Designation: E1174 – 21

Standard Test Method for Evaluation of the Effectiveness of 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, health, and 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

3. Terminology

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

3.2 *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.

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

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, n*—an applied inoculum of bacterial culture that has characteristics which allow it to be readily identified.

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.

[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 Oct. 1, 2021. Published October 2021. Originally approved in 1987. Last previous edition approved in 2013 as E1174 – 13. DOI: 10.1520/E1174-21.

[2] This version of the standard has 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.

3.2.6 *transient 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.

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

5. Significance and Use

5.1 The procedure is used to test the antimicrobial effectiveness of handwashing formulations. The test formulations 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 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 °C ± 2 °C) or *E. coli* (35 °C ± 2 °C). The former temperature range is required to ensure 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 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 regulate and moniter water at a temperature of 40 °C ± 2 °C.

7. Reagents and Materials

7.1 *Bacteriological Pipettes*—10.0 mL 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 mL to 200 mL capacity and tight closures can 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 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. Add 200 g of the soft soap to 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 should be utilized. If directions are not available, use directions provided in this test method.

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

7.7 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, 1.0 g isooctylphenoxypolyethoxyethanol, and appropriately validated 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 is 75 mL after sterilization at 121 °C.[6]

7.8 *Dilution Fluid*—Sterile Butterfield's Buffer[7], or other suitable diluent, 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 medium appropriately validated to support growth of the test organism, including appropriate neutralizing additives, if needed.

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

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

[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.

[6] 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 International*, 17th Ed. 2000. Chap. 6, p. 10. Gaitherburg, MD.