



Standard Test Method for Evaluation of Surgical Hand Scrub Formulations¹

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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 56³

AATCC 147–2004 Antibacterial Assessment of Textile Materials: Parallel Streak Method⁴

JIS Z 2801 :2000, Antimicrobial Products—Test for Antimicrobial Activity and Efficacy⁵

USP 32 United States Pharmacopeia, Chapter 61 “Microbial Limits Test”, 2009⁶

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.

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.

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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>.

3.1.8 *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 $30 \pm 2^\circ\text{C}$.

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 $40 \pm 2^\circ\text{C}$.

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 [11.1.3](#)).

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,¹ which you may attend.

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

7.8 Sampling Fluid⁸—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 0.1 N NaOH. Dispense to achieve a final volume of 75 ± 1 mL and sterilize.

7.9 Dilution Fluid—Sterile Butterfield's buffer⁹ 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 160¹⁰ 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.

Subject No.	Post Scrub Sampling Time, h		
	0-h	3-h	6-h
1	left hand	right hand	
2	left hand		right hand
3	right hand	left hand	
4	right hand		left hand
5		left hand	right hand
6		right hand	left hand

If only 0 h and 6 h post scrub samples are collected the 0 h will be randomized to the right or left hand.

9.2.1 The number of subjects per block may vary but must be divisible by two and by the number of sampling times in order to assign equal number of left and right hands to each sampling time.

9.3 No sooner than 24 h and no longer than 96 h after completion of the baseline determination, subjects perform scrub with the test formulation. The starting interval should be same for all subjects participating in the study. According to the random sampling plan, determine the bacterial populations on the subjects' hands at the assigned sampling times after scrubbing. Determine bacterial population by sampling hands and enumerating the bacteria in the sampling fluid as specified in Sections **13** and **14**.

9.4 If measurement of cumulative effect is desired, the hands are sampled one more time after performing 11 additional scrubs with the active test formulation over a 5 day period. Repeat the treatment procedure with the test formulation one additional time after the sampling on Day 1 and treat three additional times on Day 2, Day 3 and Day 4 with at least a 1-h interval between scrub treatments. On day 5 perform one scrub treatment prior to sampling.

9.5 In summary, measurement of immediate activity is made following a single scrub. Persistent activity may be measured by collecting samples after 3 and or 6 h of glove wear or other selected times after the immediate sampling. If measurement of cumulative activity is desired the subjects are to scrub a total of twelve times with the test formulation, twice on Day 1 and three times per day on Days 2, 3, and 4, and once on Day 5.

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,¹ which you may attend.

Collect the samples following single scrubs on Days 1 and 5. This mimics typical usage and permits determination of reduction immediately after a single use and after repeated uses.

9.6 The schedule for scrubbing and sampling is shown in the following table. Samples collected immediately after the first scrub are used to measure the immediate reduction; optionally samples collected after scrub 12 are used to measure cumulative activity.

Day	Scrubs	Sample
1	2 (1 before and 1 after sample)	1
2	3	0
3	3	0
4	3	0
5	1	1
Totals	12	2

10. Washing Technique for Baseline Determinations

10.1 Subjects clean under fingernails with nail stick and clip fingernails to ± 2 -mm free edge. Remove all jewelry from hands and arms.

10.2 Rinse hands including two-thirds of forearm under running tap water for 30 s. Maintain hands higher than elbows during this procedure and steps outlined in 10.3-10.5. Adjust water temperature to $40 \pm 2^\circ\text{C}$ and the water flow rate to 4 L per minute.

NOTE 7—This may be accomplished by placing a 2000 mL glass beaker or flask under each spigot to be used for subjects' hand washing. Allow the water to flow into the beaker. Adjust the water flow at each spigot accordingly, so that the beaker fills within 30 s.

10.3 Perform a cleansing wash of hands and forearms with cleansing wash formulation for 30 s using water as required to develop lather.

10.4 Rinse hands and forearms for 30 s under tap water thoroughly removing all lather.

10.5 Place gloves (see 7.5) used for sampling on right and left hands and secure gloves at wrist.

10.6 Sample hands for recoverable bacteria as described in Section 13.

11. Surgical Scrub Technique to Be Used Prior to Bacterial Sampling

11.1 *For Water-aided Test Formulations:*

11.1.1 Repeat 10.1 and 10.2.

11.1.2 Perform scrub with test formulation in accordance with directions furnished with the active test formulation(s) and/or the negative controls.

NOTE 8—If no instructions are provided with the active test formulation, use the 10-min scrub procedure in 11.1.3.

11.1.3 *Ten-Minute Scrub Procedure:*

11.1.3.1 Dispense prescribed amount of formulation into hands.

11.1.3.2 Set and start timer for 5 min (time required for the steps in 11.1.3.3-11.1.3.7).

11.1.3.3 With hands, distribute formulation over hands and lower two-thirds of forearms.

11.1.3.4 If scrub brush is to be used, pick up with fingertips and pass under tap to wet, without rinsing formulation from hands.

11.1.3.5 Alternately scrub right hand and lower two-thirds of forearm and left hand and lower two-thirds of forearm.

11.1.3.6 Rinse both hands, the lower two-thirds of both forearms, and the brush for 30 s.

11.1.3.7 Place brush in sterile dish within easy reach.

11.1.3.8 Repeat 11.1.3.1-11.1.3.7 so that each hand and forearm is washed twice. The second wash and rinse should be limited to the lower one-third of the forearms and the hands.

11.1.3.9 Perform final rinse. Rinse each hand and forearm separately for 1 min per hand.

11.1.3.10 Immediately (within less than 30 s), place gloves used for sampling on right and left hands and secure gloves at wrist.

11.1.4 Sample hands as described in Section 13 at assigned sample times.

11.2 *For Leave-on Test Formulations that are Not Water-aided:*

11.2.1 Repeat 10.1.

11.2.2 Perform application of test formulation in accordance with directions furnished with the active test formulation(s) and/or the negative controls.

NOTE 9—If no instructions are provided with the active test formulation, use the three-application procedure in 11.2.3.

11.2.3 *Three-Application Scrub Procedure:*

11.2.3.1 Dispense prescribed amount of formulation into the palm of one hand, dip fingernails of opposite hand into the product, and work in under the nails. Spread the remaining product evenly over the hand and lower two-thirds of the forearm, paying particular attention to nails, cuticles, and interdigital spaces. The product is allowed to air dry completely.

11.2.3.2 Dispense prescribed amount of formulation into the palm of remaining hand, dip fingernails of opposite hand into the product, and work in under the nails. Spread the remaining product evenly over the hand and lower two-thirds of the forearm, paying particular attention to nails, cuticles, and interdigital spaces. The product is allowed to air dry completely.

11.2.3.3 Dispense prescribed amount of product into either of the subject's hands. Spread the product evenly over both hands up to the wrist, paying particular attention to the nails, cuticles, and interdigital spaces. The product is allowed to air dry completely.

11.2.3.4 Immediately after drying (within less than 30 s), place gloves used for sampling on right and left hands and secure gloves at wrist.

11.2.4 Sample hands as described in Section 13 at assigned sample times.

12. Surgical Scrub Technique When Bacterial Samples Are Not Specified

12.1 Perform technique as described in Section 11, except omit 11.1.3.10. Subjects dry hands with clean paper towels after final rinse of hands.

13. Bacterial Recovery

13.1 At each specified sampling time, (for example, immediate, 3h, 6h) aseptically add 75 mL of sampling fluid with

neutralizer (see 7.8) to the gloved hand to be sampled and secure the glove above the wrist.

13.2 Within one minute of donning gloves, uniformly massage all surfaces of the hand for $1 \text{ min} \pm 5 \text{ s}$, paying particular attention to the fingers and flipping the hand after 30 s to ensure both the palm and back of the hand are thoroughly massaged.

13.3 Aseptically retrieve a 3 to 5 mL sample of the fluid in the glove by pulling the glove away from the wrist, inserting a pipet into the finger region of the glove, and withdrawing the fluid.

13.4 Rinse hands under running tap water to remove residual sampling fluid.

13.5 The first dilution of sampling fluid is to be made in dilution fluid with appropriate neutralizer within 1 min and 10 s of completing the massage. The plating of the recovered sampling solution is completed within 30 min after sampling.

14. Enumeration of Bacteria in Sampling Fluid

14.1 Enumerate the bacteria in the sampling fluid by microbiological techniques such as surface inoculation technique (spread plating or spiral plating) or pour-plate technique.

14.2 Prepare sample dilutions in Dilution Fluid (see 7.9). Use Soybean-Casein Digest Agar (see 7.10). Plate in duplicate.

14.3 Incubate plated sample at $30 \pm 2^\circ\text{C}$ for 48 to 72 h before reading. Standard plate counting procedures are to be used.

14.4 Calculate for each hand sampled at each sampling time the average number of colony forming units (CFUs) recovered.

15. Determination of Reduction Obtained

15.1 For each post-treatment sampling time determine changes from baseline counts obtained with the test formulation.

15.2 To determine the activity of the test formulation, all counts of colony forming units per hand should be converted to common (base10) logarithms. At each sampling time $\log_{10}$ reductions should be calculated.

16. Method of Statistical Analysis

16.1 Prior to initiating the statistical analyses, the subjects' first and second baseline count for each hand are to be examined to determine if they meet the qualification criterion ($>1.0 \times 10^5$ CFU/hand).

16.2 *Check for Significant Difference Between Right and Left Hand Bioburdens at Baseline*—The source data for the baseline analysis are the 3-day average $\log_{10}$ values for the right and left hands of each subject. Potential differences between right and left hand bioburdens at the baseline are examined using a two-factor, subject $\times$ hand, analysis of variance procedure.

16.3 *Activity*—The mean $\log_{10}$ reductions and the 95 % confidence intervals for the test formulation(s) after 1 or 5 days of usage are to be calculated for each sampling time. $\log_{10}$ reductions for each subject are calculated as average baseline $\log_{10}$ of a hand minus $\log_{10}$ of the post-treatment count for that hand.

16.3.1 *Persistent Activity (Within-treatments)*—Analysis of variance techniques are to be performed to evaluate differences between sampling intervals in a given day. $\log_{10}$ reduction values from baseline are used in this analysis.

16.3.2 *Cumulative Activity (Within-treatments)*—Analysis of variance techniques are to be performed to calculate differences between similar sampling times on different test days. (that is, comparing 6 h, Day 5 to 6 h, Day 1). Log reduction values from baseline are used in this analysis.

17. Internal Reference Standard

17.1 To measure the validity of the test method within a study an internal reference formulation and a negative control should be evaluated.

18. Precision and Bias

18.1 A precision and bias statement can not be made for this test method at this time.

19. Keywords

19.1 antimicrobial; efficacy; glove juice; handwash; healthcare; surgical scrub

APPENDIX

(Nonmandatory Information)

X1. SAMPLE SIZE CALCULATIONS

X1.1 Sample size calculations should be done to determine the number of subjects necessary to find statistically significant differences (reductions) from baseline. The number of subjects required depends on the statistical confidence required for the expected results, the variability encountered in the data collection (that is, variability in reductions from baseline), and the expected efficacy of the test product (that is, its expected reduction from baseline). This number of subjects (n) can be estimated from the following equation:

$$n > S^2 \left[\frac{(Z_{\alpha/2} + Z_{\beta})^2}{D^2} \right]$$

where:

- S^2 = estimate of variance (of reduction from baseline based on in-house data pool or published data),
- $Z_{\alpha/2}$ = cumulative probability of the standard normal distribution = 1.96 for $\alpha = 0.05$,
- Z_{β} = power of the test = 0.842 for $\beta = 0.80$, and
- D = expected efficacy (expected reduction from baseline).

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