Designation: E2755 – 10

StandardTest Method for Determining the Bacteria-Eliminating Effectiveness of Hand Sanitizer Formulations Using Hands of Adults[1]

This standard is issued under the fixed designation E2755; 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 hand sanitizers (also known as hand rubs, hygienic hand rubs, or hand antiseptics) 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 (see 21 CFR Parts 50 and 56).

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 8.2.

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

E2276 Test Method for Determining the Bacteria-Eliminating Effectiveness of Hygienic Handwash and Handrub Agents Using the Fingerpads of Adults

2.2 *Other Standards:*

AATCC Test Method 147 2004 Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method[4]

21 CFR Parts 50 and 56 Protection of Human Subjects; Institutional Review Boards[5]

3. Terminology

3.1 *Definitions:*

3.1.1 *antiseptic, n*—a material for use on living tissue that either destroys microorganisms or suppresses their growth.

3.1.2 *hand sanitizer, n*—an antimicrobial gel, foam, liquid, spray, or wipe, used on hands that are not visibly soiled to reduce the number of transient microorganisms, which is applied by rubbing, and does not require a post-treatment water rinse. Such agents may also be referred to as hand rubs, hygienic hand rubs, or hand antiseptics.

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 microbicide, often achieved through physical (for example, filtration or dilution) or chemical means, or both.

3.1.5 *resident microorganisms, n*—microorganisms that survive and multiply on the skin, forming a stable population.

3.1.6 *test bacteria, n*—an applied inoculum of bacteria that has characteristics which allow it to be readily identified. Test bacteria are used to simulate a transient topical microbial contaminant. This may also be referred to as a test organism, marker organism, simulant, or contaminant.

3.1.7 *test material, n*—a product or formulation which incorporates an antimicrobial ingredient(s).

3.1.8 *transient microorganisms, n*—microorganisms that contaminate the skin but do not form a stable population.

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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[2] *CDC-NIH, Biosafety in Microbiological and Biomedical Laboratories*, 5th ed., U.S. Department of Health and Human Services, U.S. Government Printing Office, Washington, DC, 2007.

[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.

[5] 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.

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 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 Section 11).

4.2 Subjects' hands are artificially contaminated with 0.2 mL of a high-titer suspension of the test bacteria which is distributed over all surfaces of the hands and fingers to produce a minimum baseline recovery level of 10^8 cfu/hand. Because *Serratia marcescens* is relatively sensitive to drying, the high titer suspension is prepared by growing in broth with vigorous aeration, followed by a 10-fold concentration with centrifugation. *Staphylococcus aureus* is more resistant to drying and is therefore not concentrated after growth with vigorous aeration in broth.

4.3 Test material effectiveness is measured by comparing the number of test bacteria recovered from contaminated hands after use of the test material to the number recovered from contaminated hands not exposed to the test material. Activity of the test material is measured following a single application and may also be measured after multiple consecutive contamination/application cycles in a single day.

5. Significance and Use

5.1 Hand hygiene is considered one of the most important measures for preventing the spread of infectious microorganisms. Hand sanitizers reduce the microbial load on the hands without the use of soap and water, and are thus an important tool in the practice of good hand hygiene. Hand sanitizers are recommended for use on hands that are not visibly soiled. They are formulated to be applied full strength to dry hands, "rubbed in" until dry, and are not rinsed off.

5.2 This test method is specifically designed to evaluate the bacteria-eliminating activity of hand sanitizers from experimentally-contaminated hands. It is intended to be an alternative to Test Method E1174, which was designed primarily to evaluate antimicrobial handwashing agents that are lathered with the aid of water and then rinsed off. When using Test Method E1174 to evaluate hand sanitizers, inadequate drying of the hands after contamination dilutes the test product and can compromise activity, leading to an underestimation of effectiveness. By applying a higher titer test bacteria suspension in a smaller volume, soil load on the hands is minimized and hands are completely dry prior to application of the test material. These modifications result in a better approximation of the in-use conditions for hand sanitizers and thus provide a more reliable indication of their performance in the field.

5.3 This test method can be used to test any form of hand sanitizer, including gels, rubs, sprays, foams, and wipes according to label directions at typical "in-use" doses.

5.4 Susceptibility to biocides can vary among different species of bacteria and major differences have been noted between gram-negative and gram-positive organisms. This test method provides the option to use either a gram-negative bacterium (*Serratia marcescens*) or a gram-positive bacterium (*Staphylococcus aureus*) as the test organism. *S. marcescens* is used as a test organism in both Test Method E1174 and Test Method E2276. *S. aureus* is a highly relevant pathogen in healthcare, institutional, and community settings. Moreover, hands are an important vehicle in the transfer of *S. aureus* between people and the environment, and in the transfer between individuals.

5.5 This test method may be used as an alternative to Test Method E2276, which limits the test bacteria to the fingerpads and does not incorporate actual use conditions such as friction during hand decontamination.

5.6 The investigator should be aware of potential health risks associated with the use of these organisms and precautions similar to those referenced in Section 8 should be taken.

6. Apparatus

6.1 *Centrifuge*—For the sedimentation of *S. marcescens* for concentration.

6.2 *Centrifuge Tubes*—Sterile, for sedimentation of *S. marcescens* for concentration.

6.3 *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.

6.4 *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 the antibacterial activity.

6.5 *Handwashing Sink*—Sufficient in size to permit handwashing without the touching of hands to sink surface or other subjects.

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

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

6.6 *Incubator*—Capable of maintaining temperatures of 35 ± 2°C and 25 ± 2°C. The latter temperature ensures adequate pigment production for *S. marcescens* on solid media.

6.7 *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.

6.8 *Sampling Containers*—Sterile or sterilizable containers having tight closures and sufficient capacity to hold 75 mL sampling solution (see 7.7).

6.9 *Shaking Incubator*—Rotary platform shaking incubator capable of maintaining 35 ± 2°C and capable of shaking at 250 r/min. Alternatively, use an incubator capable of maintaining 35 ± 2°C and able to accommodate a portable rotary shaker, capable of shaking at 250 r/min.

6.10 *Sterilizer*—Any steam sterilizer capable of processing culture media and reagents.

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

6.12 *Tourniquets*—Children's size or any style capable of securing gloves to the wrist.

6.13 *Vortex Mixer*—Any vortex that will ensure proper mixing of culture tubes.

7. Reagents and Materials

7.1 *Antibiotic Ointment*—A topical, triple-antibiotic ointment for application to the hands after the final decontamination.

7.2 *Cleansing Wash*—A mild, proven non-antimicrobial liquid soap. May be purchased commercially or prepared according to the instructions provided in Test Method E1174.

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

7.4 *Culture Media:*

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

7.4.2 *Agar Plating Media:*

7.4.2.1 *S. aureus Plating Medium*—HardyCHROM (trademark), *Staph aureus*, available from Hardy Diagnostics, is recommended. Other indicator media for *S. aureus* or MRSA may be appropriate but should be validated prior to use.

NOTE 1—*S. aureus* forms smooth, deep pink to fuchsia-colored colonies. The growth of most other organisms, including *Staphylococcus epidermidis* are partially to completely inhibited.

7.4.2.2 *S. marcescens Plating Medium*—Soybean-casein digest agar (tryptic soy agar) is recommended.

7.5 *Dilution Fluid*—Sterile Butterfield's buffered phosphate diluent[6] (or other suitable diluent) adjusted to pH 7.2 ± 0.1 and containing an effective inactivator for the test material, if necessary.

NOTE 2—Inactivator is only required if neutralization of the test material cannot be achieved upon dilution into the sampling solution (see 7.7).

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

7.7 *Sampling Solution*—Dissolve 0.4 g KH_2PO_4, 10.1 g Na_2HPO_4, 1.0 g isooctylphenoxypolyethoxyethanol (for example, Triton X-100), and appropriately validated neutralizers, if necessary, in distilled 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 portions into sterile sampling containers (see 6.8).[7]

NOTE 3—A neutralizer validation should be conducted according to Test Methods prior to the study. Test Methods E1054 provides a list of neutralizers appropriate for commonly used antimicrobial agents. In some cases (for example, some alcohol-based hand sanitizers) neutralization is achieved by dilution alone.

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

8. Test Bacteria

8.1 *Serratia marcescens* (ATCC 14756). This strain forms a stable red pigmentation at 25°C.

8.2 *Staphylococcus aureus* (ATCC 6538 (methicillin-sensitive) or ATCC 33591 (methicillin-resistant)) is an alternative test bacteria. *S. aureus* is differentiated from resident microorganisms (including *Staphylococcus epidermidis*) with chromogenic indicator medium (see 7.4.2.1). (**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 the test has been completed, decontaminate the subject's hands and follow proper procedures to reduce infection risk (12.1-12.4). If an infection occurs, provide the antibiotic sensitivity profile to the attending clinician.)

9. Preparation of Test Bacteria Suspension

9.1 *Method 1 (for S. marcescens):*

9.1.1 A homogeneous bacterial suspension is used to inoculate the subjects' hands. Prepare a stock culture of *S. marcescens* (ATCC 14756) by inoculating approximately 5 mL of soybean-casein digest broth (see 7.4.1) from a cryogenic stock or lyophilized vial or pellet and incubate for 25 ± 1 h at 35 ± 2°C. Inoculate the appropriate volume of soybean-casein digest broth with 1 mL of the stock culture of *S. marcescens*/125 mL of broth to yield the volume necessary to complete the study (that is, 2 mL per hand contamination (see 11.3) per test subject). The volume of the broth culture should not exceed about one fourth of the capacity of the Erlenmeyer flask to ensure adequate aeration. Incubate for 25 ± 1 h at 35 ± 2°C with shaking at 250 r/min to yield a titer of approximately 1.0×10^{10} cfu/mL.

NOTE 4—The frozen or lyophilized stock should be at least two but no more than four 24-h soybean-casein digest broth (see 7.4.1) transfers from the original ATCC culture.

9.1.2 Transfer the culture to appropriate sized sterile centrifuge tubes or bottles and centrifuge at conditions appropriate to sediment the culture completely (recommended conditions are 7000 G for 10 min). Decant the supernatant and resuspend the pellet to one-tenth the original volume with soybean-casein digest broth (see 7.4.1) to yield a homogeneous suspension containing between 5.0×10^{10} and 1.0×10^{11} cfu/mL.

9.2 *Method 2 (for S. aureus):*

9.2.1 Use a homogeneous bacterial suspension to inoculate the subjects' hands. Prepare a stock culture of *S. aureus* (AATCC 6538 or ATCC 33591) by inoculating approximately 5 mL of soybean-casein digest broth (see 7.4.1) from a frozen stock or lyophilized vial and incubate for 25 ± 1 h at 35 ± 2°C (see Note 4). Inoculate the appropriate volume of soybean-casein digest broth with 1 mL of stock culture of *S. aureus*/125 mL of broth to yield the volume necessary to complete the study (that is, 0.2 mL per hand contamination (see 11.3) per test

[6] Horowitz, W., (Ed.), *Official Methods of Analysis of the AOAC International*, 18th Ed., Sec. 6.3.03 A.(f), Chapter 6, p. 10. AOAC International, Gaithersburg, MD, 2000.

[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.