Designation: E 1174 – 00

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

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:*

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[3]

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 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

[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.

Current edition approved August 10, 2000. Published November 2000. Originally published as E 1174 – 87. Last previous edition E 1174 – 94.

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

[3] *Annual Book of ASTM Standards*, Vol 11.04.

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 ± 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.[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.)

7.7 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4 and 1.0 g isooctylphenoxypolyethoxyethanol[7] 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 Buffer[9] 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. MUG[10]).

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°C ± 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 5 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 milliliter of stock culture/100mLs of broth to yield the volume necessary to complete the study. Incubate for 24 ± 4 hours at 35 ± 2°C.

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[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] 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-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 grams/L.

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 hours. The suspension may not vary more than $\pm$ 0.5 $\log_{10}$ cfu/mL over an 8 hour period.

9. Subjects

9.1 Recruit a sufficient number of healthy adult human volunteers who have no clinical evidence of dermatosis, 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 treated pools, hot tubs, or spas should be avoided. Subjects are to be provided with a kit of nonantimicrobial personal care products for exclusive use during the test 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 second cleansing wash (7.4) in the same manner that is described for the test and control formulations. This procedure removes oil and dirt and familiarizes the panelists with the washing technique.

10.2 *Hand Contamination*—A liquid suspension of the test organism containing a minimum of 1×10^8 cfu/mL is used. See Table 1.

10.2.1 A 1.5mL 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 $\pm$ 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 $\pm$ 5 s.

TABLE 1 Hand Contamination with Test Organism Suspension

Volume	Spread Time	Dry Time
1.5 mL	20 sec	30 sec
1.5 mL	20 sec	30 sec
1.5 mL	20 sec	90 sec

10.2.2 To continue the contamination of the hands, an additional 1.5mL aliquot of the test organism suspension is dispensed into the hands, distributed over the hands for 20 $\pm$ 5 seconds, and air dried for 30 $\pm$ 5 seconds.

10.2.3 To complete the contamination, a final 1.5mL aliquot of test organism suspension is dispensed into the hands, distributed over the hands for 20 $\pm$ 5 seconds, and air dried for 90 $\pm$ 5 seconds (Table 1).

NOTE 2—The hands may still be wet after the 90 seconds.

10.2.4 The total test organism suspension applied to the hands is 4.5 mL. Contamination may take approximately 5 minutes. 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 below illustrates a typical test. The number of repeated test washes may be reduced or eliminated at the discretion of the investigator.

TABLE 2 Hand Contamination 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
Test Wash 2–10	yes	Test Formulation	no
Test Wash 11	yes	Test Formulation	Plate Recovered Sampling Solution with Neutralizer

10.4 *Baseline Recovery*—A baseline sample is taken after contamination to determine the number of marker organisms surviving on the hands. Bacterial sampling will follow the 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 above 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. Spread over hands and lower ⅓of forearms.

NOTE 3—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 with 40 $\pm$ 2°C tap water.

11.2.3 Wash in a vigorous manner for 30 $\pm$ 5 seconds 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 be added to maintain lather.

11.2.4 Rinse thoroughly from fingertips to elbows under 40 $\pm$ 2°C tap water for 30 $\pm$ 5 seconds. Caution should be exercised to avoid contact with the sink and fixtures to eliminate recontamination from the sink surfaces.

11.2.5 Subject's hands and forearms are lightly patted dry with paper toweling.

NOTE 4—After washes requiring sampling, the hands are not to be dried, but held upright until procedures in 12.1 are performed.

11.3 *Waterless Formulations*[11]

11.3.1 Dispense 5 mL of test material into cupped hands.

NOTE 5—The 5 ml volume has been chosen for test purposes due to the requirement for washing hands and forearms.

11.3.2 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 seconds or until dry. 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).

11.4 *Solid Formulations*

11.4.1 Sparingly wet contaminated hands and forearms with 40 ± 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 ± 3 seconds. Place product aside.

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

11.4.5 Rinse thoroughly from fingertips to elbows under 40 ± 2°C tap water for 30 ± 5 seconds. Caution should be exercised to avoid contact with the sink and fixtures to eliminate contamination from the sink surfaces.

11.4.6 Subject's hands and forearms are lightly patted dry with paper toweling.

11.5 *Other Product Forms*

11.5.1 Use standardized amount (e.g. 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 5 minutes after specified washes (10.3), 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 Uniformly massage all surfaces of the hand for 1 min ± 5 seconds.

12.3 Aseptically retrieve a 3-5 mL sample of the fluid in the glove by pulling the glove away from the wrist, inserting a pipet into the finger region of the glove, and withdrawing the fluid.

12.4 The dilution and plating of the recovered sampling solution is completed within 30 minutes after sampling.

[11] An alternative test methodology may be found in European Standard CEN-1500: Chemical Disinfectants and Antiseptics - Hygenic Handrub - Test Method and Requirements (phase2/step2), July, 1997.

13. Enumeration of Bacteria in Sampling Solution

13.1 *S. marcescens*

13.1.1 Enumerate the *S. marcescens* in the recovered sampling solution (12.3) using standard microbiological techniques, such as membrane filtration or spread plating. The pour plate technique is not recommended because subsurface *S. marcescens* colony forming units may not exhibit the red pigment.

13.1.2 Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.8). Use soybean-casein digest agar (7.9) with suitable inactivator as recovery medium.

13.1.3 Incubate prepared plates 48 ± 4 h at 25 ± 2°C. Standard plate counting procedures are used to count only the red pigmented *S. marcescens*.

13.2 *E. coli*

13.2.1 Enumerate the *E. coli* in the sampling solution using standard microbiological techniques, such as membrane filtration, pour or spread plating. Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.8). Use soybean-casein digest agar (7.9) with suitable inactivator and indicator (MUG[10]) as recovery medium.

13.2.2 Incubate prepared plates 48 ± 4 hour at 35 ± 2°C. Standard plate counting procedures are used to count only the fluorescent (MUG[10]) *E. coli* colonies. Fluorescent colonies are counted using long-wave UV light.

14. Determination of Reduction

14.1 Convert plate counts (cfu/hand) to $\log_{10}$. Average left and right hands.

14.2 Determine Log_{10} Reductions at each recovery interval/wash using the following formula:

$$\text{Log}_{10}\ \text{Reduction at Sampling Interval} = \text{Log}_{10}\ \text{Baseline Recovery} - \text{Log}_{10}\ \text{Sampling Interval} \quad (1)$$

15. Comparison of Test Material

15.1 It may be desirable to compare the test material with other test formulations. If this is the case, an equivalent number of panelists should be assigned to each formulation on a random basis. All test parameters will be equivalent for products, although the wash procedure for an established product may be different. Both products should be run concurrently.

16. Precision and Bias

16.1 A precision and bias statement cannot be made for this test method at this time.

17. Keywords

17.1 antimicrobial; contaminant; efficacy; handwash; healthcare; marker organism; simulant