This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

Designation: E2946 – 21

Standard Test Method for Determining the Bacteria-Reducing Effectiveness of Food-Handler Handwash Formulations Using Hands of Adults[1]

This standard is issued under the fixed designation E2946; 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 activity of food-handler handwashes against transient bacterial flora on the hands.

1.2 Performance of this procedure requires the knowledge of regulations pertaining to the protection of human subjects (**1**)[2].

1.3 This test method should be performed by persons with training in microbiology, in facilities designed and equipped for work with potentially infectious agents at biosafety level 2 (**2**).

1.4 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* For more specific precautionary statements see 8.1.1.

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

E1054 Test Practices for Evaluation of Inactivators of Antimicrobial Agents

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

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *AATCC Standards:*[4]

Test Method 147 Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method

3. Terminology

3.1 *Definitions*—See Terminology E2756.

3.2 *food-handler handwash, n*—a water or non-water-aided formulation used for handwashing by personnel in food service settings intended to kill or remove transient bacteria, or both from the hands.

3.3 *non-medicated/non-antimicrobial soap, n*—a soap or detergent that is mild to the skin and does not contain any germicidal chemicals.

3.4 *test material, n*—a product or formulation that incorporates one or more antimicrobial ingredients.

4. Summary of Test Method

4.1 This test method is performed using adult subjects who have provided written informed consent, and whose hands have been determined to be free of 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 test procedures (Section 11).

4.2 Subjects contaminate hands with *E. coli* incorporated into a medium or heavy organic soil (beef broth or hamburger, respectively), which then is distributed over all surfaces of the hands and fingers to result in a minimum baseline recovery population of $\geq 10^7$ colony forming units (CFU)/hand.

4.3 Test material effectiveness is measured by comparing the number of challenge bacteria recovered from contaminated hands after a single application of the test material to the number recovered from contaminated hands not exposed to the test material.

[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] The boldface numbers in parentheses refer to a list of references at the end of this standard.

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

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5. Significance and Use

5.1 Hand hygiene is considered one of the most important measures for preventing the spread of infectious microorganisms and is critical for reducing the incidence of food-borne disease. Food-handling settings are unique in that moderate to heavy soil load present on hands often can influence the ability of a product to remove or kill microorganisms (**3**, **4**). Test methods are needed for assessing the efficacy of hand hygiene products under conditions representative of those encountered in a food-handling environment.

5.2 This test method is specifically designed to evaluate the effectiveness of food-handler products to kill and remove bacteria from experimentally-contaminated hands under conditions of moderate to heavy organic soil load. The inclusion of soils typical of food service setting makes this a methodology more appropriate than Test Method E1174, which was designed to evaluate healthcare personnel hand washes and does not include an option to include soil (**4**).

6. Apparatus

6.1 *Colony Counter*—Any of several types may be used; for example, Quebec colony counters and similar devices. Automated, computerized plater/counter systems may also be used.

6.2 *Gloves,* sterile, loose-fitting, unlined, powder-free gloves possessing no antimicrobial properties. Perform a zone of inhibition test, such as AATCC Test Method 147, to evaluate for antibacterial activity.

6.3 *Handwashing Sink,* sufficient in size to permit handwashing without the touching of hands to sink surface or other subjects.

6.3.1 *Water Faucet(s)*—Located above the sink at a height to permit hands to be held higher than the elbow during the washing procedure.

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

6.4 *Incubator,* capable of maintaining temperatures of 35 °C ± 2 °C.

6.5 *Miscellaneous Labware*—Continuously adjustable pipetters (1 mL and 0.2 mL capacity) and sterile pipette tips, sterile serological pipettes (5.0 mL capacity), sterile culture tubes, sterile disposable Petri dishes, sterile syringes, Erlenmeyer flasks, and beakers.

6.6 *Sampling Containers,* sterile or sterilizable containers having tight closures and sufficient capacity to hold 75 mL of sampling solution (7.7)

6.7 *Sterilizer,* any steam sterilizer capable of processing culture media and reagents.

6.8 *Timer (Stop-Clock),* type that can be read for minutes and seconds.

6.9 *Tourniquets,* child size or any style capable of securing gloves to the wrist.

6.10 *Vortex Mixer*—Any vortexing device that will ensure proper mixing of culture tubes.

7. Reagents and Materials[5]

7.1 *Cleansing Wash*—Any mild, proven non-antimicrobial liquid soap. It may be purchased commercially or as an example it may be prepared according to instructions for making 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

Add linseed oil to a solution of potassium hydroxide in 15 parts water and heat up to approximately 70 °C while 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 *Chlorhexidine Skin Cleanser*—Antiseptic skin cleanser containing 4% chlorhexidine gluconate (w/v) for hand decontamination.

7.3 *Culture Media:*

7.3.1 *Broth*—Soybean-casein digest broth (tryptic soy broth) is recommended.

7.3.2 *Agar Plating Media:*

7.3.2.1 MacConkey Agar is recommended. *E. coli* (ATCC #11229) will produce purple colonies on this agar.

7.4 *Beef Broth*—Any standard, sterile liquid beef broth, such as Swanson brand beef broth or other similar product, would be suitable, low sodium or other modified versions are not recommended.

7.5 *Hamburger*—Gamma-irradiated 90% lean ground beef to ensure that the ground beef contains no contaminating microorganisms prior to use in testing; ground beef can be purchased pre-irradiated from a standard meat supplier.

7.6 *Dilution Fluid*—Sterile Butterfield's buffered phosphate diluent (**5**) (or other suitable diluent) adjusted to pH 7.2 ± 0.1 and containing an effective inactivator of the antimicrobial chemistry of the test material, if necessary.

NOTE 1—Inactivator is required only if neutralization of the test material cannot be achieved upon dilution into the Sampling Solution (7.8).

7.7 *Ethanol Solution*—70 % ethanol in water (v/v) for hand decontamination.

7.8 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, 1.0 g isooctylphenoxypolyethoxyethanol,[6] and appropriately validated neutralizers, if necessary, in distilled

[5] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[6] Sold under several Trade Names including Triton™ X-100 (The Dow Chemical Company, Midland, MI), Igepal® CA-630 (Rhodia, Inc., Cranbury, NJ) and Protachem OP-9 (Protameen Chemicals, Inc., Totowa, NJ).

water. Adjust pH to 7.8 ± 0.1 with 0.1 N HCl or 0.1 N NaOH and bring volume to 1 L with distilled water. Sterilize in an autoclave and aseptically dispense 75-mL aliquants into sterile sampling containers (6.6)(**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 can be achieved by dilution alone.

7.9 *Test Material*—Use directions provided with the test material. If directions are not provided, use the directions provided in this method.

8. Test Bacteria Species

8.1 *Escherichia coli* ATCC 11229.

8.1.1 **Warning—**Application of microorganisms to the skin may involve a health risk. Determine the antibiotic sensitivity profile of the test bacteria prior to applying to the skin. After testing has been completed, decontaminate the subjects' hands and follow proper procedures to reduce infection risk (12.1 – 12.2). If an infection occurs, provide the antibiotic susceptibility profile to the attending clinician.

9. Preparation of Test Bacteria Suspension

9.1 *Method 1(for moderate soil matrix with beef broth):* :

9.1.1 Prepare a stock culture by transferring *E. coli* (ATCC 11229) from a cryogenic stock or lyophilized vial or pellet into approximately 5 mL of soybean-casein digest broth (7.3.1) and incubate for 24 h ± 2 h at 35 °C ± 2 °C. Inoculate a volume of soybean-casein digest broth with 1 mL of the stock culture per 1000 mL of broth to yield a volume of suspension sufficient to complete the study. The volume of the suspension should not exceed one half of the capacity of the Erlenmeyer flask. Incubate for 24 h ± 2 h at 35 °C ± 2 °C to yield a titer of approximately 1.0×10^8 CFU/mL.

NOTE 3—The frozen or lyophilized stock should be at least two, but no more than four, 24 h soybean-casein digest broth transfers from the original ATCC culture.

9.1.2 Transfer the culture to appropriately sized sterile centrifuge tubes or bottles and centrifuge to sediment the culture completely. Recommended conditions are 4750 rpm ± 50 rpm for 30 min. Decant the supernatant and resuspend the pellet in a volume of beef broth equal to the original culture volume (7.4) to yield a homogeneous challenge suspension containing approximately 1.0×10^8 CFU/mL.

9.1.3 Swirl or shake suspension prior to withdrawal of each aliquot used for testing. Assay the suspension for the number of bacteria at the beginning and at the end of the use period. Do not use a suspension for more than 8 h. The population should not vary by more than ± 0.5 $\log_{10}$ CFU/mL over the 8 h period.

9.2 *Method 2 (for heavy soil matrix with hamburger):*

9.2.1 Prepare a stock culture by transferring *E. coli* ATCC 11229 from a cryogenic stock or lyophilized vial or pellet into approximately 5 mL of soybean-casein digest broth (7.3.1) and incubate for 24 h ± 4 h at 35 °C ± 2 °C. Inoculate a volume of soybean-casein digest broth with 1 mL of the stock culture per 1000 mL of broth to yield a volume of suspension sufficient to complete the study. The volume of the suspension should not exceed one half of the capacity of the Erlenmeyer flask. Incubate for 24 h ± 4 h at 35 °C ± 2 °C to yield a titer of approximately 1.0×10^8 CFU/mL.

9.2.2 Swirl or shake suspension prior to withdrawal of each aliquot used for testing, and assay it for the number of bacteria present at the beginning and at the end of the use period. Do not use a suspension for more than 8 h. The population should not vary by more than ± 0.5 $\log_{10}$ CFU/mL over the 8-h period.

9.2.3 Prior to testing, weigh approximately 4 ounce (113 g) portions of gamma-irradiated ground beef (7.5). Assay a representative sample of ground beef for viable *E. coli* at the beginning of the use period, if the *E. coli* was found to be contaminated it is recommended that subjects that used that inoculum are replaced. Immediately prior to each hand contamination, dispense 5.0 ml of the challenge suspension (9.2.1) into a ground beef portion and, using gloved hands, knead the beef portion in a manner to ensure even distribution of the suspension fluid evenly throughout the portion.

10. Subjects

10.1 Recruit a sufficient number of healthy adult human subjects who have no clinical evidence of dermatoses, cuts, lesions, hangnails, or other skin disorders on the hands or forearms. A minimum of eight (8) subjects should be used for each test material. The total number of subjects used will depend on the number of test materials, the purpose of the study, and the regulatory requirements governing the study.

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10.2 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 adult human subjects for testing and to obtain informed and written consent from those selected for the study before starting the tests.

10.3 Instruct subjects to avoid contact with antimicrobial products 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. Bathing in biocide-treated pools, hot tubs, or spas should be avoided. Harsh chemicals such as acids, bases, and solvents should also 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.

11. Procedure

11.1 *Admission to Testing*—Instruct each subject to return to the laboratory for testing after they have refrained from using antimicrobials for at least seven (7) days. Question the subject to confirm adherence to the study requirements (10.3). Inspect the subject's hands and forearms to confirm the absence of clinical signs of skin disorders as described in 10.1. Admit the subject into the test if the above criteria are met. Instruct the