Designation: E 1115 – 02

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

This standard is issued under the fixed designation E 1115; 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 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 In this method, metric units are used for all applications, except for distance, in which case inches are used and metric units follow in parentheses.

1.4 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects.[2]

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:*

D 1193 Specification for Reagent Water[3]

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[4]

2.2 *Other Documents:*

21 CFR Parts 50 and 56[5]

AATCC Test Method 147 1993 Antibacterial Assessment of Textile Materials: Parallel Streak Method[6]

Horowitz, W. (Ed.), 2000, Official Methods of Analysis of AOAC International 17th Ed., Ch 17, p. 4, Sec. 17.2.01 (m). Assoc. of Off. Anal. Chemist, Washington, D.C.

United States Pharmacopeia, 25, 2001, United States Pharmacopeial Convention, Inc., Rockville, MD. Chapter 61 "Microbial Limits Test"

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 of the subjects prior to collecting baseline samples.

3.1.3 *cleansing wash formulation*[7]—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.

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 subjects selected from a group of volunteers who have refrained from using any antimicrobials for at least two weeks 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

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[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] 21 CFR Ch. 1, Parts 50 and 56.

[3] *Annual Book of ASTM Standards*, Vol 11.01.

[4] *Annual Book of ASTM Standards*, Vol 11.05.

[5] *United States Code of Federal Regulations*.

[6] Technical Manual of the American Association of Textile Chemists and Colorists, P.O. Box 12215, Research Triangle Park, NC 27709.

[7] Johnson's Baby Wash Head– to–Toe™ Johnson's and Johnson's Inc., Skillman, NJ 08558–9418.

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.

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.

NOTE 1—The researcher should be cautioned that components of chemical neutralizer systems such as lecithin and polysorbate 80 may interfere in the determination of cumulative effect on the skin.[8,9,10]

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*—Any of several types may be used, for example, Quebec Colony Counter.

6.2 *Incubator*—Any incubator that can maintain a temperature of 30 ± 2°C may be used.

6.3 *Sterilizer*—Any suitable steam sterilizer that can produce the conditions of sterility is acceptable.

6.4 *Timer (stop-clock)*—That can be read for 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.[11]

7.2 *Bacteriological Pipets*—10.0 and 2.2 or 1.1-mL capacity.[12]

7.3 *Water-Dilution Bottles*—Any sterilizable container having a 150 to 200-mL capacity and tight closures may be used.[13]

7.4 *Cleansing Wash Formulation*—A formulation without an active ingredient.

7.5 *Gloves for Sampling*—Loose-fitting, unlined, powder-free latex gloves which do not demonstrate antimicrobial activity, or equivalent glove. An equivalent is a glove composed of any material that is unlined, does not leak and does not demonstrate antimicrobial activity. A zone of inhibition test such as AATCC Test Method 147 may be used to evaluate the antibacterial activity.

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).

7.7 *Water*—Sterile deionized water or equivalent (Specification D 1193, Type III).

7.8 *Sampling Fluid*[14]—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4 and 1.0 g isooctylphenoxypolyethoxyethanol[14] in 1 L of water. Adjust to obtain a final pH 7.8 ± 0.1. Dispense to achieve a final volume of 75 ± 1 mL into water dilution bottles, or other suitable containers, and sterilize. Optionally, the sampling fluid may be sterilized before dispensing into sterile containers. The ability of the sampling fluid to neutralize or quench the antimicrobial activity of the test formulation must be validated (see Practices E 1054). The validation test should be conducted *in vivo* in accordance with how the surgical hand scrub study is conducted. If the sampling fluid does not quench the antimicrobial activity of the test formulation and the internal reference formulation, an antimicrobial inactivator should be included if required.

7.9 *Dilution Fluid*—Butterfield's buffered phosphate diluent[15] adjusted to pH 7.2 ± 0.1 (or other suitable diluent) and containing an antimicrobial inactivator if required.

7.10 *Soybean-Casein Digest Agar, or equivalent*[16]—Containing an antimicrobial inactivator if required.

NOTE 2—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

[8] Bently, M.V., Kedor,E.R., Vianna, R.F., Collett, J.H., Influence of lecithin and urea on the in vitro permeation of hydrocortisone acetate through skin from hairless mouse. International Journal of Pharmaceutics. Vol. 146: 255–262.

[9] Kato, A., Ishibahi, Y., Effect of egg yolk lecithin on transdermal delivery of bunazosin. Journal of Pharmacy and Pharmacology. Vol 39: 399–400.

[10] Rejendran D., Sivabalan, M., Dhanaraj, S.A., Ponnusankar,S., Dube,R., Suresh, B., Trandermal delivery of prazoosin HCL with non–ionic surfactants. Indian Journal of Pharmaceutical Sciences. Vol. 150–153.

[11] Pre-sterilized/disposable plastic petri dishes are available from most local laboratory supply houses.

[12] Pre-sterilized/disposable bacteriological pipets are available from most local laboratory supply houses.

[13] Dilution bottles of 160-mL capacity having a screw-cap closure are available from Corning Glass Co., Kimble Glass Co., or most local laboratory supply houses.

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

[15] Horowitz, W. (Ed.) 2000, *Official Methods of Analysis of AOAC International* 17th Ed., Ch 17, p. 4, Sec. 17.2.01 (m). Assoc. of Off. Anal. Chemist, Washington, D.C.

[16] *United States Pharmacopeia*, 23, 1995, United States Pharmacopeial Convention, Inc., Rockville, MD. Chapter 61 "Microbial Limits Test."

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[17] or any equivalent may be used.

8. Subjects

8.1 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),
$Z_{\alpha/2}$ = 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).

8.2 Recruit a sufficient number 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.

8.3 Instruct the subjects to avoid contact with antimicrobials (other than the test formulation as dispensed for each scrub) for at least two weeks prior to obtaining the first baseline sampling and for the duration of 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, is not permitted. Subjects should be 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 two weeks, 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 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 three additional times on Day 2, Day 3 and Day 4 with at least a 1-h interval between scrubs. On day 5 perform one scrub 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. 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.

[17] E-Z Scrub 160, Cat. No. 371603, Manufactured by Becton Dickinson Div., Franklin Lakes, NJ 07417-1884.

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 Volunteers 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 40 ± 2°C for 30 s. Maintain hands higher than elbows during this procedure and steps outlined in 10.3-10.5.

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 as described in Section 13.

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

11.1 Repeat 10.1 and 10.2.

11.2 Perform scrub with test formulation in accordance with directions furnished with the active test formulation.

NOTE 3—If no instructions are provided with the active test formulation, use the 10-min scrub procedure in 11.3.

11.3 *Ten-Minute Scrub Procedure*:

11.3.1 Dispense prescribed amount of formulation into hands.

11.3.2 Set and start timer for 5 min (time required for the steps in 11.3.3-11.3.7).

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

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

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

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

11.3.7 Place brush in sterile dish within easy reach.

11.3.8 Repeat 11.3.1-11.3.6 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.

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

11.3.10 Place gloves used for sampling on right and left hands and secure gloves at wrist.

11.4 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 11.3.10. Subjects dry hands with clean paper towels after final rinse of hands.

13. Sampling Techniques

13.1 At each specified sampling time, (for example, immediate, 3h, 6h) aseptically add 75 mL of sampling fluid with neutralizer (see 7.6) to the gloved hand to be sampled and secure the glove above the wrist.

13.2 After adding sampling fluid, uniformly massage all surfaces of hand for 1 min.

13.3 After massaging, aseptically sample the fluid from the glove for bacterial enumeration.

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

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. The initial dilution of the sampling fluid must be completed within 30 s of sampling. All serial dilutions and plating must be completed within 30 min of sampling. Prepare sample dilutions in Dilution Fluid (see 7.9). Use Soybean-Casein Digest Agar (see 7.10). Plate in duplicate. Incubate plated sample at 30 ± 2°C for 48 to 72 h before reading. Standard plate counting procedures are to be used. Calculate for each hand sampled at each sampling time the average number of colony forming units (CFU) 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).

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 × hand, analysis of variance procedure.

16.3 *Activity*—The mean $\log_{10}$ reductions and the 95 % confidence intervals for the test articles 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 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; surgical scrub