



Designation: E2784 – 10

Standard Test Method for Evaluation of the Effectiveness of Handwash Formulations Using the Paper Towel (Palmar) Method of Hand Contamination¹

This standard is issued under the fixed designation E2784; 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 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 In this test method, SI units are used for all applications, except for distance in which case inches are used and SI 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 8.5.

2. Referenced Documents

2.1 ASTM Standards:³

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E1174 Test Method for Evaluation of the Effectiveness of Health Care Personnel Handwash Formulations

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 on 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.

2.2 Other Standards:

AATCC Test Method 147 Antibacterial Assessment of Textile Materials: Parallel Streak Method⁴

3. Terminology

3.1 Definitions:

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 procedure intended to remove soil or residue. 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*—the process for inactivating or quenching the activity of a microbiocide. Often achieved through chemical or physical means (for example, filtration or dilution).

3.1.5 *resident microbial skin flora, n*—microorganisms that survive and multiply on the skin, forming a stable population.

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

3.1.7 *test organism, n*—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 microbial skin flora, n*—microorganisms that contaminate the skin but do not normally form a stable population.

4. Summary of Test Method

4.1 This test method is conducted on a group of volunteer subjects 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

⁴ Technical Manual of the American Association of Textile Chemists and Colorists, P.O. Box 12215, Research Triangle Park, North Carolina 27709.

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 in a single day using a neutralization recovery method.

4.2 Alternative test organisms which may be used are *Escherichia coli*, *Shigella flexneri*, and *Staphylococcus aureus*. Culture media and incubation conditions appropriate for the alternative organisms should be employed.

4.3 The investigator should be aware that there may be health risks associated with the use of the test organisms and precautions similar to those referenced in 8.5 should be undertaken.

5. Significance and Use

5.1 This procedure has been designed to evaluate handwash products using a palmar surface only contamination method. This method is an alternative contamination procedure to that listed in Test Method E1174. The current contamination procedure in Test Method E1174 describes a standardized procedure for contaminating the entire hand, palmar surface and back, directly using a marker organism. The contamination procedure in Test Method E1174 does not necessarily represent real world hand contamination. During routine activities it is only the palmar surface, comprising palms, fingers, and finger pads of the hands that becomes contaminated by contact with transient microorganisms. These microorganisms can then be transferred to food or objects. Methods to measure the amount of microorganisms transferred to food or objects can be found in Fischler et al⁵ and Fuls et al⁶ and will be developed into a future ASTM standard.

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* ($25 \pm 2^\circ\text{C}$ —this temperature is required to ensure pigment production for *S. marcescens*); *S. aureus*, *E. coli*, *S. flexneri* ($35 \pm 2^\circ\text{C}$).

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 subjects to wash without touching hands to sink surface or other subjects.

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. Faucet should maintain a flow rate of 4 L per minute, as determined in (10.3).

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

6.6 *Vortex Mixer*—Any suitable vortex mixer capable of mixing sample and diluent.

6.7 *Sterile Bacteriological Pipets*—1.1, 2.2, 5.0, and 30.0 mL capacity.

6.8 *Adjustable or Fixed Volume Pipets and Sterile Tips*—0.1 mL and 1.0 mL capacity.

6.9 *Sampling Containers*—Any sterile or sterilizable container having tight closures and sufficient capacity to hold 75 mL sampling solution (7.3).

6.10 *Sterile Container*—Any sterile or sterilizable container having the capacity to culture the amount of inoculum required for testing.

6.11 *Gloves*—Loose-fitting, unlined, powder-free gloves which possess no antimicrobial properties, or equivalent. Perform a zone of inhibition test, such as AATCC Test Method 147, to evaluate the antibacterial activity. (Plastic bags (6.12) with low bioburden may be used in place of gloves.)

6.12 *Plastic Bags*—May be used in place of gloves (6.11). Bags should be approximately 29 by 31 cm, possess no antimicrobial properties, and have a low bioburden. Perform a zone of inhibition test, such as AATCC Test Method 147, to evaluate the antibacterial activity.

6.13 *Wrist Ties or Tourniquets*—Any item which will allow the plastic bags (6.12) or gloves (6.11) to be secured to the subject's wrist.

6.14 *Sterile Paper Towel Pouches*—Each pouch consists of two single-ply paper towels, each of which measures approximately 20 by 32 ± 5 cm, encased in aluminum foil.

6.14.1 Fold two single-ply paper towels in half, lengthwise to form a rectangle approximately 20 by 16 cm. Place one paper towel inside of the other.

6.14.2 Place the paper towels in a piece of aluminum foil which has been folded in half, widthwise. The aluminum foil should measure approximately 38 by 23 cm, after folding. Aluminum foil which is rated as “Heavy Duty” and has a minimum thickness of 0.2 mm is recommended to minimize the risk of tearing during handling. Fold the edges of the aluminum foil together to form a pouch ensuring that the paper towels remain flat. Sterilize the pouch by autoclaving.

6.15 *Sterile Centrifuge Tubes*—Minimum of 50 mL capacity.

7. Reagents and Materials

7.1 *Cleansing Wash*—A mild, proven, non-antimicrobial soft soap. The formula in Table 1 can be used if a mild, non-antimicrobial soft soap is not commercially available.

7.1.1 Add linseed oil to a solution of potassium hydroxide in 15 parts water and heat up to approximately 70°C while

⁵ Fischler, et al, “Effect of Hand Wash Agents on Controlling the Transmission of Pathogenic Bacteria from Hands to Food,” *Journal of Food Protection*, Vol 70, No. 12, 2007, pp. 2873–2877.

⁶ Fuls, et al, “Alternative Hand Contamination Techniques to Compare the Activities of Antimicrobial and Nonantimicrobial Soaps Under Different Test Conditions,” *Applied and Environmental Microbiology*, Vol 74, No. 12, June 2008, pp. 3739–3744.

TABLE 1 Formula for Mild, Non-Antimicrobial Liquid 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

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.

7.2 Test Material—Manufacturer directions for use of the test material should be utilized. If directions are not available, use the directions provided in this test method.

7.3 Sampling Solution—Dissolve 0.4 g monopotassium phosphate (KH_2PO_4), 10.1 g disodium hydrogen phosphate (Na_2HPO_4), 1.0 g isooctylphenoxypolyethoxyethanol (for example, Triton X-100), and appropriately validated neutralizers in 1 L distilled water. Adjust pH with 0.1 *N* hydrochloric acid (HCl) or 0.1 *N* sodium hydroxide (NaOH). Sterilize in an autoclave. Final pH after sterilization is 7.8 ± 0.1 . Dispense so that final volume after sterilization is 75 mL.⁷ Perform Test Method **E1054** to determine what neutralizers are required.

7.4 Dilution Fluid—Sterile Butterfield’s buffered phosphate diluent⁸ or other suitable diluent with appropriately validated neutralizers. Perform Test Method **E1054** to determine what neutralizers are required. The addition of neutralizer is only required if inactivation of the test material cannot be achieved upon dilution into the sampling solution (7.3). Adjust pH with 0.1 *N* HCl or 0.1 *N* NaOH. Final pH after sterilization 7.2 ± 0.1 .

7.5 Soybean-Casein Digest Agar—Sterile tryptic soy agar or other solid media appropriately validated to support growth of *Serratia* species. With appropriate neutralizers if required per Test Method **E1054**.

7.6 Hektoen Enteric Agar—Used for the recovery and growth of *Shigella* species. With appropriate neutralizers if required per Test Method **E1054**.

7.7 Mannitol Salt Agar—Sterile. Used for the recovery and growth of *Staphylococcus* species. With appropriate neutralizers if required per Test Method **E1054**.

7.8 Soybean-Casein Digest Agar with MUG⁹—Sterile tryptic soy agar with MUG, used for the indication, recovery and growth of *Escherichia* species or other solid media appropriately validated to support the growth of the test organism. With appropriate neutralizers if required per Test Method **E1054**.

7.9 Broth—Sterile soybean-casein digest broth (tryptic soy broth) or other liquid media appropriate to support growth of the test organism.

7.10 Ethanol or Isopropyl Solution—70 % ethanol or isopropyl alcohol in water (v/v) for hand decontamination.

7.11 Antibiotic Ointment—A topical triple-antibiotic ointment for application to the hands after the final decontamination.

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

7.13 Physiological Saline—Sterile. Used to dilute inoculum if lower levels are desired.

8. Test Organisms

8.1 *Serratia marcescens* (ATCC 14756) is to be used as the test organism. This is a strain having stable pigmentation at 25°C. The plating agar should be soybean-casein digest agar (7.5).

8.2 *Escherichia coli* (ATCC 11229) is an alternative test organism. When *E. coli* is used, the plating agar should be soybean-casein digest agar with MUG (7.8) or another suitable indicator.

8.3 *Shigella flexneri* (ATCC 700930) is an alternative test organism. When *S. flexneri* is used, the plating agar should be Hektoen enteric agar (7.6).

8.4 *Staphylococcus aureus* (ATCC 6538) is an alternative test organism. When *S. aureus* is used, the plating agar should be Mannitol salt agar (7.7).

8.5 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 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, a decontamination procedure should be performed (Section 13).

8.6 Preparation of Test Organism Suspension:

8.6.1 *Serratia marcescens* (ATCC 14756)—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 (7.9) transfers from the original ATCC culture, but there should be no more than five transfers removed from the ATCC culture. From the stock, inoculate the appropriate volume of

⁷ 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.

⁸ 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.

⁹ *United States Pharmacopeia 32*: 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.