Designation: E2784 – 10 (Reapproved 2015)

Standard Test Method for Evaluation of the Effectiveness of Handwash Formulations Using the Paper Towel (Palmar) Method of Hand Contamination[1]

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

2.2 *Other Standards:*

AATCC Test Method 147 Antibacterial Assessment of Textile Materials: Parallel Streak Method[4]

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

[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.

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

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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 al[5] and Fuls et al[6] 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 ± 2°C—this temperature is required to ensure pigment production for *S. marcescens*); *S. aureus*, *E. coli*, *S. flexneri* (35 ± 2°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 ± 2°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 diluent[8] 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 MUG*[9]—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 lypholized, 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-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 g/L.

soybean-casein digest broth (7.9) using 1.0 mL of the stock culture per 100 mL of broth to yield the volume necessary to complete the study. Incubate for 24 ± 4 h at 25 ± 2°C. Broth should develop a red pigment.

8.6.2 *Escherichia coli (ATCC 11229), Shigella flexneri (ATCC 700930), Staphylococcus aureus (ATCC 6538)*—A homogeneous culture is used to inoculate the hands. The stock culture 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 soybean-casein digest broth (7.9) using 1.0 mL of the stock culture per 100 mL of broth to yield the volume necessary to complete the study. Incubate for 24 ± 4 h at 35 ± 2°C.

8.7 Following the steps in 8.6.1 or 8.6.2 will result in an inoculum of 5.0×10^8 to 1.0×10^9 CFU/mL. If a lower inoculum level is desired, dilute the inoculum from steps 8.6.1 or 8.6.2 with a suitable volume of physiological saline (7.13) not more than 8 hours prior to start of test.

8.8 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 h. The suspension may not vary more than ± 0.5 $\log_{10}$ CFU/mL over an 8-h period.

8.9 Not more than 8 h prior to start of test, transfer 30 mL of suspension (8.6) into 50 mL centrifuge tubes (6.15). Swirl or gently shake the suspension before the withdrawal of each aliquot.

8.10 Each subject will require two 30 mL centrifuge tubes of suspension per hand contamination (1 for the left hand, and 1 for the right hand).

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 the 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 non-antimicrobial 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 *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 (9.2). Inspect the subject's hands and forearms to confirm the absence of clinical signs of skin disorders (9.1). Admit the subject into the test if each of the above criteria is met. Instruct the subject to remove all jewelry from their hands and arms, push or roll long sleeves to above the elbow, 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 a 30 s cleansing wash (7.1). This procedure removes oil and dirt and familiarizes the subjects with the washing technique.

10.2.1 Instruct the subject to sparingly wet contaminated hands by rapidly passing them one time through the 40 ± 2°C tap water. This process should be performed in less than 1 s.

10.2.2 Dispense 5 mL of test material into cupped hands. Instruct the subject to spread the test material over hands and lower third of forearms.

NOTE 1—The 5 mL volume has been chosen for test purposes due to the requirement for washing hands and forearms. Amount of test product used may vary based on use directions.

10.2.3 Instruct the subject to wash all surfaces of the hands and the lower third of the forearm in a vigorous manner for 30 ± 5 s. 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.

10.2.4 Instruct the subject to rinse thoroughly from fingertips to elbows under 40 ± 2°C tap water for 30 ± 5 s. Instruct subject to avoid rubbing hands and forearms during the rinsing process. Caution should be exercised to have the subject avoid contact with the sink and fixtures to eliminate recontamination from the sink surfaces.

10.3 For this and all other washes and rinses, the water temperature is adjusted to 40 ± 2°C and the water flow rate to 4 L per minute. This may be accomplished by placing a 2000 mL glass beaker or flask under each spigot to be used for subjects' hand washing. Allow the water to flow into the beaker. Adjust the water flow at each spigot, so that the beaker fills within 30 s.

10.4 *Hand Contamination*—A liquid suspension of the test organism containing between 5.0×10^8 and 1.0×10^9 CFU/mL is used (8.6).

10.4.1 Open two sterile paper towel pouches (6.14) and place side by side on a stable surface. Placement should allow the subject to place each hand approximately shoulder width apart onto the towels. See Fig. 1.

10.4.2 Vortex the aliquot of test organism (8.9) prior to dispensing.

10.4.3 Dispense a 30 mL aliquot (8.9) of the test organism evenly onto each paper towel not more than 2 min prior to hand contamination. Allow test organism to disperse evenly over the towel surface.

10.4.4 Instruct the subject to center each hand directly above the individual paper towels, then press down firmly for 5 ± 1 s. Ensure that the entire palm, fingers and finger pads are in contact with the saturated towel.

10.4.5 The hands are then held motionless away from the body and allowed to air dry for 90 ± 5 s.

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

10.4.6 Discard the towels and aluminum foil.

10.5 *Contamination Schedule*—The subjects' hands are contaminated with the test organism prior to the baseline bacterial

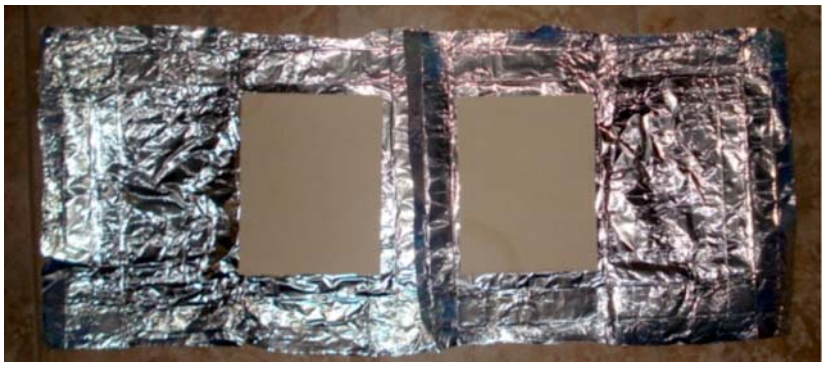

FIG. 1 Placement of Paper Towels

sample collection and prior to the wash with the test material. Table 2 illustrates a typical test.

10.6 *Baseline Recovery*—A baseline sample is taken after the first 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 shows the contamination and recovery schedule for the overall study.

11.2 *Liquid Formulations:*

11.2.1 Instruct the subject to sparingly wet contaminated hands by rapidly passing them one time through the 40 ± 2°C tap water. This process should be performed in less than 1 s.

11.2.2 Dispense 5 mL of test material into cupped hands within 10 s of completing the drying step in 10.4.5. Instruct the subject to spread the test material over hands and lower third of forearms.

NOTE 3—The 5 mL volume has been chosen for test purposes due to the requirement for washing hands and forearms. Amount of test product used may vary based on use directions.

11.2.3 Instruct the subject to wash all surfaces of the hands and the lower third of the forearm in a vigorous manner for 30 ± 5 s. 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.

TABLE 2 Hand Contaminations and Recovery Schedule

Name	Contamination	Type of Wash	Recovery
Cleansing Wash	No	Cleansing Wash	No
Baseline	Yes	None	Plate Recovered Sampling Solution with Neutralizer
Cleansing Wash	No	Cleansing Wash	No
Test Wash	Yes	Test Formulation	Plate Recovered Sampling Solution with Neutralizer
Decontamination	No	Reduce or remove all remaining test organisms	No

11.2.4 Instruct the subject to rinse thoroughly from fingertips to elbows under 40 ± 2°C tap water for 30 ± 5 s. Instruct subject to avoid rubbing hands and forearms during the rinsing process. Caution should be exercised to have the subject avoid contact with the sink and fixtures to eliminate recontamination from the sink surfaces.

11.3 *Solid Formulations:*

11.3.1 Instruct the subject to sparingly wet contaminated hands by rapidly passing them one time through the 40 ± 2°C tap water. This process should be performed in less than 1 s.

11.3.2 Wet the product.

11.3.3 Instruct the subject to rub the product between the hands and on the forearms for 15 ± 3 s. Have the subject place the product aside. This should be performed within 10 s of completing the drying step in 10.4.5.

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

11.3.5 Instruct the subject to rinse thoroughly from fingertips to elbows under 40 ± 2°C tap water for 30 ± 5 s. Instruct subject to avoid rubbing hands and forearms during the rinsing process. Caution should be exercised to have the subject avoid contact with the sink and fixtures to eliminate recontamination from the sink surfaces.

11.4 *Other Product Forms:*

11.4.1 Use standardized amount (for example, weight, volume) of test material in accordance with use directions.

11.5 After washing, the hands are not to be dried, but held upright until procedures in 12.1 are performed.

12. Bacterial Recovery

12.1 Within 1 min after specified washes (Section 11), place bags (6.12) used for sampling on the right and left hand of the subject. Add 75 mL of sampling solution (7.3) with neutralizer to each bag and secure bags above the wrist using wrist ties (6.13).

12.2 Within 1 min of donning bags, uniformly massage all surfaces of the hand for 1 min ± 5 s, paying particular attention to the fingers. Flip the hand after 30 s to ensure both the palm and back of the hand are thoroughly massaged.

12.3 Within 1 min of completing the massage, aseptically retrieve a 5 mL sample of the fluid in the bag by pulling the bag away from the wrist, inserting a pipet into the bag and withdrawing the fluid.

12.4 The first dilution is to be made in dilution fluid with appropriate neutralizer (7.4) within 10 s of removing the 5 mL from the bag. The plating of the recovered sampling solution is completed within 30 min after sampling.

13. Hand Decontamination

13.1 Upon completion of testing, rinse the hands and forearms of subject with 70 % ethanol or isopropyl alcohol (7.10) for at least 30 s and allow to air dry.

13.2 Instruct subject to perform a 4 min wash with a 4 % chlorhexidine gluconate skin cleanser (7.12). Have the subject use a scrub brush during the first minute of the wash.

13.3 Apply a topical, antibiotic ointment (7.11) to the subject's hands and forearms. Instruct subject to rub the ointment in.

13.4 Prior to leaving, a follow up visit to the test site should be scheduled for each subject. Each subject should be instructed to examine their hands daily until the follow up visit to ensure that any delayed adverse events, such as primary skin infections, are reported to the Study Investigator. All test subjects should be instructed to examine their hands daily until the final scheduled visit for 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.

14. Enumeration of Bacteria in Sampling Solution

14.1 *S. marcescens:*

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

14.1.2 Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.4).

14.1.3 Use soybean-casein digest agar (7.5) as recovery medium.

14.1.4 Incubate prepared plates 48 ± 4 h at 25 ± 2°C.

14.1.5 Standard plate counting procedures are used to count only the red pigmented *S. marcescens* colonies.

14.2 *E. coli:*

14.2.1 Enumerate the *E. coli* in the recovered sampling solution (12.3) using standard microbiological techniques, such as pour or spread plating.

14.2.2 Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.4).

14.2.3 Use soybean-casein digest agar with indicator MUG (7.8) as recovery medium.

14.2.4 Incubate prepared plates 18 to 24 h at 35 ± 2°C.

14.2.5 Standard plate counting procedures are used to count only the *E. coli* colonies.

14.3 *S. aureus:*

14.3.1 Enumerate the *S. aureus* in the recovered sampling solution (12.3) using standard microbiological techniques, such as pour or spread plating.

14.3.2 Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.4).

14.3.3 Use Mannitol salt agar (7.7) as recovery medium.

14.3.4 Incubate prepared plates 18 to 24 h at 35 ± 2°C.

14.3.5 Standard plate counting procedures are used to count only the *S. aureus* colonies.

14.4 *S. flexneri:*

14.4.1 Enumerate the *S. flexneri* in the recovered sampling solution (12.3) using standard microbiological techniques, such as pour or spread plating.

14.4.2 Prepare dilutions of the recovered sampling solution (12.3) in dilution fluid (7.4).

14.4.3 Use Hektoen enteric agar (7.6) as recovery medium.

14.4.4 Incubate prepared plates 18 to 24 h at 35 ± 2°C.

14.4.5 Standard plate counting procedures are used to count only the *S. flexneri* colonies.

15. Determination of Reduction

15.1 Convert plate counts (CFU/hand) to $\log_{10}$. Average left and right hands for each sampling interval.

15.2 Determine $\log_{10}$ reductions at each recovery interval/wash using the following formula: Log_{10} Reduction at Sampling Interval = Log_{10} Baseline Recovery – Log_{10} Sampling Interval

16. Comparison of Test Material

16.1 It may be desirable to compare the test material with other test formulations. If this is the case, an equivalent number of subjects 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.

17. Precision and Bias

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

18. Keywords

18.1 antimicrobial; contaminant; efficacy; handwash; healthcare; marker organism; palmar; paper towel; simulant; transfer

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