



Standard Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations¹

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1. Scope

1.1 This test method is designed to determine the effectiveness of antimicrobial handwashing agents for the reduction of transient microbial flora when used in a handwashing procedure.

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

1.3 This test method may be used to evaluate topical antimicrobial handwash formulations.

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

1.5 The values stated in SI units are to be regarded as standard; except for distance, in which case inches are used and metric units follow in parentheses.

1.6 *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

E2755 Test Method for Determining the Bacteria-Eliminating Effectiveness of Hand Sanitizer Formulations Using Hands of Adults

3. Terminology

3.1 Definitions:

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 Antimicrobial Agents.

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² *Federal Register*, Vol 46, No. 17, Jan. 27, 1991.

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.

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

3.1.2 *cleansing wash, n*—a non-antimicrobial wash intended to remove gross soil or residues from the hands of the panelists prior to the conduct of the study and as noted throughout the study. This may also be referred to as a cosmetic wash.

3.1.3 *healthcare personnel handwash, n*—a cleanser or waterless agent intended to reduce transient bacteria on the hands.

3.1.4 *neutralization, n*—a process which results in quenching the antimicrobial activity of a test material. This may be achieved through dilution of the test material(s) to reduce the antimicrobial activity, or through the use of chemical agents, called neutralizers, to eliminate antibacterial activity.

3.1.5 *resident microorganisms, n*—microorganisms that live and multiply on the skin, forming a permanent population.

3.1.6 *test formulation, n*—a formulation which incorporates antimicrobial ingredient(s).

3.1.7 *test organism*—an applied inoculum of an organism that has characteristics which allow it to be readily identified. The test organism is used to simulate a transient topical microbial contaminant. It may also be referred to as a marker organism, bacterial simulant, or bacterial contaminant.

3.1.8 *transient microorganisms*—organisms from the environment that contaminate but do not normally colonize the skin.

4. Summary of Test Method

4.1 This test method is conducted on a group of volunteer panelists who have refrained from using topical antimicrobial formulations for at least one week prior to the initiation of the test. Activity of the test material is measured by comparing the number of test organisms recovered from artificially contaminated hands after use of a handwashing formulation to the number recovered from contaminated hands not exposed to the test formulation. The method describes specific procedures to be followed using *Serratia marcescens* as the test organism. The activity of the test material is measured following a single wash and may be measured following multiple washes in a single day using a neutralization recovery method.

4.2 An alternative test organism is *Escherichia coli*. Culture media and incubation conditions appropriate for this organism should be employed. The investigator should also be aware that there may be health risks associated with the use of this organism and precautions similar to those referenced in 8.2 should be undertaken.

5. Significance and Use

5.1 The procedure may be used to test the effectiveness of antimicrobial handwashing agents. The test formulations may be designed for frequent use to reduce the transient bacterial flora on hands. Alcohol-based hand rubs and other leave-on formulations used without the aid of water may be tested using Test Method E2755.

6. Apparatus

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

6.2 *Incubator*—Any incubator capable of maintaining the following temperatures: *S. marcescens* ($25 \pm 2^\circ\text{C}$) or *E. coli* ($35 \pm 2^\circ\text{C}$). This temperature is required to ensure pigment production for *S. marcescens*.

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

6.4 *Timer* (Stop-clock)—One that can be read for minutes and seconds.

6.5 *Handwashing Sink*—A sink of sufficient size to permit panelists to wash without touching hands to sink surface or other panelists.

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

6.6 *Tap Water Temperature Regulator and Temperature Monitor*—To monitor and regulate water temperature of $40 \pm 2^\circ\text{C}$.

7. Reagents and Materials

7.1 *Bacteriological Pipettes*—10.0 and 2.2-mL or 1.1-mL capacity.

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

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

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

7.3 *Erlenmeyer Flask*—2-L capacity for culturing test organism.

7.4 *Cleansing Wash*—A mild, non-antimicrobial soft soap.

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 *Test Material*—Directions for use of the test material may be utilized. If directions are not available, use directions provided in this test method.

7.6 *Gloves*—Loose-fitting, unlined, powder-free gloves which possess no antimicrobial properties, or equivalent.⁴ (Plastic bags with low bioburden may be used in place of gloves.)

7.7 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4 , 10.1 g Na_2HPO_4 , and 1.0 g isooctylphenoxypolyethoxyethanol and with appropriately validated neutralizers in 1 L distilled water. Adjust pH to 7.8 with 0.1 N HCl or 0.1 N NaOH. Dispense so that final volume after sterilization is 75 mL, sterilized at 121°C .⁵

7.8 *Dilution Fluid*—Sterile Butterfield's Buffer⁶ or other suitable diluent, adjusted to pH 7.2 with effective neutralizer for the test material. Adjust pH with 0.1 N HCl or 0.1 N NaOH. See Test Methods E1054.

7.9 *Agar*—Soybean-casein digest agar or other solid media appropriately validated to support growth of the test organism with appropriate neutralizers if needed.

7.10 *Broth*—Soybean-casein digests broth or other liquid media appropriate to support growth of the test organism.

8. Test Organism

8.1 *Serratia marcescens* (ATCC 14756) is to be used as the test organism. This is a strain having stable pigmentation at 25°C .

8.2 *Escherichia coli* (ATCC 11229) are an alternative test organism. When *E. coli* is used, the plating agar should include a suitable indicator (for example, MUG⁷). (**Warning**—The application of microorganisms to the skin may involve a health risk. Prior to applying the test organism to the skin, the antibiotic susceptibility profile of the strain should be determined. If the strain is not susceptible to gentamicin, do not use it. If an infection occurs, the antibiotic sensitivity profile should be made available to the attending clinician. Following the

⁴ A zone of inhibition test such as AATCC Test Method 90-1965 may be used to evaluate antimicrobial properties of gloves, AATCC Test Methods, American Association of Textile Chemists and Colorist, 1968 Technical Manual, Section B-75.

⁵ 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.

⁶ Horowitz, W., (Ed.), *Official Methods of Analysis of the AOAC*, 17th Ed., Sec. 6.3.03 A.(f), Chapter 6, 2000, p. 10. Official Methods of Analysis of AOAC International, Gaithersburg, MD.

⁷ *United States Pharmacopeia* 28; United States Pharmacopeial Convention, Inc., Rockville, MD, Chapter entitled "Microbial Limits Test." The MUG (4-methylumbelliferyl- β -D-gluconide) substrate is hydrolyzed by β -D-gluconidase to yield a fluorescent end product, 4-methylumbelliferone. β -D-gluconidase is possessed by *E. coli* (ATCC 11229). MUG is incorporated into the appropriate growth medium at 0.05 g/L.

subject's last contamination and wash with the formulation, the subject's hands are to be sanitized by scrubbing with 70% isopropanol solution or equivalent. The purpose of this alcohol scrub is to destroy residual test organisms on the skin.)

8.3 Preparation of Test Organism Suspension

8.3.1 *S. marcescens*—A homogeneous culture is used to inoculate the hands. The stock culture, frozen or lyophilized, should be at least two 24-h soybean-casein digest broth (7.10) transfers from the original ATCC culture, but there should be no more than four transfers removed from the ATCC culture. From the stock culture of *Serratia marcescens* (ATCC 14756), inoculate the appropriate volume of soybean-casein digest broth (7.10) with 0.1 mL of stock culture of *S. marcescens*/100 mL of broth to yield the volume necessary to complete the study. Incubate for 24 ± 4 h at $25 \pm 2^\circ\text{C}$. Broth should develop a red pigment.

8.3.2 *E. coli*—A homogeneous culture is used to inoculate the hands. The stock culture should be at least two 24-hour broth transfers from the original ATCC culture, but no more than five transfers removed from the ATCC culture. From the stock culture of *Escherichia coli* (ATCC 11229), inoculate the appropriate volume of soybean-casein digest broth (7.10) with 0.1 mL of stock culture/100 mL of broth to yield the volume necessary to complete the study. Incubate for 24 ± 4 h at $35 \pm 2^\circ\text{C}$.

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

9. Subjects

9.1 Recruit a sufficient number of healthy adult human volunteers who have no clinical evidence of dermatoses, open wounds, hangnails, or other skin disorders.

9.2 Instruct subjects to avoid contact with antimicrobial products (other than the test material as dispensed for each test wash) 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, also such materials as acids, bases, and solvents. Bathing in biocide treated pools, hot tubs, or spas should be avoided. Subjects are to be provided with a kit of nonantimicrobial personal care products for exclusive use during the test and rubber gloves to be worn when contact with antimicrobial or harsh chemicals cannot be avoided.

10. Procedure

10.1 After subjects have refrained from using antimicrobial formulations for at least 7 days, they perform a 30 s cleansing wash (7.4) in the same manner that is described for the test and control formulations. This procedure removes oil and dirt and familiarizes the panelists with the washing technique. For this and all other washes and rinses, the water temperature is adjusted to $40 \pm 2^\circ\text{C}$ and the water flow rate to 4 L per minute. 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.

10.2 *Hand Contamination*—A liquid suspension of the test organism containing between 5.0×10^8 and 1.0×10^9 cfu/mL is used. See Table 1.

10.2.1 A 1.5 mL aliquot of the test organism suspension is dispensed into the subjects' cupped hands. This aliquot is rubbed over the entire surfaces of the hands for 20 ± 5 s (front and back) not reaching above the wrist. The hands are then held motionless away from the body and allowed to air dry for approximately 30 ± 5 s.

10.2.2 To continue the contamination of the hands, an additional 1.5 mL aliquot of the test organism suspension is dispensed into the hands, distributed over the hands for 20 ± 5 s, and air dried for 30 ± 5 s.

10.2.3 To complete the contamination, a final 1.5 mL aliquot of test organism suspension is dispensed into the hands, distributed over the hands for 20 ± 5 s, and air dried for 90 ± 5 s (Table 1).

NOTE 3—The hands may still be wet after the 90 s.

10.2.4 The total test organism suspension applied to the hands is 4.5 mL. Contamination may take approximately 5 min. This method of contamination minimizes the loss of test organism while spreading.

10.3 *Contamination Schedule*—The subjects' hands are contaminated with the test organism prior to the baseline bacterial sample collection and prior to each washing with the test material. Table 2 illustrates a typical test. The number of repeated test washes may be reduced or eliminated at the discretion of the investigator.

10.4 *Baseline Recovery*—A baseline sample is taken after contamination to determine the number of marker organisms surviving on the hands. Bacterial sampling will follow the procedures outlined in Section 12.

11. Wash and Rinse Procedure

11.1 Conduct the test in accordance with the use directions for the test material. If test material directions are not available, the wash and rinse procedure described as follows should be used. Table 2 shows the contamination and recovery schedule for the overall study.

11.2 Liquid Formulations:

11.2.1 Dispense 5 mL of test material into cupped hands within 10 s of completing the drying step in 10.2.3. Spread over hands and lower third of forearms.

TABLE 1 Hand Contamination with Test Organism Suspension^A

Volume	Spread Time	Dry Time
1.5 mL	20 s	30 s
1.5 mL	20 s	30 s
1.5 mL	20 s	90 s

^A Alterations in volume and frequency of hand contamination of the test organism suspension for waterless formulations may be used but must be validated to yield an inoculum equivalent to 10.2.

TABLE 2 Hand Contaminations and Recovery Schedule

Name	Contamination	Type of Wash	Recovery
Cleansing Wash	No	Cleansing Wash	No
Baseline	Yes	No	Plate Recovered Sampling Solution with Neutralizer
Cleansing Wash	No	Cleansing Wash	No
Test Wash 1	Yes	Test Formulation	Plate Recovered Sampling Solution with Neutralizer
Cleansing Wash	No	Cleansing Wash	No
Test Wash 2–10	Yes	Test Formulation	No
Test Wash 11	Yes	Test Formulation	Plate Recovered Sampling Solution with Neutralizer

NOTE 4—The 5 mL volume has been chosen for test purposes due to the requirement for washing hands and forearms.

11.2.2 Sparingly wet contaminated hands by rapidly passing them one time through the tap water. This process should be performed in less than 1 s.

11.2.3 Wash in a vigorous manner for 30 ± 5 s all surfaces of the hands and the lower third of the forearm. Caution should be exercised to retain the test material in the hands. If the lather becomes too dry, a small amount of water may be added to maintain lather.

11.2.4 Rinse thoroughly from fingertips to elbows under $40 \pm 2^\circ\text{C}$ tap water for 30 ± 5 s. Caution should be exercised to avoid contact with the sink and fixtures to eliminate recontamination from the sink surfaces and to avoid rubbing hands and forearms during the rinsing process.

11.2.5 Subject's hands and forearms are lightly patted dry with paper toweling.

NOTE 5—After washes requiring sampling, the hands are not to be dried, but held upright until procedures in 12.1 are performed.

11.3 Leave-On (non-water aided) Formulations:

11.3.1 Dispense 3 mL of test material into cupped hands within 10 s of completing the drying step in 10.2.3.

11.3.2 Within 10 s, distribute test material over all surfaces of the hands and the lower third of the forearms. Continue rubbing in a vigorous manner for 30 ± 5 s. Caution should be exercised to retain the test material in the hands.

11.3.3 Subject's hands may be held upright and motionless prior to Bacterial Recovery (Section 12).

NOTE 6—When testing leave-on formulations, users should consider Test Method E2755, which is designed specifically for evaluating the efficacy of leave-on formulations, as an alternative to this test method.

11.4 Solid Formulations:

11.4.1 Sparingly wet contaminated hands and forearms with $40 \pm 2^\circ\text{C}$ tap water.

11.4.2 Wet the product.

11.4.3 Rub the product between the hands and on the forearms for 15 ± 3 s. Place product aside.

11.4.4 Lather lower third of forearms and hands for an additional 30 ± 5 s. If the lather becomes too dry, a small amount of water may be added to maintain lather.

11.4.5 Rinse thoroughly from elbows to fingertips under $40 \pm 2^\circ\text{C}$ tap water for 30 ± 5 s. Caution should be exercised to avoid contact with the sink and fixtures to eliminate contamination from the sink surfaces.

11.4.6 Subject's hands and forearms are lightly patted dry with paper toweling.

11.5 Other Product Forms:

11.5.1 Use standardized amount (for example, weight, volume) of test material in accordance with use directions.

11.6 After washes requiring sampling, the hands are not to be dried, but held upright until procedures in 12.1 are performed.

12. Bacterial Recovery

12.1 Within one minute after specified washes (10.3 and Table 2), place gloves (7.6) used for sampling on the hands. Add 75 mL of sampling solution (7.7) with neutralizer to each glove and secure gloves above the wrist.

12.2 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.

12.3 Within one minute of completing the massage, 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.

12.4 The first dilution is to be made in dilution fluid with appropriate neutralizer within 10 s of removing the 3 to 5 mL from the glove. The plating of the recovered sampling solution is completed within 30 min after sampling.

13. Enumeration of Bacteria in Sampling Solution

13.1 *S. marcescens*:

13.1.1 Enumerate the *S. marcescens* in the recovered sampling solution (12.3) using standard microbiological techniques, such as membrane filtration or spread plating. The pour plate technique is not recommended because subsurface *S. marcescens* colony forming units may not exhibit the red pigment.

13.1.2 Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.8). Use soybean-casein digest agar (7.9) with suitable inactivator as recovery medium.

13.1.3 Incubate prepared plates $48 \pm 4 \text{ h}$ at $25 \pm 2^\circ\text{C}$. Standard plate counting procedures are used to count only the red pigmented *S. marcescens*.

13.2 *E. coli*:

13.2.1 Enumerate the *E. coli* in the sampling solution using standard microbiological techniques, such as membrane filtration, pour or spread plating. Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.8). Use soybean-casein digest agar (7.9) with suitable inactivator and indicator (MUG⁷) as recovery medium.

13.2.2 Incubate prepared plates 18 to 24 hours at $35 \pm 2^\circ\text{C}$. Standard plate counting procedures are used to count only the *E. coli* colonies.

14. Determination of Reduction

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

14.2 Determine $\log_{10}$ reductions at each recovery interval/wash using the following formula:

$$\log_{10} \text{Reduction at Sampling Interval} = \quad (1)$$

$$\log_{10} \text{Baseline Recovery} - \log_{10} \text{Sampling Interval}$$

15. Comparison of Test Material

15.1 It may be desirable to compare the test material with other test formulations. If this is the case, an equivalent number

of panelists should be assigned to each formulation on a random basis. All test parameters will be equivalent for products, although the wash procedure for an established product may be different. Both products should be run concurrently.

16. Precision and Bias

16.1 A precision and bias statement cannot be made for this test method at this time.

17. Keywords

17.1 antimicrobial; artificial contaminant; efficacy; hand-wash; healthcare; marker organism; transient microorganism

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