Designation: E2870 – 13

Standard Test Method for Evaluating Relative Effectiveness of Antimicrobial Handwashing Formulations using the Palmar Surface and Mechanical Hand Sampling[1]

This standard is issued under the fixed designation E2870; 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 and is designed to determine the relative effectiveness of antimicrobial handwashing agents in reducing transient microorganisms using a controlled handwash.

1.2 Knowledge of microbiological techniques is required for these procedures.

1.3 This test method is used to evaluate topical antimicrobial handwashing formulations.

1.4 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects (21 CFR Parts 50 and 56).

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

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 8.1.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

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

E2755 Test Method for Determining the Bacteria-Eliminating Effectiveness of Hand Sanitizer Formulations Using Hands of Adults

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E2784 Test Method for Evaluation of the Effectiveness of Handwash Formulations Using the Paper Towel (Palmar) Method of Hand Contamination

2.2 *Other Standards:*

AATCC Test Method 147 Antibacterial Assessment of Textile Materials: Parallel Streak Method[3]

21 CFR Part 50 Protection of Human Subjects[4]

21 CFR Part 56 Institutional Review Boards[4]

3. Terminology

3.1 *Definitions*—For definitions of terms used in this document, see 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 *reference formulation, n*—formulation against which the activity of the test formulation is compared, for example, a handwash without an active ingredient or a handwash with a different active ingredient than the test formulation. This formulation is not considered a standard.

3.2.3 *test material, n*—a product or formulation which incorporates antimicrobial ingredients(s).

3.2.4 *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.

4. Summary of Test Method

4.1 This test method uses adult subjects who have provided a written informed consent and whose hands have been

[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] 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] Available from American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709, http://www.aatcc.org.

[4] Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, http://www.access.gpo.gov.

determined to be free from any apparent damage at the time of participation in the study. Subjects are to refrain from use of any antimicrobials for at least one week prior to the initiation of the test procedure (see 9.3).

4.2 The test compares the activity of the two handwash formulations simultaneously on the same subject under standardized and controlled conditions. Hands of the subjects are artificially contaminated with *Escherichia coli*. One hand of the subject is washed with the reference formulation and the other with the test formulation. The *E. coli* cells remaining on the hands are recovered by the glove juice method of sampling using a mechanical scrubber. In other methods, comparisons between two products are made by testing two equivalent groups of subjects. The objective of this test method is to determine the relative difference between two products tested on the same subjects and not to determine absolute reductions in organism levels. By testing both products at the same time on the same subjects with the same bacterial inoculum, variability is reduced.

4.3 Effectiveness of the test material is determined after a single wash by comparing the numbers of viable test organisms recovered after treatment with it and with the reference formulation. As an example, a cleanser with an active ingredient (test formulation) can be compared to the cleanser without an active ingredient (reference formulation) to determine the effect the active ingredient has on product performance.

4.4 No baseline sampling of the hands is performed in this test method. The inoculum volume to the palms is very small. A volume of 100 µL is applied to each palm and the palms and fingers are rubbed together. Spillage and loss do not occur, and organisms are evenly distributed across the palmar surface after rubbing. As the objective of this test method is to determine the relative difference between products and not absolute reductions, baseline sampling is not performed.

NOTE 1—If an investigator wanted to compare the effect of washing with a product to not washing, this test could be conducted with one hand serving as a baseline sample and the second hand treated with the test product.

4.5 The investigator should be aware that there may be health risks associated with the use of the test organism and precautions similar to those referenced in 8.1 should be undertaken.

5. Significance and Use

5.1 Hand hygiene is important for preventing the spread of many types of infections.

5.2 During routine activities, it is primarily the palmar surface, comprising palms, fingers, and finger pads, of the hands that may become contaminated with transient microorganisms. The contamination could then be transferred to articles touched or handled or to other parts of the body. Palmar contamination is used in Test Method E2784.

5.3 In Test Method E1174, incomplete drying of the experimentally contaminated hands dilutes the applied test product, thus compromising its activity. Application of a smaller volume of the microbial test suspension keeps the soil load to a reasonable level while allowing the hands to become visibly dry prior to application of the test material and reference formulation. These modifications are aimed at producing a better approximation of in-use conditions and a more realistic assessment of the test substance, thus providing a more reliable indication of product performance.

5.4 Unlike Test Methods E1174, E2755, and E2784, this test method enables a direct comparison between two formulations on the same subject. The test method also uses a mechanical scrubbing machine in conjunction with the glove juice technique for more efficient recovery of viable test bacteria from the palms. The mechanical sampling results in greater recovery of bacteria from the palms than conventional recovery methods, such as massaging.

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 temperature: 35 ± 2°C.

6.3 *Shaker Incubator*—Any incubator capable of maintaining the following temperature: 35 ± 2°C and capable of shaking the culture medium at 120 to 140 r/min.

6.4 *Sterilizer*—Any suitable steam sterilizer capable of producing the conditions of sterilization.

6.5 *Timer (Stop-Clock)*—Type that can be read for minutes and seconds.

6.6 *Handwashing Sink*—A sink of sufficient size to permit subjects to wash without touching hands to sink surface or other subjects.

6.6.1 *Water Faucet(s)*—To be located above the sink at a height which permits the hands to be higher than the elbow during the washing procedure. Faucet should maintain a flow rate of 3 L/min as determined in 10.4.

6.6.2 *Water Temperature Regulator and Temperature Monitor*—To set and maintain the water temperature at 40 ± 2°C.

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

6.8 *Mechanical Scrubber*[5]—To mechanically sample the palms for test bacteria (see Fig. 1). The machine contains two artificial metallic paddles covered with an artificial turf for a smooth and nonslip surface. The drive mechanism is powered by a 48V DC geared motor and has a variable speed from 50 to 150 r/min produced by an electronic speed regulator. The specialized eccentric mechanism produces 12.7 cm long (total stroke-length) horizontal reciprocating silent movement. Both paddles face upwards, parallel to the palms, and during the test, the complete system works to simulate the live activity of mechanically sampling the hands.

[5] The sole source of supply of the apparatus known to the committee at this time is Trishul Equipment, Shiva Industrial Estate, Unit No. 107, First Floor, Lake Road, Bhandup West, Mumbai-400078, India. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

FIG. 1 Mechanical Scrubber

6.9 *Spectrophotometer*—An instrument that can measure optical density at a wavelength of 620 nm.

6.10 *Adjustable or Fixed Volume Pipets and Sterile Tips*—1 mL capacity and 0.1 mL capacity.

6.11 *Sampling Containers*—Any sterile or sterilizable container having a tight closure and sufficient capacity to hold 75 mL of sampling solution (7.2).

6.12 *Centrifuge*—For the sedimentation of *E. coli* for concentration.

6.13 *Sterile Centrifuge Tubes*—Minimum of 15-mL capacity.

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

6.15 *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 (6.16) with low bioburden may be used in place of gloves.)

6.16 *Plastic Bags*—May be used in place of gloves. Bags should be approximately 30 × 18 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.

6.17 *Wrist Ties or Tourniquets*—Any item which will allow the gloves (6.15) or plastic bags (6.16) to be secured to the subject's wrists.

6.18 *Sterile Tissues or Paper Towels*—Any sterile tissue or paper towel that can be used to dry hands.

7. Reagents and Materials

7.1 *Test Substances*—Follow the manufacturer's directions for use of the test material and reference formulation. If directions are not available, use the directions provided in this test method (10.5).

7.2 *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 distilled water. Adjust pH to 7.8 ± 0.1 with 0.1 *N* hydrochloric acid (HCl) or 0.1 *N* sodium hydroxide (NaOH) and bring volume to 1 L with distilled water. Sterilize in an autoclave and aseptically dispense 40-mL and 35-mL portions into sterile sampling containers (6.11).[6]

NOTE 2—A neutralizer validation should be conducted according to Test Method E1054 prior to the study. Test Method E1054 provides a list of neutralizers appropriate for commonly used antimicrobial agents. In some cases, neutralization may be achieved by dilution alone.

7.3 *Dilution Fluid*—Sterile Butterfield's buffered phosphate diluent[7] (or other suitable diluent) adjusted to pH 7.2 ± 0.1 and containing an effective inactivator for the test material, if necessary.

NOTE 3—Inactivator is only required if neutralization of the test material cannot be achieved upon dilution into the sampling solution (see 7.2).

7.4 *Soybean-Casein Digest Agar with MUG* (4–methylumbelliferyl-b-D-glucuronide)—Sterile tryptic soy agar with MUG (0.5 g/L), 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, according to Test Method E1054.

NOTE 4—The MUG substrate is hydrolyzed by b-D-glucuronidase to yield a fluorescent end product, 4-methylumbelliferone. b-D-glucuronidase is possessed by *E. coli* (ATCC 10536).

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

7.6 *Soybean-Casein Digest Agar*—Sterile tryptic soy agar for growth of *Escherichia* species or other solid media appropriately validated to support the growth of the test organism.

7.7 *Physiological Saline*—Sterile. Used to prepare the final inoculum.

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

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

8. Test Organism

8.1 *Escherichia coli* (ATCC 10536) is the test organism. The plating agar should be soybean casein digest agar with MUG (7.4) or another suitable indicator. (**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 contamination and wash with the formulation, a decontamination procedure should be performed (Section 11)).

8.2 *Preparation of Test Organism Suspension:*

8.2.1 A homogeneous culture of *E. coli* (ATCC 10536) is used to inoculate the hands. The stock culture should be at least

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

[7] Butterfield, C. T., "The Selection of a Dilution Water for Bacteriological Examinations," *J. Bacteriol*, Vol 23, 1931, pp. 355–368.

two 24-h soybean-casein digest broth or agar slant transfers from the original ATCC culture, but should be no more than four transfers removed from the ATCC culture.

8.2.2 The stock culture, maintained on soybean-casein digest agar slants, should not be more than one month old. From the slant, inoculate a loopful of the culture into 50 mL of soybean-casein digest broth.

8.2.3 Incubate the broth culture for 16 to 18 h at 35 ± 2°C on a shaker incubator set to 120 to 140 r/min.

8.2.4 After incubation, add 1 mL of this overnight culture to fresh 40-mL soybean-casein digest broth.

8.2.5 Incubate the 40-mL soybean-casein digest broth culture for 4 h at 35 ± 2°C on a shaker incubator set to 120 to 140 r/min. This culture is used for the test.

8.2.6 The culture is centrifuged at 5000 r/min (corresponding g value is 3214) and washed twice with physiological saline (7.7).

8.2.7 The pellet is suspended in physiological saline and the optical density (OD) at 620 nm is adjusted in order to obtain an inoculum of 8×10^7 to 1×10^8 CFU/mL of the *E. coli* culture. If a lower inoculum is desired, dilute the inoculum with physiological saline.

8.2.8 Prepare the inoculum not more than 1 h prior to start of test.

8.2.9 Using standard microbiological techniques, enumerate the suspension for number of organisms at the beginning and end of the use period. Do not use a suspension for more than 8 h. The suspension may not vary more than ± 0.5 $\log_{10}$ CFU/mL over an 8-h period.

8.2.10 Swirl or shake the suspension before the withdrawal of each aliquot. Each subject will require 0.2 mL of suspension.

9. Subjects

9.1 Recruit a sufficient number of healthy adult human volunteers who have no clinical evidence of dermatoses, open wounds, cuts, burns, hangnails, or other skin disorders. The total number of subjects used will depend on the number of test substances, the purpose of the study, and the regulatory requirements governing the study.

9.2 It is the responsibility of the user of this test method to obtain the necessary approval from an Institutional Review Board (IRB) or Independent Ethics Commission (IEC) for the use of humans for testing and to obtain informed and written consent from those selected for the study before starting the test.

9.3 Instruct subjects to avoid contact with antimicrobial products for the duration of the test and for at least one week (7 days) prior to the test. This restriction includes antimicrobial-containing antiperspirants, deodorants, shampoos, lotions, and soaps. Bathing in biocide-treated pools, hot tubs, or spas should be avoided. Harsh chemicals such as acids, bases, and solvents also should be avoided. Subjects may not use topical or systemic antimicrobials, antibiotics, or steroids other than for contraception or post-menopausal indications, and must agree to abstain from these materials until the completion of the study. Provide subjects with a kit of non-antimicrobial personal care products for exclusive use during the test and include rubber gloves to be worn when contact with antimicrobial or harsh chemicals cannot be avoided.

10. Procedure

10.1 *Admission to Testing*—Instruct each subject to return to the laboratory for testing after they having refrained from using antimicrobials for at least 7 days. Question the subject to confirm adherence to the study requirements (see 9.3). Inspect the subject's hands and forearms to confirm the absence of clinical signs of skin disorders as described in 9.1. Subjects' hands must be free from visible soil and contamination. Admit the subject into the test if all of the above criteria are met. Instruct the subjects to remove all jewelry from their hands and arms and to clip their fingernails to a uniform length (free edge ≤1 mm).

10.2 After subjects have refrained from using antimicrobial formulations for at least 7 days, they perform two washes with 70 % alcohol. 10 ± 1 mL of the alcohol is poured into the subjects' palms, and the subjects rub their palms and the back of the hands for 15 s. The alcohol is allowed to air dry, and the step is repeated one more time. Subjects who accidentally spill the majority of alcohol down the sink may be provided with an additional 10 ± 1 mL of the alcohol to ensure adequate decontamination of the hands. Traces of alcohol are removed by having the subjects wash their hands thoroughly with sterile water for 25 ± 5 s, and the hands are dabbed dry with sterile tissues. Instruct the subjects to exercise caution by avoiding contact with the sink and fixtures, eliminating the chance of recontamination from the sink surfaces. Also instruct subjects to avoid rubbing hands and forearms during the rinsing process.

10.3 *Hand Contamination*—100 µL of the *E. coli* suspension containing between 8×10^7 and 1.0×10^8 CFU/mL is applied onto each palm of the subject. Subjects should be instructed to spread the suspension across the palms and the fingertips uniformly by rubbing the palms and fingertips against each other for 15 ± 1 s. Only the palmar surfaces are contaminated and the subjects should not spread the inoculum on the back of their hands. The hands are allowed to air-dry for 30 ± 5 s.

10.4 For washes and rinses, the water temperature is adjusted to 40 ± 2°C and the water flow rate is 3 L/min. To adjust the flow rate, place a 1500-mL glass beaker or flask under each water faucet and allow the water to flow into the beaker. Adjust the flow rate at each faucet accordingly so that the beaker or flask fills within 30 s.

10.5 *Product Application (Wash and Rinse Procedures)*—Conduct the test in accordance with the use directions for the test substances. If directions are not available, the wash and rinse procedure should be as follows:

10.5.1 *Solid Cleansing Formulations:*

10.5.1.1 Instruct subject to sparingly wet a contaminated hand by rapidly passing one hand once through tap water. This process should be performed in less than 1 s.

10.5.1.2 The investigator will wet the product by passing it under running tap water once.

10.5.1.3 The investigator, wearing a low bioburden glove, will rub the product on one of the subject's hands back and forth from the palm to the fingertips (not the back of the hand) five (5) times. One rub will be comprised of one back and forth motion. The subject's palm must remain flat and outstretched during the washing procedure.

10.5.1.4 The investigator will place 10 ± 1 mL water in the subject's palms and will lather the palmar surface of the subject's hand by rubbing back and forth across the entire palm and fingers for 15 ± 1 s.

10.5.1.5 Instruct the subject to rinse off the washed hand for 20 ± 2 s under a gentle stream of water having a flow rate of 3 L/min.

10.5.1.6 The procedure is repeated with the other palmar surface. One hand is washed with a reference formulation while the other hand is washed with the test formulation. The two formulations must be randomized between left and right hand, and the order of handwashing (left and right palmar surfaces) must be balanced between the two formulations.

10.5.1.7 After washing, the hands are not to be dried, but held upright until procedures in 10.6 are performed. Caution should be exercised to have the subject avoid contact with the sink and fixtures to eliminate recontamination from the sink surfaces.

10.5.2 *Liquid Cleansing Formulations:*

10.5.2.1 Instruct subject to sparingly wet a contaminated hand by rapidly passing one hand once through tap water. This process should be performed in less than 1 s.

10.5.2.2 Dispense 0.75 g of the test material into the palm of one hand of the subject.

10.5.2.3 The investigator, wearing a low bioburden wetted glove, will place 10 ± 1 mL water in the subject's palms and will lather the palmar surface of the subject's hand by rubbing back and forth across the entire palm and fingers for 15 ± 1 s. The subject's palm must remain flat and outstretched during the washing procedure.

10.5.2.4 Instruct the subject to rinse off the washed hand for 20 ± 2 s under a gentle stream of water having a flow rate of 3 L/min.

10.5.2.5 The procedure is repeated with the other palmar surface. One hand is washed with a reference formulation while the other hand is washed with the test formulation. The two formulations must be randomized between left and right hand, and the order of handwashing (left and right palmar surfaces) must be balanced between the two formulations.

10.5.2.6 After washing, the hands are not to be dried, but held upright until procedures in 10.6 are performed. Caution should be exercised to have the subject avoid contact with the sink and fixtures to eliminate recontamination from the sink surfaces.

10.5.3 *Other Cleansing Product Forms:*

10.5.3.1 Use standardized amount (for example, weight or volume) of test material in accordance with use directions. In case manufacturer's directions are not available, 0.75 g may be used. The two formulations must be randomized between left and right hand, and the order of handwashing (left and right palmar surfaces) must be balanced between the two formulations.

10.5.3.2 After washing with water, the hands are not to be dried, but held upright until procedures in 10.6 are performed. Caution should be exercised to have the subject avoid contact with the sink and fixtures to eliminate recontamination from the sink surfaces.

10.6 *Bacterial Recovery:*

10.6.1 Within 1 min after the specified washes, the subject's hands are placed in low bioburden plastic bags containing 40 mL of sampling solution. Situate the bags on the hands so that the fingers are approximately 1 to 1.5 cms from the bottom of the bag.

10.6.2 Secure the plastic bags containing the sampling solution above the wrist using wrist ties or tourniquets.

10.6.3 Instruct the subject to rest his/her arms across the top of the mechanical scrubber so that the palms are placed with slight pressure across the paddles (see Fig. 2). The hands must remain flat and in contact with the paddles, and the sampling solutions should not be allowed to pool in the corners of the plastic bags. Operate the instrument at 150 ± 10 r/min for 1 min ± 5 s to ensure uniform recovery of bacteria.

10.6.4 The entire sampling solution from each bag should be transferred to separate sterile sample containers.

10.6.5 The recovery process is repeated with a 35-mL aliquot of the sampling solution (10.6.3).

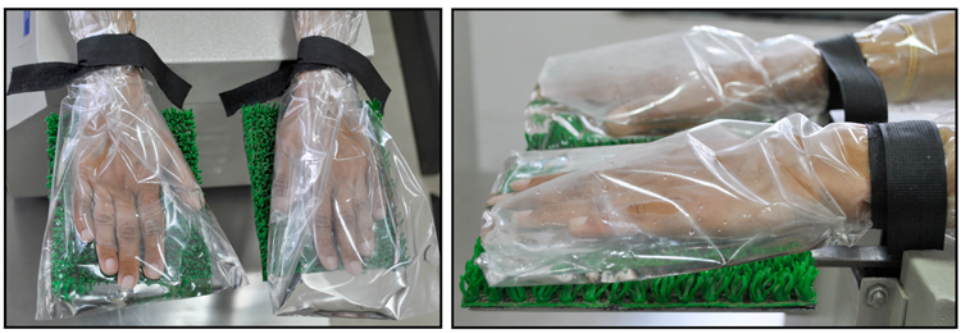

FIG. 2 Placement of Hands on Mechanical Scrubber Panels

10.6.6 The samples from the same hand are pooled in the same container. The plating of the recovered sampling solution should be completed within 30 min after sampling.

11. Hand Decontamination

11.1 Upon completion of testing, rinse the hands and forearms of subject with 70 % ethanol or isopropyl alcohol (7.8) for at least 30 s and allow to air dry.

11.2 Instruct subject to perform a 1-min wash with a 4 % chlorhexidine gluconate skin cleanser (7.9).

11.3 Prior to leaving, a follow up visit to the test site should be scheduled for each subject. All test subjects should be instructed to examine their hands daily until the final scheduled visit for any delayed adverse events, such as the presence of pimples, blisters, or raised, red itching bumps surrounded by erythema and/or edema that may be indicative of a skin infection. Subjects, who notice such lesions, should be instructed to call the test site immediately.

12. Enumeration of Bacteria in Sampling Solution

12.1 Enumerate the *E. coli* in the sampling solution using standard microbiological techniques, such as pour or spread plating or membrane filtration.

12.2 Prepare dilutions of the recovered sampling solution in dilution fluid.

12.3 Use soybean-casein digest agar with MUG and a suitable neutralizer, if necessary, as recovery medium.

12.4 Incubate prepared plates for 18 to 24 h at 35 ± 2°C. Standard plate counting procedures are used to count only the *E. coli* colonies.

13. Determination of Difference between Test and Reference Formulations

13.1 Convert plate counts (CFU/hand) to $\log_{10}$ for each hand.

13.2 Determine the mean number of *E. coli* bacteria on the palms for the two products and test for significance using an appropriate statistical method. The difference in activity between the two formulations can be determined using the following formula:

$$\log_{10}\text{Recovery of Test Material} - \log_{10}\text{Recovery of Reference Formulation} \quad (1)$$

14. Precision and Bias

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

15. Keywords

15.1 antimicrobial; antisepsis; contaminants; efficacy; *Escherichia coli*; handwash; infection control; mechanical scrubbers; palmar contamination