

# Standard Test Method for Determining the Bacteria-Reducing Effectiveness of Food-Handler Handwash Formulations Using Hands of Adults<sup>1</sup>

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  $(\varepsilon)$  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 and health practices and determine the applicability of regulatory limitations prior to use. For more specific precautionary statements see 8.1.1.

#### 2. Referenced Documents

2.1 ASTM Standards:<sup>3</sup>

E1054 Test Methods 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:<sup>4</sup>

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 for use in food service settings intended to kill or remove transient bacteria, or both from the hands.
- 3.3 *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.

## 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

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<sup>&</sup>lt;sup>2</sup> The boldface numbers in parentheses refer to a list of references at the end of this standard.

<sup>&</sup>lt;sup>3</sup> 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.

<sup>&</sup>lt;sup>4</sup> Available from American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709, http://www.aatcc.org.

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  $\pm$  2°C.
- 6.4 *Incubator*, capable of maintaining temperatures of 35  $\pm$  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<sup>5</sup>

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  $7.2 \pm 0.1$  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.

S Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, DC. For Suggestions on the testing of reagents not listed by the American Chemical Society, see Annual 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.

7.8 Sampling Solution—Dissolve 0.4 g  $KH_2PO_4$ , 10.1 g  $Na_2HPO_4$ , 1.0 g isooctylphenoxypolyethoxyethanol,<sup>6</sup> and appropriately validated neutralizers, if necessary, in distilled water. Adjust pH to 7.8  $\pm$  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

9.1.1 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  $24 \pm 2$  h at  $35 \pm 2$ °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

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

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  $\pm$  2 h at 35  $\pm$  2°C to yield a titer of approximately  $1.0\times10^8$  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  $4750 \pm 50$  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  $1.0 \times 10^8$  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  $\pm~0.5~\log_{10}$  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  $24 \pm 4$  h at  $35 \pm 2$ °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  $24 \pm 4$  h at  $35 \pm 2$ °C to yield a titer of approximately  $1.0 \times 10^8$  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  $\pm$  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.
- 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.

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



#### 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 subject to remove all jewelry from their hands and arms and to clip their fingernails to a uniform length (free edge ≤ 1mm).
- 11.2 Cleansing Wash—Instruct the subject to perform a 30-s cleansing wash (7.1). This procedure removes oil and dirt from the hands and forearms. For this and all other hand washes and rinses, maintain the water temperature at  $40 \pm 2^{\circ}\text{C}$  and the water flow rate at approximately 4 L per min. To adjust the flow rate, place a 2000-mL glass beaker or flask under each water faucet and allow the water to flow into the beaker. Adjust the water flow at each faucet accordingly, so that the beaker fills within 30 s.
- 11.2.1 Have subject thoroughly wet their hands and forearms under tap water.
- 11.2.2 Dispense 5 mL of the cleansing wash (7.1) into the subject's cupped hands and instruct subject to spread over hands and lower third of forearms.
- 11.2.3 Instruct subject to wash all surfaces of the hands and the lower third of the forearm in a vigorous manner for 30  $\pm$  5 s. If the lather becomes too dry, add a small amount of water to maintain lather.
- 11.2.4 Instruct subject to rinse thoroughly from elbows to fingertips under tap water for  $30 \pm 5$  s, and to exercise caution to avoid contact with the sink and fixtures, eliminating the chance of recontamination from the sink surfaces. Also instruct subject to avoid rubbing hands and forearms during the rinsing process.
- 11.2.5 Hand subject a clean, dry paper towel and instruct them to lightly pat their hands and forearms dry.
- 11.3 *Hand Contamination*—Use test bacteria prepared as directed (9.1 or 9.1.1).
- 11.3.1 With moderate (broth) soil matrix—Dispense a total of 4.5 ml of the beef broth suspension (9.1.2) into subject's cupped hands in three (3) aliquots of 1.5 ml. Each 1.5 ml aliquot should be distributed over the entire surface of the hands (front and back), not reaching above the wrists, for 20 s. Following distribution of the inoculum, the hands will be held motionless, away from the body, and allowed to air-dry for 30 s. This sequence will be repeated with the remaining two (2) aliquots, except that after the final aliquot application, the hands should air-dry for a total of 90 s.
- 11.3.2 With heavy (hamburger) soil matrix—Subjects will handle and knead the portion of ground beef containing *E. coli* (9.2.3) for approximately 2 min. Technicians will dispose of any remaining ground beef. Following distribution of the ground beef, the hands will be held motionless, away from the body, and allowed to air-dry for 90 s.

Note 4—Subjects should not touch their clothing, face, or other objects with their hands during the test period. This prevents contamination of the subject and the environment with the test bacteria.

- 11.4 Contamination, Product Application, and Recovery Schedule—Perform a total of 2 hand contaminations. The subject's hands are contaminated with the test bacteria prior to the baseline recovery (11.5) and prior to test material application (11.6). Table 1 illustrates a typical design. The test material is evaluated after a single contamination/application cycle. Sample the hands for baseline recovery (11.5) immediately after the first hand contamination and then conduct a cleansing wash (11.2) after baseline recovery and prior to the second hand contamination. Contaminate hands and apply test material (11.6). To determine the test bacteria remaining after test material application, recover bacteria from the hands as described in 11.7.
- 11.5 Baseline Recovery—Recover the test bacteria surviving on the hands after the initial hand contamination (11.3) following the procedures outlined in 11.7 and enumerate according to Section 13. This represents the Baseline Recovery, which is typically between 7.5 log<sub>10</sub> and 8.5 log<sub>10</sub> CFU/hand. For a valid test it cannot be less than 7.0 log<sub>10</sub> CFU/hand.
- 11.6 Wash and Rinse procedure—Conduct the test in accordance with the use directions for the test material. If test material directions are not available, use the appropriate test material application procedure described as follows. Hands should be positioned lower than the elbows throughout the wash/rinse procedure.
- 11.6.1 Dispense 5 ml of test material into cupped hands within 10 s of completing the contamination procedure described in 11.3. Spread over hands up to wrists.
- 11.6.2 Sparingly wet contaminated hands by rapidly passing them one time through the tap water. This process should be performed in less than 1 s.
- 11.6.3 Wash in a vigorous manner for  $30 \pm 5$  s all surfaces of the hands up to the wrists. Caution should be exercised to retain the test material in the hands. If the lather becomes too dry, a small amount of water may be added to maintain lather.
- 11.6.4 Rinse thoroughly from fingertips to above the wrists under 40°C  $\pm$  2°C tap water for 30  $\pm$  5 s. Caution should be exercised to avoid contact with the sink and fixtures to eliminate contamination from the sink surfaces and to avoid rubbing hands and forearms during the rinsing process.
- 11.6.5 Hands are not to be dried, but held lower than the elbows until procedures in 11.7 are performed.

TABLE 1 Hand Contamination, Product Application and Recovery Schedule

Name	Contamination	Type of Application	Recovery
Cleansing Wash	No	Cleansing Wash	No
Baseline	Yes	None	Plate Recovered Sampling Solution with Neutralizer
Cleansing Wash	No	Cleansing Wash	No
Test Application 1	Yes	Test Material	Plate Recovered Sampling Solution with Neutralizer

#### 11.7 Bacterial Recovery:

11.7.1 Within one minute after specified test material applications (Table 1), place sterile gloves (6.2) on the subject's hands. Add 75 mL of sampling solution (7.8) with neutralizer to each glove, and secure gloves above the wrist with a tourniquet.

Note 5-Sterile bags can be used in place of gloves if desired.

- 11.7.2 Within one minute of donning gloves, thoroughly and uniformly massage all surfaces of the subject's hands and fingers for 1 min  $\pm$  5 s.
- 11.7.3 Within one minute of completing the massage, aseptically retrieve a 5-mL sample of the sampling solution from the glove by pulling the glove away from the wrist, inserting a pipette into the finger region of the glove, and withdrawing the fluid.
- 11.7.4 Within 10 s, prepare the first dilution (13.1.2 13.2) in dilution fluid with an appropriate neutralizer, if required. Complete the plating of the recovered sampling solution within 30 min after sampling.

#### 12. Hand Decontamination

- 12.1 Upon completion of testing, have subject rinse their hands and forearms for 1 min with 70% ethanol (7.7) and air-dry.
- 12.2 Supervise subject performing a 4-min wash with a 4% chlorhexidine gluconate handwash (7.2). Have the subject use a scrub brush during the first minute of the wash.

#### 13. Enumeration of Bacteria

13.1 Enumerate the *E.coli* in the recovered sampling solution (11.7.3) using standard microbiological techniques, such as spread-plating, pour-plating, or spiral-plating.

- 13.2 Prepare dilutions of the recovered sampling solution (11.7.3) in dilution fluid (7.6). Use MacConkey agar (7.3.2) with suitable neutralizer, if necessary, as the recovery medium.
- 13.3 Incubate prepared plates  $24 \pm 2$  h, if insufficient growth is observed incubate for an additional  $24 \pm 2$  h at  $30 \pm 2$ °C. Standard counting procedures are used to count only the *E. coli* colonies.

#### 14. Determination of Reduction

- 14.1 Convert plate counts (CFU/hand) to  $\log_{10}$  and average the data from the left and right hands.
- 14.2 Determine  $\log_{10}$  reductions using the following formula:

 $Log_{10}$  Reduction Post-Treatment =  $Log_{10}$  Baseline Population -  $Log_{10}$  Population Post-Treatment (1)

## 15. Comparison of Test Material

15.1 When comparing different test materials, assign an equivalent number of test subjects to each test material on a random basis. Use equivalent test parameters for all of the test materials and evaluate all test materials concurrently. Product application procedures for commercial products may be different.

#### 16. Precision and Bias

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

#### 17. Keywords

17.1 antimicrobial; contaminant; efficacy; *E. coli*, moderate contamination, heavy contamination; food-handler handwash; handwash; hand antiseptic

#### REFERENCES

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