Designation: ~~E1153 – 03 (Reapproved 2010)~~$^{\varepsilon 1}$ E1153 – 14

Standard Test Method for Efficacy of Sanitizers Recommended for ~~Inanimate~~ Inanimate, Hard, Nonporous 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.

~~ε[1] NOTE—A warning note was moved into the text editorially in May 2010.~~

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

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 Standards[3]

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.

---

[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.

Current edition approved ~~April 1, 2010~~April 1, 2014. Published ~~May 2010~~May 2014. Originally approved 1987. Last previous edition approved in ~~2003~~2010 as ~~E1153 – 03.~~E1153 – 03(2010)$^{\varepsilon 1}$. DOI: ~~10.1520/E1153-03R10.~~10.1520/E1153-14.

[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.

3.2.4 *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.

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 ~~has application~~ intended for use as a non-food contact sanitizer or as a one-step ~~cleaner/sanitizer.~~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, ~~nonchipped.~~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 ~~4.2.1~~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 ~~approximate~~same size.

5.7 *Flasks or Containers:*

5.7.1 Appropriate sizes with closures for preparation of culture medium and sterile ~~distilled~~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)~~ 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~~ 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 ~~37 ± 2°C.~~25 to 32°C or 35 to 39°C, or both.

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

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

5.19 *Membrane Filters,* ~~of 0.22 µm pore size.~~Compatible with the test organism (for example, 0.45 µm pore size).

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

5.21 ~~*Forceps.*~~ *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.

6.2.2.2 *Solution 2*—Dissolve 56.03 g sodium bicarbonate ($NaHCO_3$) 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 ($CaCO_3$~~flask and add~~) 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~~sterile distilled water;~~ 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 ~~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:~~ or deionized water.

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

6.2.3 The final pH of synthetic hard water should be from 7.6 to ~~8.2~~.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 ~~6~~(5(*c*) or by ~~commercial available kit.~~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 ~~37 ± 2°C~~35 to 39°C in which the inoculated nonporous square surfaces will

---

[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.~~6.

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

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

(*c*) ~~Page 139–140, Section 960.09A.~~Method 960.09 Section Sections D and E.