Designation: ~~E1174 – 13~~ E1174 – 21

Standard Test Method for Evaluation of the Effectiveness of ~~Health Care~~ Healthcare Personnel Handwash Formulations[1]

This standard is issued under the fixed designation E1174; 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 effectiveness of antimicrobial handwashing agents for the reduction of transient microbial skin flora when used in a handwashing procedure.[2]

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.[3]

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~~ safety, health, and ~~health~~environmental practices and determine the applicability of regulatory limitations prior to use.*For more specific precautionary statements see 8.2.

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[4]

E1054 Practices for Evaluation of Inactivators of Antimicrobial Agents

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

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

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[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 E35.15Antimicrobial Agents.

Current edition approved ~~Feb. 15, 2013~~Oct. 1, 2021. Published ~~February 2013~~October 2021. Originally approved in 1987. Last previous edition approved in ~~2006~~2013 as ~~E1174 – 06.~~E1174 – 13. DOI: ~~10.1520/E1174-13.~~10.1520/E1174-21.

[2] ~~Federal Register,~~This version ~~Vol 46, No. 17,~~of the standard has ~~Jan. 27, 1991.~~been revised to align with current United States Food and Drug Administration requirements for healthcare personnel handwash formulations. The procedure for multiple washes present in earlier versions of this standard has been moved to Appendix X1 of this version.

[3] 45CFR Part 46 Protection of Human Subjects (Effective July 19, 2018)

[4] 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. Terminology

3.1 *Definitions*—For definitions of terms used in this method, refer to Terminology E2756.

3.2 ~~*Definitions:*~~*Definitions of Terms Specific to This Standard:*

3.2.1 *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.~~

3.2.2 *healthcare personnel handwash, n*—a cleanser or waterless agent intended to reduce transient ~~bacteria~~microorganisms 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.~~

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

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

3.2.5 *test ~~organism~~—organism, n*—an applied inoculum of ~~an organism~~bacterial culture 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.~~

3.2.5.1 *Discussion*—
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.

3.2.6 *transient ~~microorganisms~~—microorganisms, n*—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.~~wash.

4.2 An alternative test organism that can be used is *Escherichia ~~coli.~~coli,* if deemed appropriate. 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~~bacterium and precautions similar to those referenced in 8.2 should be undertaken.

5. Significance and Use

5.1 The procedure ~~may be~~ is used to test the antimicrobial effectiveness of ~~antimicrobial~~ handwashing ~~agents.~~formulations. The test formulations ~~may be~~generally are 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~~should 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~~(25 °C ± ~~2°C)~~2 °C) or *E. coli* ~~(35 ± 2°C). This temperature~~ (35 °C ± 2 °C). The former temperature range is required to ensure ~~pigment production for~~ pigment-production by *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~~that 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~~regulate and ~~regulate~~moniter water at a temperature of ~~40~~40 °C ± ~~2°C.~~2 °C.

7. Reagents and Materials

7.1 *Bacteriological Pipettes*—~~10.0~~10.0 mL and ~~2.2-mL~~2.2 mL or ~~1.1-mL~~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~~150 mL to 200 mL capacity and tight closures ~~may~~can be used.

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

7.3 *Erlenmeyer Flask*—~~2-L~~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~~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~~Add 200 g of the soft soap ~~in~~to 1 L of ~~water. Dispense~~water, dispense in to appropriate containers, and sterilize in an autoclave.

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

7.6 *Gloves*—Loose-fitting, unlined, powder-free gloves ~~which~~that possess no antimicrobial properties, or equivalent.[5] (Plastic bags with low bioburden ~~may~~can be used in place of gloves.)

[5] 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.

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~~ neutralizing additives in 1 L of 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.~~mL after sterilization at 121 °C.[6]

7.8 *Dilution Fluid*—Sterile Butterfield's Buffer[7], or other suitable diluent, ~~adjusted to pH 7.2 with effective neutralizer~~ that includes neutralizing additives proved effective for the test material. Adjust to pH approximately 7.2 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~~medium appropriately validated to support growth of the test ~~organism with appropriate neutralizers~~ organism, including appropriate neutralizing additives, if needed.

7.10 *Broth*—Soybean-casein ~~digests broth~~digest broth, or other liquid ~~media appropriate~~ medium appropriately validated to support growth of the test organism.

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

7.12 *Chlorhexidine Skin Cleanser*—Antiseptic skin cleanser containing 4 % chlorhexidine gluconate (w/v) for hand decontamination.

7.13 *Antibiotic Ointment*—A topical, triple-antibiotic ointment for application to the hands and forearms after the final decontamination. If necessary, consult with a physician prior to use.

8. Test Organism

8.1 *Serratia marcescens* (ATCC 14756) is to be used as the test organism. This is a strain ~~having~~ that produces stable pigmentation at ~~25°C.~~25 °C. (**Warning—**The application of this bacterium to the skin may involve a health risk to healthy humans. Prior to application, the antibiotic susceptibility profile of the strain should be determined. The strain must be susceptible to gentamicin. If an infection occurs, the antibiotic sensitivity profile must be made available to the attending physician. Following the subject's last contamination and wash with the formulation, the subject's hands are to be decontaminated as described in Section 14. The purpose of this decontamination is to destroy residual test bacteria on the skin.)

8.2 *Escherichia coli* (ATCC 11229) ~~are~~is an alternative test organism. When *E. coli* is used, the plating agar should include a suitable indicator (for example, MUG[8]). (**Warning—**The application of this bacterium to the skin may involve a health risk to healthy humans. Prior to application, the antibiotic susceptibility profile of the strain should be determined. If the strain is not susceptible to gentamicin, it must not be used. If an infection occurs, the antibiotic sensitivity profile must be made available to the attending physician. Following the subject's last contamination and wash with the formulation, the subject's hands are to be decontaminated as described in Section 14. The purpose of this decontamination is to destroy residual test bacteria on the skin.~~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 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~~Bacterium Suspension*

8.3.1 *S. marcescens*—A homogeneous culture is to be used to inoculate the hands. The stock culture, frozen or ~~lyopholized,~~ lyophilized, should be at least two ~~24-h~~24 h soybean-casein digest broth (7.10) transfers from the original ATCC culture, but ~~there~~

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

[7] ~~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, Gaitherburg, MD.~~Horowitz, W., (Ed.), *Official Methods of Analysis of the AOAC International*, 17th Ed. 2000. Chap. 6, p. 10. Gaitherburg, MD.

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

~~should be~~ no more than four transfers removed from the ATCC culture. From the stock ~~culture of~~ culture, ~~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~~24 h ± 4 h at ~~25~~25 °C ± 2°C. Broth should develop a red pigment.

8.3.2 *E. coli*—A homogeneous culture is to be 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~~four 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~~24 h ± 4 h at ~~35~~35 °C ± 2°C.

8.4 Swirl or shake suspension before the withdrawal of each ~~aliquot.~~ aliquot for testing. Assay the suspension for number of ~~organisms~~bacteria 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 ±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.~~disorders on the hands and forearms.

9.2 Instruct subjects to avoid contact with antimicrobial products (other than the test material as dispensed for ~~each~~the 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~~and such materials as acids, bases, and solvents. Bathing in biocide treated pools, hot tubs, or spas ~~should~~ must also be avoided. Subjects are to be provided with a kit of nonantimicrobial personal care products for exclusive use during the ~~test~~ pre-test period 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 as that ~~is~~ described for the test ~~and control formulations.~~ (and control, if used) formulation. 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 ± 2°C~~and maintained at 40 °C ± 2 °C and the water flow rate ~~to~~at 4 L per minute. ~~This may be accomplished~~The flow rate can be adjusted by placing a 2000 mL glass beaker or flask under each spigot to be used for subjects' hand ~~washing. Allow~~washing allowing the water to flow into the ~~beaker. Adjust~~ beaker, and adjusting the water flow at each spigot ~~accordingly, so~~ such that the beaker fills within 30 s.

10.2 *Hand Contamination*—A liquid suspension of the test organism containing between 5.0 cfu/mL × 10^8 cfu/mL and ~~1.0~~ 4.0 cfu/mL × 10^9 cfu/mL for *S. marcescens* and 1.0 CFU/mL × 10^8 and 1.0 CFU/mL × 10^9 CFU/mL for *E. coli* 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~~20 s ± 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~~30 s ± 5 s.

10.2.2 ~~To continue the contamination of the hands, an additional~~ A second 1.5 mL aliquot of the test organism suspension is dispensed into the hands, distributed over the hands for ~~20~~20 s ± 5 s, and air dried for ~~30~~30 s ± 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~~20 s ± 5 s, and air dried for ~~90~~90 s ± 5 s (Table 1).

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~~
Cleansing Wash	No	Decontamination	No
~~Test Wash 2–10~~	~~Yes~~	~~Test Formulation~~	~~No~~
~~Test Wash 11~~	~~Yes~~	~~Test Formulation~~	~~Plate Recovered Sampling Solution with Neutralizer~~

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

10.2.4 The total volume of 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.~~ testing sequence.

10.4 *Baseline Recovery*—A baseline sample is taken after contamination to determine the number of ~~marker~~challenge organisms surviving on the hands. Bacterial sampling ~~will follow the~~ is performed according to 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.

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~~30 s ± 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~~can be added to maintain lather.

11.2.4 For rinsing, arms are held over the sink flexed upright from the elbows. Rinse thoroughly from ~~fingertips~~elbows to ~~elbows~~fingertips under ~~40~~40 °C ± ~~2°C~~2 °C tap water for ~~30~~30 s ± 5 s. Caution should be exercised to avoid contact with the sink and ~~fixtures to eliminate recontamination from the sink surfaces~~ fixtures, and to avoid rubbing between hands and forearms during the rinsing process.

11.2.5 Subject's hands and forearms are then lightly patted dry with paper toweling. Dispose of paper toweling according to standard operating procedures for handling biohazardous materials.

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~~40 °C ± ~~2°C~~2 °C tap water.

11.4.2 Wet the product.

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

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

11.4.5 For rinsing, arms are held over the sink flexed upright from the elbows. Rinse thoroughly from elbows to fingertips under ~~40~~40 °C ± ~~2°C~~2 °C tap water for ~~30~~30 s ± 5 s. Caution should be exercised to avoid contact with the sink and fixtures ~~to eliminate contamination from the sink surfaces.~~and to avoid rubbing between hands and forearms during the rinsing process.

11.4.6 Subject's hands and forearms are then lightly patted dry with paper toweling. Dispose of paper toweling according to standard operating procedures for handling biohazardous materials.

~~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 min ± 5 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~~5 mL to ~~5 mL~~10 mL sample of the fluid ~~in~~from the glove by pulling the glove away from the wrist, and inserting a pipet into the finger ~~region of the glove, and withdrawing the fluid.~~region. The volume of sample should be sufficient to allow for the dilution and plating of the sample. Dispense the sample into a test tube.

12.4 The ~~first dilution~~ 5 mL to 10 mL sample aliquot is to be ~~made~~ plated and/or diluted in dilution fluid ~~with appropriate~~