



Designation: E1153 – 14

Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate, Hard, Nonporous Non-Food Contact Surfaces¹

This standard is issued under the fixed designation E1153; 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 used to evaluate the antimicrobial efficacy of sanitizers on precleaned, inanimate, hard, nonporous, non-food contact surfaces against *Staphylococcus aureus*, or *Klebsiella pneumoniae* or *Enterobacter aerogenes*, or a combination thereof. Appropriate modifications to the method may be required when testing organisms not specified herein. When utilizing test surfaces not described herein (see Test Method E2274) or when evaluating spray-based or towelette-based antimicrobial products, modifications may also be required.

1.2 This test method may also be used to evaluate the antimicrobial efficacy of one-step cleaner-sanitizer formulations recommended for use on lightly soiled, inanimate, nonporous, non-food contact surfaces.

1.3 It is the responsibility of the investigator to determine whether Good Laboratory Practices (GLP) are required and to follow them where appropriate (see section 40 CFR, 160 or as revised.)

1.4 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 may involve hazardous materials, chemicals and microorganisms and should be performed only by persons who have had formal microbiological training. *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.*

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. Referenced Documents

2.1 *ASTM Standards*:²

D1193 Specification for Reagent Water

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2274 Test Method for Evaluation of Laundry Sanitizers and Disinfectants

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Federal Standard*:

40 CFR, Part 160, Good Laboratory Practice Standards³

3. Terminology

3.1 Terms used in this test method are defined in Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard*:

3.2.1 *accuracy, n*—a measure of the degree of conformity of a value generated by a specific procedure to the assumed or accepted true value, and includes both precision and bias.

3.2.2 *ambient temperature, n*—temperature of the environment in which a test method is performed.

3.2.3 *antimicrobial, adj*—describes an agent that kills or inactivates microorganisms or suppresses their growth or reproduction.

3.2.4 *bias, n*—a systematic error that contributes to the difference between the mean of a large number of test results and an accepted reference value.

3.2.5 *cleaner-sanitizer, n*—a physical or chemical agent that removes soil from an object and reduces numbers of microorganisms on non-food contact surfaces.

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 the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.

3.2.6 *carrier, n*—a surrogate surface or matrix that facilitates the interaction of test microorganisms and treatment(s).

3.2.7 *efficacy, n*—the proven performance of a product established under defined conditions of testing.

3.2.8 *inoculum, n*—the viable microorganisms used to contaminate a sample, device or surface, often expressed as to number and type.

3.2.9 *neutralization, n*—the process for inactivating or quenching the activity of a microbiocide, often achieved through physical (for example, filtration or dilution) or chemical means.

3.2.10 *precision, n*—the closeness of agreement between independent test results obtained under prescribed conditions.

3.2.11 *reproducibility, n*—the precision of test results obtained in different laboratories performing the same test procedure under specifically defined conditions.

3.2.12 *sanitizer, n*—chemical or physical agent(s) used to reduce the number of microorganisms to a level judged to be appropriate for a defined purpose and/or claim.

4. Significance and Use

4.1 This test method shall be used to determine if a chemical intended for use as a non-food contact sanitizer or as a one-step cleaner-sanitizer provides percent reductions of the selected test organisms on treated carriers as compared to control.

5. Apparatus

5.1 *Balance*—A calibrated balance with a platform to accommodate a 100-mL volumetric flask. This balance should be sensitive to 0.01 g.

5.2 *Nonporous Test Surfaces*, pre-cleaned.

5.2.1 *Borosilicate Glass Squares*, 25 by 25 by 2 mm slides, or 18 mm by 36 mm slides, nonchipped. 3 in. by 1 in. (76 mm by 25 mm) nonchipped slides may be used for towelette applications

5.2.2 *Glazed Glass or Stainless Steel*, of appropriate type, approximately same size as in 5.2.1.

5.3 *Glass Culture Tubes*, recommended sizes: 18 to 20 by 150 mm and 25 by 150 mm without lip.

5.4 *Culture Tube Closures*, appropriate sized nontoxic closures.

5.5 *Pipets or Dispensing Syringes*, (or both), appropriately calibrated and sterile.

5.6 *Bacteriological Transfer Loop*, 4 mm inside diameter loop of platinum or platinum alloy wire or sterile, disposable plastic loops of same size.

5.7 *Flasks or Containers*:

5.7.1 Appropriate sizes with closures for preparation of culture medium and sterile deionized water.

5.7.2 *Volumetric*, 100 and 1000 mL, sterile.

5.8 *Petri dishes*, recommended sizes: 50 by 9 mm plastic, and 100 by 15 mm, glass and plastic; sterile.

5.9 *Jars*, ointment jars, (for example polypropylene) 2 oz (60 mL), recommended, with nontoxic lids, sterile.

5.10 *Graduated Cylinders*, recommended sizes; 100 and 500 mL.

5.11 *Flaming Apparatus*—A bunsen burner or other appropriate heat sterilizer.

5.12 *Mixer*—A “vortex” mixer is recommended.

5.13 *Timer*—A reliable stopwatch or laboratory timer capable of measuring elapsed time in seconds and minutes.

5.14 *pH Meter*—A reliable, standardized pH meter to determine pH of culture media.

5.15 *Desiccator*, recommended size: 200 mm inside diameter with approximately 125-mm chamber depth from inside plate to cover flange, glass.

5.16 *Incubator*, capable of maintaining temperature of 25 to 32°C or 35 to 39°C, or both.

5.17 *Sterilizer*, steam sterilizer and hot air oven ($\geq 180 \pm 2^\circ\text{C}$ for ≥ 2 h).

5.18 *Colony Counter*—Any one of several types may be used, for example Quebec.

5.19 *Membrane Filters*, Compatible with the test organism (for example, 0.45 μm pore size).

5.20 *Filter Assembly*, autoclavable or pre-sterilized.

5.21 *Forceps* (may be autoclave sterilized prior to use).

5.22 *Refrigerator*, capable of maintaining 2 to 8°C.

6. Reagents and Materials

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.⁴ Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 *Water for Dilution of Product Under Test*:

6.2.1 *Water*, sterile, deionized or distilled, equivalent to or better than Type 3, see Specification **D1193**.

6.2.2 *Association of Official Analytical Chemists (AOAC) Synthetic Hard Water*:^{5(c)}

6.2.2.1 *Solution 1*—Dissolve 31.74 g magnesium chloride (MgCl_2) (or equivalent of hydrates) and 73.99 g calcium chloride (CaCl_2) in boiled distilled or deionized water and dilute to 1 L. Sterilize by autoclaving.

⁴ *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 *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

⁵ “Official Methods of Analysis of the Association of Official Analytical Chemists,” Association of Official Analytical Chemists, Washington, DC, Chapter 6.

(a) Method 955.11 Section A. (a).

(b) Method 955.11 Section A. (c).

(c) Method 960.09 Section Sections D and E.

6.2.2.2 *Solution 2*—Dissolve 56.03 g sodium bicarbonate (NaHCO₃) in boiled distilled or deionized water and dilute to 1 L. Sterilize by membrane filtration.

6.2.2.3 Place the desired amount of Solution 1 in a sterile 1-L volumetric flask, or other appropriate volumetric vessel. Each 1 mL of Solution 1 will give a water equivalent to ca. 100 ppm of hardness calculated as calcium carbonate (CaCO₃) by the equation below. (For example, 4 mL of solution 1 would be added to the flask to target 400 ppm hardness in 1L of water.) Add approximately 600 mL or ¾ of the total water volume of sterile distilled or deionized (reagent grade) water free of substances that interfere with analytical methods; then add 4 mL of Solution 2 and dilute to exactly 1 L with sterile distilled or deionized water.

$$\begin{aligned} \text{Total hardness as ppm CaCO}_3 & \quad (1) \\ = & [2.495 \times \text{ppm Ca}] + [4.115 \times \text{ppm Mg}] \end{aligned}$$

6.2.3 The final pH of synthetic hard water should be from 7.6 to 8.0.

6.2.4 The synthetic water to be used for the testing should be analyzed chemically for hardness at the time of test. Analysis may be performed by the method described in footnote 5(c) or by commercially available kit. The water must be used within 24 h of preparation but may be refrigerated at 2 to 8°C prior to use. The solution must be analyzed for hardness on the day of use.

6.2.5 All water used for preparation of test solutions shall be sterile.

6.3 *Sanitizing Solutions*—Freshly prepared solutions of sanitizers (for example, used within 8 h of dilution) shall be used in all tests.

6.4 *Neutralizing Solutions*—Solutions appropriate to inactivate sanitizing solutions shall be used in accordance with Practices E1054.

6.5 *Culture Media*.⁵

6.5.1 *Nutrient Broth*.^{(5(a))}

6.5.2 *Nutrient Agar*.^{(5(b))}

6.5.3 *Tryptic Soy Broth*, per manufacturer's instructions

6.5.4 Other appropriate growth medium or subculture agar may be used where appropriate for the test organism (prepared per manufacturer's instructions or purchased commercially).

6.6 *Soil, Fetal Bovine Serum*, aseptically derived and maintained.

7. Preparation of Apparatus

7.1 *Constant Humidity Chamber (Desiccator)*:

7.1.1 At least one day prior to use, fill the lower portion of a large size desiccator with about 500 mL of glycerin solution having a refractive index of 1.4529 at 25°C (approximately 86.5 % glycerin in distilled water will provide this refractive index). This will provide a constant 40 to 41 % relative humidity at 35 to 39°C in which the inoculated nonporous square surfaces will be dried prior to treatment with the sanitizer. Replace the porcelain floor plate of the desiccator and store at 35 to 39°C to allow to come to equilibrium. Alternatively, a humidity controlled incubator set to 35 to 39°C

may be used to achieve drying conditions appropriate for maximum survival of the test organism.

7.2 *Test Squares*:

7.2.1 Test squares shall be dipped in acetone or 70 to 95 % ethyl or isopropyl alcohol, rinsed with distilled or deionized water, and air dried before sterilization.

7.2.2 Place test squares into a large, glass dish and sterilize in a hot air oven for ≥2 h at ≥180°C.

7.2.3 After sterilization, place each square into separate 50 by 9 mm or 100 by 15 mm sterile plastic Petri dishes using sterile technique.

8. Test Organisms

8.1 *Klebsiella pneumoniae* American Type Culture Collection (ATCC) 4352 or *Enterobacter aerogenes* American Type Culture Collection (ATCC) 13048 and *Staphylococcus aureus* ATCC 6538.

8.2 *Maintenance of Test Organisms*—Maintain stock cultures on nutrient agar. Incubate 2 days at 35 to 39°C for *K. pneumoniae* and *S. aureus* or 25 to 32°C for *E. aerogenes*, then refrigerate at approximately 2 to 8°C for up to one month (for example, up to 31 days). To prepare the test inocula, transfer each culture for at least 3 days (transfers) as described in 9.1. Stock slant cultures used for inoculation should not be more than five passages removed from the ATCC cultures (USP XXIII).⁶ Information on long term culture maintenance and storage is found in “Manual of Methods for General Bacteriology”⁷ and “ATCC Catalogue of Bacteria and Bacteriophages”.⁸

9. Preparation of Inocula

9.1 *K. pneumoniae* and *S. aureus* are grown in nutrient broth. *E. aerogenes* is grown in tryptic soy broth. From stock cultures, (no more than 1 month old), inoculate tubes containing 10 mL of appropriate broth, and incubate for 24 ± 2h at 35 to 39°C for *K. pneumoniae* and *S. aureus* or 25 to 32°C for *E. aerogenes*. Using a 4 mm inside diameter transfer loop, transfer a loopful of the culture into fresh broth. Make at least three consecutive daily transfers prior to use as an inoculum. The final transfer is incubated for 48 h to 54 h, and this culture is used for the test. Cultures may be appropriately adjusted (by dilution with growth medium or centrifuge-concentration) to ensure appropriate population control recovery. Refer to 13.3.2 for the population control recovery requirements.

9.2 *Inocula for Testing Sanitizers for Use on Pre-cleaned Surfaces*—Thoroughly mix 48 to 54 h culture of test organism on “vortex” mixer, then allow the culture to settle for ≥15 min. Remove the upper two thirds of this suspension by aspiration or decanting and use this as the inoculum for testing non-food surface sanitizers for use on precleaned surfaces.

9.3 *Inocula for Testing Formulations as One-Step Cleaner-sanitizers or Sanitizers for Use on Lightly Soiled Surfaces*—

⁶ Sterility Tests (71), United States Pharmacopeia (USP) XXII.

⁷ *Manual of Methods for General Bacteriology*, 1981, P. Gerhardt (ed. in chief) ASM Microbiology, Washington, DC.

⁸ Associated Concentrates, Inc., 32-60 61st St., Woodside, NY 11377.