Designation: E1153 – 03

# Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces[1]

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, nonporous, non-food contact surfaces.

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) is required and to follow them where appropriate (see section 40 CFR, 160) or as revised.

1.4 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.*

## 2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

2.2 *Federal Standard:*

40 CFR, Part 160, Good Laboratory Practice Standards[3]

## 3. Significance and Use

3.1 This test method shall be used to determine if a chemical has application as a non-food contact sanitizer or as a one-step cleaner/sanitizer.

## 4. Apparatus

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

4.2 *Nonporous Test Surfaces*, pre-cleaned.

4.2.1 *Borosilicate Glass Squares*, 25 by 25 by 2 mm slides, nonchipped.

4.2.2 *Glazed Glass or Stainless Steel*, of appropriate type, approximately same size as in 4.2.1.

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

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

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

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

4.7 *Flasks or Containers*:

4.7.1 Appropriate sizes with closures for preparation of culture medium and sterile distilled water.

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

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

4.9 *Jars*, ointment jars, 2 oz (60 mL) with nontoxic lids, sterile.

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

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

4.12 *Mixer*—A "vortex" mixer is recommended.

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

4.14 *pH Meter*—A reliable pH meter to determine pH of culture media.

[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.

Current edition approved April 10, 2003. Published July 2003. Originally approved 1987. Last previous edition approved in 1994 as E1153 – 94. DOI: 10.1520/E1153-03.

[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.

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

4.16 *Incubator*, capable of maintaining temperature of 37 ± 2°C.

4.17 *Sterilizer*, steam sterilizer and hot air oven (180 ± 2°C for 2 h).

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

4.19 *Membrane Filters*, of 0.22 µm pore size.

4.20 *Filter Assembly*, autoclavable.

4.21 *Forceps*.

## 5. Reagents and Materials

5.1 *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.

5.2 *Water for Dilution of Product Under Test*:

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

5.2.2 *Association of Official Analytical Chemists (AOAC) Synthetic Hard Water:*[5(c)]

5.2.2.1 *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 water and dilute to 1 L. Sterilize by autoclaving.

5.2.2.2 *Solution 2*—Dissolve 56.03 g sodium bicarbonate ($NaHCO_3$) in boiled distilled water and dilute to 1 L. Sterilize by membrane filtration.

5.2.2.3 Place the desired amount of Solution 1 in a sterile 1-L flask and add approximately 600 mL sterile distilled water; then add 4 mL of Solution 2 and dilute to exactly 1 L with sterile distilled water. Each millilitre of Solution 1 will give a water equivalent to 100 ppm of hardness calculated as calcium carbonate ($CaCO_3$) by the following equation:

$$\text{Total hardness as ppm } CaCO_3 = [2.495 \times \text{ppm Ca}] + [4.115 \times \text{ppm Mg}] \quad (1)$$

5.2.3 The pH of synthetic hard water should be from 7.6 to 8.2.

5.2.4 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 6(*c*) or by commercial available kit.

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

5.3 *Sanitizing Solutions*—Freshly prepared solutions of sanitizers shall be used in all tests.

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

5.5 *Culture Media:*[5]

5.5.1 *Nutrient Broth.*[(5(a))]

5.5.2 *Nutrient Agar.*[(5(b))]

5.6 *Soil*, Bovine Serum, aseptically derived and maintained.

## 6. Preparation of Apparatus

6.1 *Constant Humidity Chamber (Desiccator)*:

6.1.1 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 37 ± 2°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 37 ± 2°C to allow to come to equilibrium.

6.2 *Test Squares*:

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

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

6.2.3 After sterilization, place each square into separate 50 by 9 mm sterile plastic petri dishes using sterile technique.

## 7. Test Organisms

7.1 *Klebsiella (K.) pneumoniae* American Type Culture Collection (ATCC) 4352 and *Staphylococcus (S.) aureus* ATCC 6538.

7.2 *Maintenance of Test Organisms*—Maintain stock cultures of *K. pneumoniae* and *S. aureus* on nutrient agar. Incubate 2 days at 37 ± 2°C, then refrigerate at 5 to 7°C. Transfer culture every 3 days. Stock 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]

## 8. Preparation of Inocula

8.1 *K. pneumoniae and S. aureus—K. pneumoniae* and *S. aureus* are grown in nutrient broth. From stock cultures, (no more than 30 days old), inoculate tubes containing 10 mL of appropriate broth, and incubate for 24 h at 37 ± 2°C. Using a 4 mm inside diameter transfer loop, transfer a loopfull of the

[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: Disinfectants, 15th ed., 1990.

(*a*) Page 133, Section 955.11 A. (a).

(*b*) Page 133, Section 955.11 A. (c).

(*c*) Page 139–140, Section 960.09A.

[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.