with antimicrobial products (other than the test formulation(s) as dispensed for each scrub) for the duration of the test and for at least one week prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions, dishwashing liquids and soaps, and also such materials as acids, bases, and solvents. Bathing in biocide treated pools, hot tubs, or spas should be avoided. Subjects are provided with a kit of non-antimicrobial personal care products for exclusive use during the test and rubber gloves to be worn when contact with antimicrobials agents cannot be avoided.

9. Procedure

9.1 After subjects have refrained from using antimicrobials for at least one week, perform wash with cleansing wash formulation (see 7.4) using methodology outlined in 10.1-10.4. Subjects are not to have washed their hands on this day 2 h prior to baseline determination. After washing, determine first estimate of baseline bacterial population by sampling hands and enumerating the bacteria in the sampling fluid. This is Day 1 of "Baseline Period." Repeat this baseline determination procedure on Days 3 and 7, Days 3 and 5, or Days 5 and 7 of "Baseline Period" to obtain three estimates of baseline population. After obtaining the first and second estimates of the baseline populations, select subjects who exhibited at each sampling time counts $\geq 1 \times 10^5$ per hand. The three estimates of the baseline population obtained for each of the selected subjects are averaged to obtain the mean baseline counts.

9.2 A basic random bacterial recovery sampling plan should be followed. The number of subjects and sampling times depend on the test formulation but must establish the onset and extent of the bacterial suppression and the duration of suppression below the baseline counts. Equal numbers of subjects should be assigned per sampling time, test formulation and hand. A typical balanced randomization plan for testing a block of six subjects follows with sampling at 0 h, 3 h (optional), and 6 h.

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[7] The sole source of supply of the apparatus (Ansell #579500, sterile, Encore Acclaim Latex Surgical Gloves) known to the committee at this time is PSS Medical, Inc. (Cat #105613). 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, which you may attend.

[8] Peterson, A. F., "The Microbiology of the Hands: Evaluating the Effects of Surgical Scrubs," *Developments in Industrial Microbiology*, Vol 14, 1973, pp. 125–130.

[9] Horowitz, W. (Ed.) , 2006 *Official Methods of Analysis of AOAC International,* 2006, 18th Ed., Revision 1, Ch 17, p. 4, Sec. 17.2.01 (m). AOAC, Washington, D.C.

[10] The sole source of supply of the apparatus known to the committee at this time is E-Z Scrub 160, Cat. No. 371603, manufactured by Becton Dickinson Div., Franklin Lakes, NJ 07417–1884. 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, which you may attend.