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Standard Test Method for Evaluation of Health Care Personnel Handwash Formulations by Utilizing Fingernail Regions¹

This standard is issued under the fixed designation E 1327; 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.

^{ε1} NOTE—Section 13 was added editorially in March 1995.

1. Scope

1.1 This test method covers the determination of ability of antimicrobial handwashing agents to give reductions of transient microbial flora (bacterial flora) when used in a hand washing procedure.

1.2 A knowledge of microbiological techniques is required for these procedures.

1.3 *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 hazard statements, see Note 1.

2. Referenced Documents

2.1 ASTM Standards:

E 1054 Practices for Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products²

3. Summary of Test Method

3.1 This test method, involving an improved method of recovering bacteria from hands, is used to study the effects of antimicrobial health care personnel handwash products. The group of volunteer panelists need not refrain from using topical antimicrobials (such as deodorant soaps) before participating in the study. All subjects wash their hands with a nonantimicrobial hand soap prior to testing to remove any residual hand lotions and to lower the numbers of resident skin flora. Activity of products is measured by comparing the numbers of marker bacteria recovered from artificially contaminated fingernail regions after use of the handwashing formulations to the numbers recovered from the artificially contaminated but unwashed fingernail regions. Broth cultures of *Serratia marcescens* (a red pigmented bacterial species) and *Escherichia coli* (which produces fluorescent colonies on a special agar medium) are used as test bacteria. A spore suspension of *Bacillus*

subtilis may be utilized to study (1) degree of physical removal by handwashing techniques, and (2) the recovery and precision aspects of the test method.

4. Significance and Use

4.1 The procedure should be used to test the degerming effectiveness of antimicrobial hand washing products used by health care personnel that are intended for frequent use, and that are intended to reduce the level of contamination acquired through contact with contaminated objects or people.

4.2 Performance of these procedures requires the knowledge of regulations pertaining to the protection of human subjects.³

5. Apparatus

5.1 *Colony Counter*—Any of several types may be used, for example, Quebec Colony Counter.

5.2 *Incubators*—One incubator capable of maintaining a temperature of $25 \pm 2^\circ\text{C}$ (this temperature required to ensure pigment production of *Serratia*); a second incubator capable of maintaining $37 \pm 2^\circ\text{C}$ is used for *E. coli* and *B. subtilis* incubation is acceptable.

5.3 *Water Bath*—Capable of maintaining temperature of $80 \pm 2^\circ\text{C}$ for heat shocking of *B. subtilis* spores is needed.

5.4 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization is acceptable.

5.5 *Timer*—Any stop-watch that can be read in minutes and seconds is required.

5.6 *Handwashing Sink*—A sink of sufficient size to permit panelists to wash without touching hands to sink surface or other panelists is needed.

5.6.1 *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.

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

5.7 *Quad Petri plates*, 100 by 15 mm, plastic, sterile, disposable.⁴

¹ This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial and Antiviral Agents.

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² *Annual Book of ASTM Standards*, Vol 11.05.

³ *Federal Register*, Vol 46, No. 17, Jan. 27, 1981.

⁴ Presterilized disposable quad plastic petri plates, the two sizes of glass petri plates and other equipment are available from most local laboratory supply houses.

5.8 *Small Petri Plates*, 60 by 15 mm, glass.⁴

5.9 *Large Petri Plates*, 150 by 15 mm, glass.⁴

5.10 *Tooth Brushes*:

5.10.1 *Young Size*.⁵

5.10.2 *Battery Operated*.⁶

5.11 *Ultraviolet Lamp*, having separate short wave and long wave bulbs.⁷

5.12 *Germicidal Lamp Monitor Strips*.⁸

6. Reagents and Materials

6.1 *Bacteriological Pipettes*, 10.0 mL, sterile.

6.2 *Pipettors and Pipette Tips*, Eppendorf, MLA or similar types.

6.3 *Disposable Analyzer Cusp*, 2 mL, plastic, not sterile.

6.4 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4 , 10.1 g Na_2PO_4 and 1.0 g isooctylphenoxypolyethoxyethanol⁹ in 1 L distilled water. Adjust pH to 7.8 with 0.1 N HCl or 0.1 N NaOH. Dispense in 100 mL-volumes and sterile for 20 min at 121°C.

6.5 *Dilution Fluid*—The sampling fluid may be used for dilutions or use Butterfields sterile phosphate buffered water¹⁰ adjusted to pH 7.2 with suitable inactivator for the antimicrobial. Adjust pH with 0.1 N HCl or 0.1 N NaOH (see Practices E 1054).

6.6 *Agar*, Tryptic soy agar or equivalent. Include the appropriate inactivator if needed.

6.7 *Agar with MUG*—Tryptic soy agar with 60 to 80 $\mu\text{g}/\text{mL}$ 4-methylumbelliferyl- β -D-glucuronide (MUG) is required.

6.8 *Test Formulations*—Directions for use of test formulation should be included if available. If these are not available, liquid antimicrobial soap formulations are tested by same routine as the nonantimicrobial control (10.5); alcoholic lotion type formulations are rubbed to dryness and then sampled for survivors (10.7).

6.9 *Nonantimicrobial Control Soap*, a liquid castile soap or other liquid soap containing no antimicrobials.

6.10 *Broth*—Tryptic soy broth or equivalent is required.

7. Test Organisms

7.1 *Serratia marcescens* American Type Culture Collection, ATCC No. 14756 is to be used as a marker organism. This is a strain having stable pigmentation. Grow in tryptic soy broth at 28°C.

7.2 *Escherichia coli*, ATCC No. 11229 is used as another gram negative marker organism. Grow in tryptic soy broth at 37°C.

⁵ Oral B-30 tooth brushes, available from Oral B Laboratories, Inc., Redwood City, CA, have been found suitable for this purpose.

⁶ Battery-operated tooth brushes, Model TB-9, available from General Electric Co., Bridgeport, CT, have been found suitable for this purpose.

⁷ Combination ultraviolet lamps with individual long wave and short wave bulbs are available from most laboratory supply houses.

⁸ Strips available from Uvicide[®] Germicidal Lamp Monitor, Catalog No. UV3000, Vanguard International, Neptune, NJ 07753, or equivalent, have been found suitable for this purpose.

⁹ Triton X-100, available from Rohm and Haas Co., Philadelphia, PA, has been found suitable for this purpose.

¹⁰ Butterfield's Phosphate Buffer. *Journal of the Association of Official Analytical Chemists*, Vol 22, No. 625, 1939.

7.3 *Bacillus subtilis*, ATCC No. 19659. Grow in tryptic soy broth at 37°C.

7.4 *Preparation of Spore Suspension*—Inoculate each surface of two tryptic soy agar plates (30 mL agar in 150-mm petri plates) with 1 mL of *B. subtilis* tryptic soy broth culture. Spread over the entire surface of the agar. Incubate for 5 to 10 days at 37°C. Suspend the growth in 20 mL of 0.1 % tryptone water¹¹ by rubbing the agar surface with a sterile rubber policeman. Add ethanol to the suspension to a final concentration of 80 % (wt/wt) and store in a refrigerator.

7.5 Other bacteria containing adequate markers to enable distinction from normal flora and of known safety may also be used for testing purposes.

NOTE 1—**Warning:** The application of microorganisms to the skin may involve a health risk. Prior to applying *S. marcescens* or other bacteria to the skin, the antibiotic sensitivity profile of the strain should be determined. If the *Serratia* strain is not sensitive to gentamycin, it should not be used. If an infection occurs, the antibiotic sensitivity profile should be made available to an attending clinician. Following the panelist's contamination and testing for the day, the panelist's hands should be decontaminated with a 70 % ethanol solution. Care should be taken to decontaminate around the fingernail regions.

7.6 *Preparation of Marker Culture Suspension*—Inoculate a 10-mL tryptic soy broth tube with each of the test bacteria and incubate each tube at the temperature indicated. When studying mixed inocula, mix equal volumes of the cultures into a sterile test tube; an equivalent volume of *B. subtilis* spore suspension (that is prepared by centrifuging the alcoholic suspension and resuspending cells in water) may be added for bacterial physical removal determinations. Keep mixed suspension on ice during the days testing.

8. Panelists

8.1 Recruit a sufficient number of healthy adult human volunteers who have no clinical evidence of dermatosis, open wounds, hangnail or other skin disorders. The number of people needed for a trial is dependent on the number of treatments within a study.

8.2 Volunteers are asked to maintain their normal use of soaps, shampoos, etc. They are asked to refrain from the use of acids, bases, solvents on the hands during the test period. Gloves should be provided for use where exposure to these agents is unavoidable.

9. Experimental Design

9.1 Each fingernail of a volunteer may be assayed separately; therefore, 10 test determinations (replicates) may be obtained from one volunteer. For the comparison of several products during a single study, a design such as a Latin Square Design may be utilized.¹² For example, to compare 5 antimicrobial test products, one nonantimicrobial product and unwashed hand control (7 total variables), 7 volunteers, (or multiples of 7) should be recruited. Each person performs one testing of product or other variable on each of 7 test days,

¹¹ Bacto tryptone (Difco) water available from Difco Laboratories, Detroit, MI, has been found suitable for this purpose.

¹² Bennett, C. A., and Franklin, N. L., *Statistical Analysis in Chemistry and the Chemical Industry*, John Wiley and Sons, New York, 1954.

according to schedule such as the following; the numbers = day for testing that variable (see Table 1).

9.1.1 *Example:* Volunteer A tests Treatment 1 on Day 1, then Treatment 2 on Day 2; Volunteer B tests Treatment 2 on Day 1, Treatment 3 on Day 2 etc.

9.1.2 —Each product or variable is tested once on each day, unless multiple numbers of volunteers are in the study.

9.1.3 The number of fingers, which are inoculated and then assayed after using the product, should be kept standard throughout. Although the number can be as high as 10, three fingers on one hand is a more convenient and cost savings approach. The ring, middle and index fingers of the left hand have been selected for several studies; however, an operator may select the number and particular fingers to assay as long as they are held constant throughout.

TABLE 1 Latin Square Design for Testing Seven Variables

Day	Volunteer						
	A	B	C	D	E	F	G
1	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇
2	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₁
3	T ₃	T ₄	T ₅	T ₆	T ₇	T ₁	T ₂
4	T ₄	T ₅	T ₆	T ₇	T ₁	T ₂	T ₃
5	T ₅	T ₆	T ₇	T ₁	T ₂	T ₃	T ₄
6	T ₆	T ₇	T ₁	T ₂	T ₃	T ₄	T ₅
7	T ₇	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆

10. Procedure

10.1 Before tests for the day, sterilize the analyzer cups by placing in suitable rack (24 well culture plates¹³ with lids are convenient) and placing the open cups under short-wave ultraviolet lamp for 15 to 30 min. To each cup is then added 0.9 mL of sterile diluent: set up sufficient cups only for each day's testing.

10.2 Place 7 mL of collection fluid into each of 21 small petri plates.

10.3 Place 0.02 mL of marker culture suspension on the region surrounding the cuticle and under the fingernails of three fingers of the left hand of a volunteer. The volunteer then holds the hand in front of an electric fan for 5 min for complete drying of the suspension.

10.4 For unwashed hand determinations, proceed directly to 10.8.

10.5 When testing nonantimicrobial soap (controls), wet both hands under flowing warm tap water ($40 \pm 2^\circ\text{C}$). Add 2.5 to 3.0 mL of the liquid soap to hands, rub hands together in normal washing manner for 15 s (no additional water), then rinse under the flowing water for 15 s to remove suds. Do not dry hands, proceed directly to 10.8.

10.6 For testing liquid antimicrobial soap formulations, follow the use directions on the label or follow the routine of 10.5. After washing, proceed to 10.8 without drying hands.

10.7 Alcoholic formulations are tested by placing the recommended volume on the hands and then rubbing the hands together until the alcohol has evaporated. Proceed to 10.8.

10.8 After performing the procedure for the day designated in the Latin Square Design, the technician scrubs with a toothbrush for 1 min each fingernail into a separate petri plate containing 7 mL of collection fluid.

NOTE 2—Although manual toothbrushes may be used for this purpose, greater uniformity between scrubblings may be obtained with less operator fatigue if an electric toothbrush such as the GE model TB-9 or another type may be used. A brush which operates longitudinally to the handle is preferred because of less splashing.

10.9 After each scrubbing, the brushes are dropped into a beaker containing 70 % ethyl or isopropyl alcohol and allowed to stand for at least 10 min. The brushes are then rinsed in sterile distilled water and allowed to dry. The brushes are not sterilized.

10.10 Perform serial 10-fold dilutions by serial transfers of 0.1 mL amounts of fluid from one well to the next. Place 0.1 mL amounts of the appropriate dilutions onto the surface of agar sections of quad plates. These drops of liquid are spread with sterile inoculation loops or needles to completely cover the quads. Allow drops to completely absorb.

10.11 Incubate inverted plates at 37°C for 12 to 18 h. Count the *E. coli* colony forming units (CFU) that fluoresce under long wave ultraviolet light. Transfer the plates to a 25°C incubator and incubate for another day.

10.12 Count the red pigmented *S. marcescens* CFU. Record the CFU per countable sections of the plates and convert values to the CFU obtained per finger by multiplying by the appropriate dilution factors.

10.13 Convert each CFU per finger determination to the \log_{10} value.

10.14 Determine the mean \log_{10} CFU per finger value, this is the mean \log_{10} value for that variable and subject for that day. These \log_{10} values are used for statistical comparisons.

10.15 If an estimation of the degree of physical removal caused by a product is desired, *B. subtilis* spores may be included in the test strain(s) inoculum. Portions of the collection fluids are heated at 80°C for 10 min to kill the vegetative test strains; the fluids are then diluted and plated on tryptic soy agar.

11. Statistical Evaluations

11.1 The completed table of data obtained from the Latin Square Design described should be analyzed by Analysis of Variance Tests.¹²

11.2 In tests where a small number of variables and large numbers of people are involved, for example, 20 people testing 2 antimicrobial products, a nonantimicrobial product and unwashed hand controls, use a Neuman-Kreul multiple range analysis¹⁴ or the parametric *t* test may be used.

12. Precision and Bias

12.1 *Precision*—The precision of this test method within one laboratory has been determined;¹⁵ precision of recovery is comparable with other accepted methods such as the glove juice technique.

¹³ Multiwell tissue culture plates, available from Becton Dickinson Labware, Lincoln Park, NJ, have been found suitable for this purpose.

¹⁴ Miller, R. G., *Simultaneous Statistical Inference*, McGraw Hill, New York, 1966.

¹⁵ Mahl, M. C., *Journal of Clinical Microbiology*, Vol 27, 1989, pp. 2295–2299.

12.2 Using *B. subtilis* spore tracers, this test method recovers almost all inoculated spores that were placed on the fingernails as inert tracers and then scrubbed into collection fluid versus placing the equivalent amount of spores in the equivalent volume of collection fluid and assaying directly.¹⁵ The precision aspects of this test method can be expressed as the coefficient of variation by using replicate spore recovery data, for example, a test where fifteen recoveries were made from the fingernails the following data were obtained:

CFU/finger	Log ₁₀ (CFU/finger)
470,000	5.67
500,000	5.70
300,000	5.48
340,000	5.53
320,000	5.51
300,000	5.48
370,000	5.57
310,000	5.49
380,000	5.58

300,000	5.48
370,000	5.57
400,000	5.60
300,000	5.48
340,000	5.57
230,000	5.36

Sample mean = 5.54,
Standard deviation = 0.084

$$\text{Coefficient of variation} = \frac{\text{Standard deviation}}{\text{Sample mean}}$$

$$\times 100 = \frac{0.084}{5.54} \times 100 = 1.5 \%$$

13. Keywords

13.1 antimicrobial soap; *Escherichia coli*; fingernail; health care personnel handwash; *Serratia marcescens*; toothbrush

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