Designation: E2755 – 15

Standard Test Method for Determining the Bacteria-Eliminating Effectiveness of Healthcare Personnel Hand Rub Formulations Using Hands of Adults[1]

This standard is issued under the fixed designation E2755; 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 activity of healthcare personnel hand rubs, (also known as hand rubs, hygienic hand rubs, hand sanitizers, or hand antiseptics) against transient microbial skin flora on the hands after a single application and after repeated applications.

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.[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 8.2.

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E1174 Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations

E2276 Test Method for Determining the Bacteria-Eliminating Effectiveness of Hygienic Handwash and Handrub Agents Using the Fingerpads of Adults

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Other Standards:*

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

21 CFR Parts 50 and 56 Protection of Human Subjects; Institutional Review Boards[5]

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 handrub, 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, or hand antiseptics.

3.2.2 *healthcare personnel handwash, n*—a cleanser or waterless agent intended to reduce transient microbial skin flora on the hands.

3.2.3 *test bacteria, n*—an applied inoculum of bacteria that has characteristics which allow it to be readily identified. Test bacteria are used to simulate a topical transient microbial contaminant. This may also be referred to as a test organism, marker organism, simulant, or contaminant.

3.2.4 *test material, n*—a product or formulation which incorporates an antimicrobial ingredient(s).

[1] This test method 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.

Current edition approved May 15, 2015. Published June 2015. originally approved in 2010. Last previous edition approved in 2010 as E2755–10. DOI: 10.1520/E2755–15

[2] *CDC-NIH, Biosafety in Microbiological and Biomedical Laboratories*, 5th ed., U.S. Department of Health and Human Services, U.S. Government Printing Office, Washington, DC, 2007.

[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 American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709, http://www.aatcc.org.

[5] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

Copyright © ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959. United States

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 apparent damage at the time of participation in the study. Subjects are to refrain from use of any antimicrobials for at least one week prior to the initiation of the test procedure (see Section 11).

4.2 Subjects' hands are artificially contaminated with 0.2 mL of a high-titer suspension of the test bacteria which is distributed over all surfaces of the hands and fingers to produce a minimum baseline recovery level of 10^8 cfu/hand. Because *Serratia marcescens* is relatively sensitive to drying, the high titer suspension is prepared by growing in broth with vigorous aeration, followed by a 10-fold concentration with centrifugation. *Staphylococcus aureus* is more resistant to drying and is therefore not concentrated after growth with vigorous aeration in broth.

4.3 Test material effectiveness is measured by comparing the number of test bacteria recovered from contaminated hands after use of the test material to the number recovered from contaminated hands not exposed to the test material. Activity of the test material is measured following a single application and after multiple consecutive contamination/application cycles in a single day. Evaluating effectiveness after multiple applications simulates repeated use of hand rubs in clinical settings and determines whether progressive build-up of non-volatile ingredients from the test material inhibits the antimicrobial action. An abbreviated test measuring activity of the test material following a single application may be used to simulate situations where high frequency use is not expected.

5. Significance and Use

5.1 Hand hygiene is considered one of the most important measures for preventing the spread of infectious microorganisms. Hand rubs reduce the microbial load on the hands without the use of soap and water, and are thus an important tool in the practice of good hand hygiene. Alcohol-based hand rubs are recommended in healthcare settings for use on hands that are not visibly soiled. They are formulated to be applied full strength to dry hands, "rubbed in" until dry, and are not rinsed off.

5.2 This test method is designed specifically to evaluate hand rubs for efficacy in eliminating bacteria from experimentally-contaminated hands. It is designed as an alternative to Test Method E1174, which was intended primarily to evaluate antimicrobial handwashing agents that are lathered with the aid of water and then rinsed off. When using Test Method E1174 to evaluate hand rubs, inadequate drying of the hands after contamination dilutes the test material and can compromise activity, to result in an underestimation of effectiveness. Additionally, because hand rubs are not rinsed after product use, activity can be further degraded by build-up of soil from the contaminating broth and inactivated challenge bacteria on the hands.

5.2.1 In this method, application to the hands of a small volume of high-titer test bacteria suspension minimizes soil load such that the skin is completely dry prior to application of the test material. Further, by applying the bacterial suspension only prior to those test material application cycles followed by sampling, excessive buildup of killed bacteria on the hands is avoided, and the potential impact of non-volatile test product ingredients on bacteria-eliminating effectiveness after ten consecutive applications can be specifically assessed.

5.3 A reference control is evaluated for each subject prior to evaluation of the test material. Data from the reference control helps to control for inter-subject variability, inter-experimental variability, and inter-laboratory variability; and enables improved statistical comparison of test materials evaluated in the same experiment.

5.4 This test method can be used to test any form of hand rubs, including gels, rinses, sprays, foams, and wipes when used according to label directions at typical "in-use" doses.

5.5 Susceptibility to biocides can vary among different species of bacteria and major differences have been noted between gram-negative and gram-positive organisms. This test method provides the option to use either a gram-negative bacterium (*Serratia marcescens*) or a gram-positive bacterium (*Staphylococcus aureus*) as the test organism. *S. marcescens* is used as a test organism in both Test Method E1174 and Test Method E2276. *S. aureus* is a highly relevant pathogen in healthcare, institutional, and community settings. Moreover, hands are an important vehicle in the transfer of *S. aureus* between people and the environment, and in the transfer between individuals.

5.6 This test method may be used as an alternative to Test Method E2276, which limits the test bacteria to the fingerpads and does not incorporate actual use conditions such as friction during hand rubbing.

5.7 The investigator should be aware of potential health risks associated with the use of these organisms and precautions similar to those referenced in Section 8 should be taken.

6. Apparatus

6.1 *Centrifuge*—For the sedimentation of *S. marcescens* for concentration.

6.2 *Centrifuge Tubes*—Sterile, for sedimentation of *S. marcescens* for concentration.

6.3 *Colony Counter*—Any of several types may be used; for example, Quebec colony counters and similar devices. Automated, computerized plater/counter systems may also be used.

6.4 *Gloves*—Sterile, loose-fitting, unlined, powder-free gloves possessing no antimicrobial properties. Perform a zone of inhibition test, such as AATCC Test Method 147, to evaluate the antibacterial activity.

6.5 *Handwashing Sink*—Sufficient in size to permit handwashing without the touching of hands to sink surface or other subjects.

6.5.1 *Water Faucet(s)*—Located above the sink at a height to permit hands to be held higher than the elbow during the washing procedure.

6.5.2 *Tap Water Temperature Regulator and Temperature Monitor*—To set and maintain the tap water temperature at 40 ± 2°C.

6.6 *Incubator*—Capable of maintaining temperatures of 35 ± 2°C and 25 ± 2°C. The latter temperature ensures adequate pigment production for *S. marcescens* on solid media.

6.7 *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, and beakers.

6.8 *Plastic Bags*—May be used in place of gloves (6.4). Bags should be approximately 29 by 31 cm, possess no antimicrobial properties, and have a low bioburden. Perform a zone of inhibition test, such as AATCC Test Method 147, to evaluate the antibacterial activity

6.9 *Sampling Containers*—Sterile or sterilizable containers having tight closures and sufficient capacity to hold 75 mL sampling solution (see 7.7).

6.10 *Shaking Incubator*—Rotary platform shaking incubator capable of maintaining 35 ± 2°C and capable of shaking at 250 r/min. Alternatively, use an incubator capable of maintaining 35 ± 2°C and able to accommodate a portable rotary shaker, capable of shaking at 250 r/min.

6.11 *Sterilizer*—Any steam sterilizer capable of processing culture media and reagents.

6.12 *Timer (Stop-Clock)*—Type that can be read for minutes and seconds.

6.13 *Tourniquets*—Children's size or any style capable of securing gloves to the wrist.

6.14 *Vortex Mixer*—Any vortex that will ensure proper mixing of culture tubes.

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 provided in Test Method E1174.

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 *S. aureus Plating Medium*—HardyCHROM (trademark), *Staph aureus*, available from Hardy Diagnostics, is recommended. Other indicator media for *S. aureus* or MRSA may be appropriate but should be validated prior to use.

NOTE 1—*S. aureus* forms smooth, deep pink to fuchsia-colored colonies. The growth of most other organisms, including *Staphylococcus epidermidis* are partially to completely inhibited.

7.4.2.2 *S. marcescens Plating Medium*—Soybean-casein digest agar (tryptic soy agar) is recommended.

7.5 *Dilution Fluid*—Sterile Butterfield's buffered phosphate diluent[6] (or other suitable diluent) adjusted to pH 7.2 ± 0.1 and containing an effective inactivator for the test material, if necessary.

NOTE 2—Inactivator is only required if neutralization of the test material cannot be achieved upon dilution into the sampling solution (see 7.7).

7.6 *Ethanol Solution*—70 % ethanol in water (v/v) for hand decontamination.

7.7 *Sampling Solution*—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, if necessary, 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. Sterilize in an autoclave and aseptically dispense 75-mL portions into sterile sampling containers (see 6.9).[7]

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.

7.8 *Test Material*—Use directions provided with the test material. If directions are not provided, use the directions given in this method.

7.9 *Reference Control*—60% isopropanol in water (v/v).

8. Test Bacteria

8.1 *Serratia marcescens* (ATCC 14756). This strain forms a stable red pigmentation at 25°C.

8.2 *Staphylococcus aureus* (ATCC 6538 (methicillin-sensitive) or ATCC 33591 (methicillin-resistant)) is an alternative test bacteria. *S. aureus* is differentiated from resident microbial skin flora (including *Staphylococcus epidermidis*) with chromogenic indicator medium (see 7.4.2.1). (**Warning—**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 (12.1 – 12.4). If an infection occurs, provide the antibiotic susceptibility profile to the attending clinician.)

9. Preparation of Test Bacteria Suspension

9.1 *Method 1 (for S. marcescens):*

9.1.1 A homogeneous bacterial suspension is used to inoculate the subjects' hands. Prepare a stock culture of *S. marcescens* (ATCC 14756) by inoculating approximately 5 mL of

[6] Horowitz, W., (Ed.), *Official Methods of Analysis of the AOAC International*, 18th Ed., Sec. 6.3.03 A.(f), Chapter 6, p. 10. AOAC International, Gaithersburg, MD, 2000.

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

soybean-casein digest broth (see 7.4.1) from a cryogenic stock or lyophilized vial or pellet and incubate for 25 ± 1 h at 35 ± 2°C. Inoculate the appropriate volume of soybean-casein digest broth with 1 mL of the stock culture of *S. marcescens*/125 mL of broth to yield the volume necessary to complete the study (that is, 0.2 mL per hand contamination (see 11.3) per test subject). The volume of the broth culture should not exceed about one fourth of the capacity of the Erlenmeyer flask to ensure adequate aeration. Incubate for 25 ± 1 h at 35 ± 2°C with shaking at 250 r/min to yield a titer of approximately 1.0×10^{10} cfu/mL.

NOTE 4—The frozen or lyophilized stock should be at least two but no more than four 24-h soybean-casein digest broth (see 7.4.1) transfers from the original ATCC culture.

9.1.2 Transfer the culture to appropriate sized sterile centrifuge tubes or bottles and centrifuge at conditions appropriate to sediment the culture completely (recommended conditions are 7000 G for 10 min). Decant the supernatant and resuspend the pellet to one-tenth the original volume with soybean-casein digest broth (see 7.4.1) to yield a homogeneous suspension containing between 5.0×10^{10} and 1.0×10^{11} cfu/mL.

9.2 *Method 2 (for S. aureus):*

9.2.1 Use a homogeneous bacterial suspension to inoculate the subjects' hands. Prepare a stock culture of *S. aureus* (AATCC 6538 or ATCC 33591) by inoculating approximately 5 mL of soybean-casein digest broth (see 7.4.1) from a frozen stock or lyophilized vial and incubate for 25 ± 1 h at 35 ± 2°C (see Note 4). Inoculate the appropriate volume of soybean-casein digest broth with 1 mL of stock culture of *S. aureus*/125 mL of broth to yield the volume necessary to complete the study (that is, 0.2 mL per hand contamination (see 11.3) per test subject). The volume of the broth culture should not exceed about one fourth of the capacity of the Erlenmeyer flask to ensure adequate aeration. Incubate for 25 ± 1 h at 35 ± 2°C with shaking at 250 r/min to yield a titer of approximately 1.0×10^{10} cfu/ml.

9.3 Swirl or shake suspension before the withdrawal of each aliquot. Assay the suspension for the number of organisms present at the beginning and at the end of the use period. Do not use a suspension for more than 8 h. The suspension should not vary more than ± 0.5 $\log_{10}$ cfu/mL over an 8-h period.

10. Subjects

10.1 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 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.

10.2 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.

10.3 Instruct subjects to avoid contact with antimicrobial products 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, and soaps. Bathing in biocide-treated pools, hot tubs, or spas should be avoided. Harsh chemicals such as acids, bases, and solvents should 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.

11. Procedure

11.1 *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 10.3). Inspect the subject's hands and forearms to confirm the absence of clinical signs of skin disorders as described in 10.1. 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).

11.2 *Cleansing Wash*—Instruct the subject to perform a 30-s cleansing wash (see 7.2). This procedure removes oil and dirt from the hands and forearms. For this and all other hand washes and rinses, adjust the water temperature to 40 ± 2°C and the water flow rate to 4 L per minute. To adjust the flow rate, place a 2000-mL glass beaker or flask under each water faucet and allow the water to flow into the beaker. Adjust the water flow at each faucet accordingly, so that the beaker fills within 30 s.

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

11.2.2 Dispense 5 mL of the cleansing wash (see 7.2) into the subject's cupped hands and instruct subject to spread over hands and lower third of forearms.

11.2.3 Instruct subject to wash all surfaces of the hands and the lower third of the forearm in a vigorous manner for 30 ± 5 s. If the lather becomes too dry, add a small amount of water to maintain lather.

11.2.4 Instruct subject to rinse thoroughly from fingertips to elbows under tap water for 30 ± 5 s. Have the subject exercise caution to avoid contact with the sink and fixtures, eliminating the chance of recontamination from the sink surfaces. Also instruct subject to avoid rubbing hands and forearms during the rinsing process.

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

11.2.6 After completing each cleansing wash, have each test subject wait five min prior to the next phase of the study. After completing the cleansing wash following use of the reference control, the wait time is extended to 20 min.

11.3 *Hand Contamination*—Use a liquid suspension of the test bacteria prepared as directed (see 9.1 or 9.2).

11.3.1 Dispense a 0.2-mL aliquot of the test bacteria suspension into the subject's cupped hands. Instruct the subject to evenly distribute the inoculum over all surfaces of both hands and fingers, not reaching above the wrist, for 30 ± 5 s, making sure that the hands are dry.

NOTE 5—Subjects should not touch their clothing, face, or other objects with their hands during the test period. This prevents contamination of the subject and the environment with the test bacteria.

11.4 *Contamination, Product Application, and Recovery Schedule*—The test material is evaluated after a single application and after ten consecutive applications. Table 1 illustrates the experimental design. The subject first completes a cleansing wash to remove oil and dirt (see 11.2). The subject's hands are then contaminated with the test bacteria (see 11.3) followed by baseline recovery (see 11.5). After a second cleansing wash to remove residual sampling solution and neutralizers, the subject's hands are again contaminated and the subject applies the reference control (see 11.6). The subject's hands are then sampled for reference control recovery followed by a cleansing wash. The subject's hands are contaminated a third time with the test bacteria, the subject applies the test material (see 11.7), hands are sampled for test material application 1 recovery, and a cleansing wash is performed. The subject then performs 9 consecutive test material applications (test applications 2-10). The subject's hands are contaminated a fourth time with the test bacteria, the subject applies the test material, and hands are sampled for test material application 11 recovery

NOTE 6—It is strongly recommended that ATCC 6538 be chosen when multiple contamination/application cycles are to be performed using *S. aureus* as the test bacteria.

NOTE 7—Alternative schedules may also be followed as long as the same schedule is followed for all test products in the study.

11.5 *Baseline Recovery*—Recover the test bacteria surviving on the hands after the initial hand contamination (see 11.3) following the procedures outlined in 11.8 and enumerate according to Section 13. This represents the baseline recovery, which is typically between 8.5 $\log_{10}$ and 9.0 $\log_{10}$cfu/hand and may not be less than 8.0 $\log_{10}$ cfu/hand.

11.6 *Reference Control Application:*

11.6.1 Dispense 1.5 mL of the reference control into the subject's cupped hands from an appropriate dispenser or syringe within 10 s of completing the contamination step in 11.3.1.

11.6.1.1 Within 10 s, instruct the subject to distribute the reference control over all surfaces of the hands, and fingers, paying attention to the nails, and continue rubbing until the product is dry. Subject should exercise caution to retain the test material in the hands.

11.6.1.2 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.8).

11.7 *Test Material Application*—Conduct the test in accordance with the use directions for the test material. If test material directions are not available, use the appropriate test material application procedure described as follows.

11.7.1 *Liquid, Gel and Spray Hand Rubs:*

11.7.1.1 Dispense 1.5 mL of test material into the subject's cupped hands from an appropriate dispenser or syringe within 10 s of completing the contamination step in 11.3.1.

11.7.1.2 Within 10 s, instruct the subject to distribute test material over all surfaces of the hands and fingers paying attention to the nails, and continue rubbing until the product is dry. Subject should exercise caution to retain the test material in the hands.

11.7.1.3 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.8).

11.7.2 *Foaming Formulations:*

11.7.2.1 Dispense approximately 1.5 mL of test material from an appropriate foaming dispenser into the subject's cupped hands within 10 s of completing the contamination step in 11.3.1.

NOTE 8—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% and 90% ethanol.

11.7.2.2 Within 10 s, instruct the subject to distribute test material over all surfaces of the hands and fingers, paying attention to the nails, and continue rubbing until the product is dry. Caution should be exercised to retain the test material in the hands.

11.7.2.3 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.8).

11.7.3 *Hand Sanitizing Wipes (Towelettes):*

11.7.3.1 Subject should remove a single towelette, or be handed a single towelette from its package, taking care not to touch the package material, and clean their fingernails for approximately 10 s, paying attention to the underside, and the cuticles.

11.7.3.2 Have the subject wipe the towelette broadly over the front and back surfaces of both hands until wet (approximately 5 s).

TABLE 1 Hand Contamination, Product Application and Recovery Schedule

Name	Contamination	Type of Application	Recovery
Cleansing Wash	No	Cleansing Wash	No
Baseline	Yes	None	Yes
Cleansing Wash	No	Cleansing Wash	No
Reference Control	Yes	Reference Control	Yes
Cleansing Wash	No	Cleansing Wash	No
Test Application 1	Yes	Test Material	Yes
Cleansing Wash	No	Cleansing Wash	No
Test Applications 2–10	No	Test Material	No
Test Application 11	Yes	Test Material	Yes

11.7.3.3 Next, the subject should scrub the fingers and thumb of each hand, wrapping the towelette around each digit to wet entire surface completely (approximately 15 s).

11.7.3.4 Subject should turn the towelette over and scrub the palms of their hands up to the wrist, then scrub the back of their hands up to the wrist (approximately 10 s). Subject continues wiping all surfaces of both hands until all liquid has evaporated.

11.7.3.5 Have subject hold hands upright and motionless prior to bacterial recovery (see 11.8).

11.8 *Bacterial Recovery:*

11.8.1 Within one minute after the initial baseline contamination reference control application and test material applications 1 and 11 (Table 1), place gloves (see 6.4) or plastic bags (see 6.8) on the subject's hands. Add 75 mL of sampling solution (see 7.7) with neutralizer to each glove and secure gloves above the wrist with a tourniquet.

11.8.2 Within one minute of donning gloves, thoroughly and uniformly massage all surfaces of the subject's hands and fingers for 1 min ± 5 s.

11.8.3 Within one minute of completing the massage, aseptically retrieve a 5-mL sample of the sampling solution from the glove by pulling the glove away from the wrist, inserting a pipette into the finger region of the glove, and withdrawing the fluid.

11.8.4 Within 10 s, prepare the first dilution (see 13.1.2) in dilution fluid with an appropriate neutralizer, if required. Complete the plating of the recovered sampling solution within 30 min after sampling.

12. Hand Decontamination

12.1 Upon completion of testing, have subject rinse their hands and forearms for 1 min with 70 % ethanol (see 7.6) and air-dry.

12.2 Supervise subject performing a 4-min wash with a 4 % chlorhexidine gluconate handwash (see 7.3). Have the subject use a scrub brush during the first minute of the wash.

12.3 Apply a topical, antibiotic ointment (see 7.1) to the subject's hands and forearms.

12.4 When *S. aureus* is the test bacteria, subject should return to the laboratory approximately 24 h following testing. Inspect hands and forearms for any signs of infection at that time.

13. Enumeration of Bacteria

13.1 *S. marcescens:*

13.1.1 Enumerate the *S. marcescens* in the recovered sampling solution (see 11.8.3) using standard microbiological techniques, such as spread plating or spiral plating. The pour plate technique is not recommended because subsurface *S. marcescens* colonies may not exhibit the red pigment.

13.1.2 Prepare dilutions of the recovered sampling solution (see 11.8.3) in dilution fluid (see 7.5). Use soybean-casein digest agar (see 7.4.2.2) with suitable neutralizer, if necessary, as the recovery medium.

13.1.3 Incubate prepared plates 48 ± 4 h at 25 ± 2°C. Count only the red pigmented *S. marcescens* using an appropriate colony counter (see 6.3).

13.2 *S. aureus:*

13.2.1 Enumerate the *S. aureus* in the recovered sampling solution (see 11.8.3) using standard microbiological techniques, such as spread plating or spiral plating. The pour plate technique is not recommended.

13.2.2 Prepare dilutions of the recovered sampling solution (see 11.8.3) in dilution fluid (see 7.5). Use an appropriate indicator medium (see 7.4.2.1) with suitable neutralizer, if necessary, as the recovery medium.

13.2.3 Incubate prepared plates 24 ± 4 h at 35 ± 2°C. Count *S. aureus* colonies using an appropriate colony counter (see 6.3) based on manufacturer's instructions for the indicator medium (see 7.4.2.1).

14. Determination of Reduction

14.1 Convert plate counts (cfu/hand) to $\log_{10}$. Average the left and right hand values for each sampling interval.

14.2 Determine $\log_{10}$ reductions for the reference control and test material at both Application I and Application 11 for each test subject using the following formulae:

$$\log_{10}\text{ Reduction for Reference Control} = \log_{10}\text{ Baseline Recovery} - \log_{10}\text{ Reference Control Recovery}$$

$$\log_{10}\text{ Reduction for Test Material}_{App1} = \log_{10}\text{ Baseline Recovery} - \log_{10}\text{ Test Material}_{App11}\text{Recovery}$$

$$\log_{10}\text{ Reduction for TestMaterial}_{App11} = \log_{10}\text{ Baseline Recovery} - \log_{10}\text{ Test Material}_{App11}\text{Recovery}$$

where:

App1 = Application 1
App11 = Application 11

15. Comparison of Different Test Materials

15.1 When comparing different test materials, assign an equivalent number of test subjects to each test material on a random basis and evaluate all test materials concurrently. Use equivalent test parameters for all of the test materials (product application procedures for commercial products may be different).

15.2 Calculate the mean difference in $\log_{10}$ reduction (ΔLR) for each Test Material and the corresponding Reference Control at both Application 1 and Application 11 for each test subject using the following formula:

$$\Delta\log_{10}\text{ Reduction}_{App1} = \log_{10}\text{ Reduction for Reference Control} - \log_{10}\text{ Reduction for Test Material}_{App1}$$

$$\Delta\log_{10}\text{ Reduction}_{App11} = \log_{10}\text{ Reduction for Reference Control} - \log_{10}\text{ Reduction for Test Material}_{App11}$$

where:

App1 = Application 1
App11 = Application 11

15.3 Perform appropriate statistics such as an unpaired T-test (for 2 test materials) or Analysis of Variance (ANOVA) (for ≥3 test materials) to compare mean ΔLog_{10}, $Reduction_{App1}$, and ΔLog_{10} $Reduction_{App11}$ between test materials to identify significant differences in test material performance.

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; *Serratia marcescens*; *Staphylococcus aureus*

ASTM International takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, at the address shown below.

This standard is copyrighted by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States. Individual reprints (single or multiple copies) of this standard may be obtained by contacting ASTM at the above address or at 610-832-9585 (phone), 610-832-9555 (fax), or service@astm.org (e-mail); or through the ASTM website (www.astm.org). Permission rights to photocopy the standard may also be secured from the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923, Tel: (978) 646-2600; http://www.copyright.com/