Designation: ~~E1174 – 06~~ E1174 – 13

Standard Test Method for Evaluation of the Effectiveness of Health Care 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 ~~covers the determination of~~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 In this test method, metric units are used for all applications, except for distance in which case inches are used and metric units follow in parentheses.~~

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:*

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

3.1.2 *cleansing ~~wash—~~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—~~handwash, n—*a cleanser or waterless agent intended to reduce transient bacteria on the hands.

3.1.4 *~~neutralization—~~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—~~microorganisms, n—*microorganisms that live and multiply on the skin, forming a permanent population.

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

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

3.1.6 *test ~~formulation—~~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 ~~may be~~ 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 ± 2°C) or *E. coli* (35 ± 2°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 ± 2°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~~

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 Buffer[6] 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, MUG[7]). (**Warning—**The application of microorganisms to the skin may involve a health risk. Prior to applying the test organism to the skin, the antibiotic ~~sensitivity~~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 Suspension*

8.3.1 *S. marcescens*—A homogeneous culture is used to inoculate the hands. The stock culture, frozen or ~~lypholized,~~ lyopholized, 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 ± 2°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 ± 2°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 ±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 ~~dermatosis,~~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

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[4] ~~The sole source of supply of the apparatus (Pharmaseal 8873C, (sterile) Flexam Latex Procedure Glove) known to the committee at this time is American Pharmaseal Laboratories, Glendale, CA 91209. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.~~ 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, Gaitherburg, MD.

[7] *United States Pharmacopeia 28*: United States Pharmacopeial Convention, Inc., Rockville, MD, Chapter entitled "Microbial Limits Test." The MUG (4-methylumbelliferyl-β-D-gluconride) 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 growth medium at 0.05 g/L.