



Designation: E 1174 – 00

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

This standard is issued under the fixed designation E 1174; 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 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.

1.4 *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 [Note 1](#).

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

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

2. Referenced Documents

2.1 *ASTM Standards*:³

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

3. Terminology

3.1 *Definitions*:

3.1.1 *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.

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

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

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

3.1.5 *test formulation*—a formulation which incorporates antimicrobial ingredient(s).

3.1.6 *neutralization*—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.

3.1.7 *cleansing wash*—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.

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

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 measured following a single wash and multiple washes in a single clay 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

¹ This test method is under the jurisdiction of ASTM Committee E35 on Pesticides 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.

organism and precautions similar to those referenced in **Note 1** 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.

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.⁴

7.2 *Water Dilution Bottles*—Any sterilizable glass container having a 150–200 mL capacity and tight closures may be used.⁵

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

7.4 *Cleansing Wash*—A mild, non-antimicrobial solid or liquid soap. (The investigator may choose to use the product vehicle.)

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.⁶ (Plastic bags with low bioburden may be used in place of gloves.)

⁴ Presterilized/disposable bacteriological pipettes are available from most local laboratory supply houses.

⁵ Milk dilution bottles of 160-mL capacity having a screw-cap closure are available from Corning Glass Co., Kimble Glass Co. or most local laboratory supply houses.

⁶ A suitable glove would be Pharmaseal 8873C, (sterile) Flexam Latex Procedure Glove from American Pharmaseal Laboratories, Glendale, CA 91209. 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 isooctylphenoxyethoxyethanol⁷ 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 .⁸

7.8 *Dilution Fluid*—Sterile Butterfield's Buffer⁹ 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 **E 1054**.

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 digest 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) is an alternative test organism. When *E. coli* is used, the plating agar should include a suitable indicator (e.g. MUG¹⁰).

NOTE 1—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 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 should be at least two 24 hour broth transfers from the original ATCC culture, but there should be no more than 5 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 milliliter of stock culture of *S. marcescens*/100mLs of broth to yield the volume necessary to complete the study. Incubate for 24 ± 4 h at $25^\circ\text{C} \pm 2^\circ\text{C}$. 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 5 transfers removed from the ATCC culture. From the stock

⁷ Triton X-100, Rohm and Haas Co., Philadelphia, PA.

⁸ Peterson, A.F., "The Microbiology of the Hands: Evaluating the Effects of the Surgical Scrubs," *Developments in Industrial Microbiology*, Vol 14, pp. 125–130, 1973.

⁹ Horowitz, W. (Ed.) 1980. *Official Methods of Analysis of the AOAC*, 13th Ed., Sec. 46.013 (m), p. 825. Assoc. of Off. Anal. Chemists, Washington, D.C. 1018 pp.

¹⁰ *United States Pharmacopeia XXII*: 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 grams/L.