Designation: E 1153 – 03

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

This standard is issued under the fixed designation E 1153; 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:*

D 1193 Specification for Reagent Water[2]

E 1054 Practices For Evaluating Inactivators of Antimicrobial Agents Used in Disinfectant, Sanitizer, Antiseptic, or Preserved Products[3]

2.2 *Federal Standard:*

40 CFR, Part 160, Good Laboratory Practice Standards[4]

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.

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).

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[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] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 11.05.

[4] Available from the Superintendent of Documents, U.S. Government Printing Office, Washington, DC 20402.

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.[5] 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 D 1193.

5.2.2 *Association of Official Analytical Chemists (AOAC) Synthetic Hard Water:*[6(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 E 1054.

5.5 *Culture Media:*[6]

5.5.1 *Nutrient Broth.*[(6(a))]

5.5.2 *Nutrient Agar.*[(6(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).[7] Information on long term culture maintenance and storage is found in "Manual of Methods for General Bacteriology"[8] and "ATCC Catalogue of Bacteria and Bacteriophages".[9]

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 culture into fresh broth. Make three consecutive daily transfers prior to use as an inoculum. The final transfer is incubated for 48 h, and this culture is used for the test.

8.2 *Inocula for Testing Sanitizers for Use on Precleaned Surfaces*—Thoroughly mix 48 h culture of test organism on

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[5] *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.

[6] "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.

[7] Sterility Tests (71), United States Pharmacopeia (USP) XXII.

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

[9] Associated Concentrates, Inc., 32-60 61st St., Woodside, NY 11377.

"vortex" mixer, then allow the culture to settle for 15 min. Decant upper two thirds of this suspension and use this as the inoculum for testing sanitizers for use on precleaned surfaces.

8.3 *Inocula for Testing Formulations as One-Step Cleaner/Sanitizers or Sanitizers for Use on Lightly Soiled Surfaces*—Thoroughly mix 48 h culture of test organism on "vortex" mixer, then allow the culture to settle for 15 min. Decant upper two thirds of this suspension and add bovine serum (for example, 19 mL of a 48 h bacterial culture and 1 mL bovine serum). Use this suspension now containing bovine serum at 5 % concentration as the inoculum for testing one-step cleaner/sanitizers or sanitizers for use on lightly soiled surfaces.

9. Preparation of Test Solutions

9.1 Prepare the sanitizer in accordance with the manufacturer's recommended dilution. Dilutions for the test may be made in sterile distilled water or in AOAC formula synthetic hard water of any hardness desired (see 5.2).

9.2 For each organism to be tested prepare 100 mL aliquots of the test solution.

10. Preparation of Neutralizer Solutions

10.1 *Quarternary and Phenolic Solutions*:

10.1.1 *Phosphate Buffer Stock Solution (0.25 M)*—Dissolve 34.0 g of potassium phosphate, monobasic (KH_2PO_4) in 500 mL distilled water; adjust the pH to 7.2 with 1*N* NaOH and dilute to 1 L.

10.1.2 *Phosphate Buffer Dilution Water*—Add 1.25 mL of 0.25 *M* phosphate buffer stock solution to 1 L water and dispense in 99 mL portions. Autoclave for 20 min at 121°C.

10.1.3 *Neutralizer Stock*—Mix 40.0 g Azolectin,[9] 280 mL polysorbate 80, and 1.25 mL phosphate stock solution buffer (see 10.1.1). Adjust to pH 7.2 with 1*N* NaOH. Dilute to 1 L with distilled water. Dispense in suitable portions and sterilize for 20 min at 121°C.

10.1.4 *Neutralizer Solution*—Mix 62.5 mL of neutralizer stock (see 10.1.3), 6.25 mL of phosphate buffer stock solution (see 10.1.1), and 381.25 mL of distilled water. Dispense 20 mL portions into 25 by 150 mm culture tubes and sterilize for 20 min at 121°C.

10.2 *Halogen Sanitizers—Neuralizer Solutions*, Dissolve 0.31 g of sodium thiosulfate and 0.30 mL of Triton X-100 in 500 mL of distilled water. Dispense 20 mL portions into 25 by 150 mm culture tubes and sterilize for 20 min at 121°C.

10.3 *Other Sanitizing Agents*—Use appropriate neutralizers (see Practices E 1054).

11. Procedures

11.1 *Inoculation of Test Squares*:

11.1.1 Inoculate each sterile glass or other nonporous surface (see 6.2.3) squares with exactly 0.02 mL of 48 h culture. Spread the inoculum to within 3 mm of the edges of the square. Prepare appropriate number of test squares, depending upon the test and its parameters.

11.1.2 Number each plate used in the order in which the squares are inoculated. Place all plates containing the inoculated squares in the 37°C constant humidity desiccator. Set the lids of the plates slightly ajar and close the desiccator lid. Allow the squares to remain at this temperature and humidity for exactly 35 min.

NOTE 1—**Caution:** Be very careful to remove the desiccator lid only long enough to place the 50 by 9 mm plates on the porcelain floor plate and set their lids ajar.

11.2 *Inoculum Count*:

11.2.1 Plate the appropriate dilutions of *K. pneumoniae* and *S. aureus* using nutrient agar. Incubate the organisms for 48 h at 37 ± 2°C. Count the colonies to determine the number of organisms per mL of culture and present at the start of the test. Cultures used for further testing must be kept at 5 to 7°C for no more than 8 h.

11.2.2 Report inoculum count for the test organisms.

11.3 *Sanitizer or Cleaner/Sanitizer Treatment of Inoculated Test Squares*:

11.3.1 Transfer five squares to five sterile 2 oz (60 mL) ointment jars using sterile forceps. Be sure to resterilize the forceps between each transfer. (Dip in 70 to 95 % ethyl or isopropyl alcohol and burn off). Mark each jar with a number corresponding to that on the plate from which the square was taken.

11.3.2 At zero time on the timer, cover inoculated square No. 1 (the first one inoculated) with exactly 5 mL of the test solution using a sterile 5 mL pipette. At exactly 1 min, cover square No. 2 with 5 mL of the test solution. Treat square No. 3 in a like manner at 2 min, square No. 4 at 3 min, and square No. 5 at 4 min.

11.3.3 At exactly 5 min on the timer, add 20 mL of appropriate neutralizer solution into jar No. 1 and rotate the jar vigorously on an even plane for approximately 50 rotations to suspend the surviving organisms. At 6 min, add 20 mL of neutralizer into jar No. 2 and rotate as in No. 1. Continue addition of neutralizer to jars No. 3, No. 4 and No. 5 at 1 min intervals, and rotate each in turn.

11.3.4 Within 30 min after the addition of the neutralizer to the test sanitizer or cleaner/sanitizer, plate in duplicate 1.0 and 0.1 mL of the neutralizer solution from each of the five jars using standard pour plate techniques. Use nutrient agar for both organism types. Incubate for 48 h, *K. pneumoniae* and *S. aureus* at 37 ± 2°C. Count the number of colonies on the plates.

11.4 *Inoculation of Control Squares*—Allow the refrigerated cultures to come to ambient temperature. Prepare three glass squares for each organism type as in 11.1.1 and 11.1.2.

11.5 *Treatment of Inoculated Control Squares*:

11.5.1 Proceed as in 11.3.1.

11.5.2 Proceed as in 11.3.2, use 5 mL sterile distilled water in place of test solutions.

11.5.3 Exactly 5 min after treating control square No. 1 with distilled water, cover it with 20 mL of the appropriate neutralizer solution used for sanitizer testing. Rotate the jar vigorously on an even plane for approximately 50 rotations to suspend the surviving organisms in the neutralizer solution. In like manner, add 20 mL of the same neutralizer to control squares No. 2 and 3 exactly 5 min after treating them with the distilled water. Agitate the jars containing these squares, as was done for the jar containing control square No. 1.

11.5.4 Dilute the neutralizer solution from each of the three control jars in phosphate buffer dilution solution to a dilution that will provide countable plates.

11.5.5 Plate dilutions in duplicate using standard pour plate techniques and nutrient agar. Incubate the plates for 48 h at 37 ± 2°C. Count the number of colonies on the plates.

12. Calculation

12.1 *Number of Viable Organisms/Millilitres in the Neutralizer Solution*—Determine the number of viable organisms in the neutralizer solution from the test squares and the control squares. Add the total number of colony forming units on each of the duplicate countable plates from each test sample and divide by 2 to obtain the number of organisms surviving treatment per millilitre of neutralizer solution.

12.2 *Number of Organisms Surviving per Square*—Multiply the number of organisms surviving per millilitre of neutralizer/sanitizer solution by 25 to provide the number of organisms surviving per square.

12.3 *Geometric Mean of Number of Organisms Surviving on Control Squares*:

12.3.1 Determine the geometric mean of the number of organisms surviving on the three inoculated control squares by the following equation:

$$\text{Geometric Mean} = \text{Antilog}\,\frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_3}{3} \quad (2)$$

where:

X = number of organisms surviving per control square.

12.3.2 An average of at least 7.5×10^5 organisms must have survived on the inoculated control squares for the test to be valid.

12.4 *Geometric Mean of Number of Organisms Surviving on Test Squares*—Determine the geometric mean of the number of organisms surviving on the five test squares by the following equation:

$$\text{Geometric Mean} = \text{Antilog}\,\frac{\text{Log}_{10}Y_1 + \text{Log}_{10}Y_2 + \text{Log}_{10}Y_3 + \text{Log}_{10}Y_4 + \text{Log}_{10}Y_5}{5} \quad (3)$$

where:

Y = number of organisms surviving per test.

12.5 *Percent Reduction*—Use the following equation to calculate the percent reduction:

$$\%\ \text{reduction} = \frac{(a - b) \times 100}{a} \quad (4)$$

where:

a = geometric mean of the number of organisms surviving on the inoculated control squares (as determined in 12.3), and

b = geometric mean of the number of organisms surviving on the test squares (as determined in 12.4).

13. Interpretation of Results

13.1 A 99.9 % reduction in the numbers of both test organisms must be obtained when the percent reduction is calculated in accordance with 12.5.

14. Report

14.1 Report the percent reduction in numbers of both test organisms obtained.

14.2 Also report the following information:

14.2.1 Name of product(s) under test.

14.2.2 Chemical composition of product(s) under test.

14.2.3 Concentration(s) of active ingredient(s) tested.

14.2.4 Water employed to dilute product (if synthetic hard water employed report hardness levels).

14.2.5 Whether or not organic load (bovine serum in inoculum) was employed.

14.2.6 Organisms employed.

14.2.7 Neutralizer and neutralizer concentration employed.

14.2.8 Number of organisms surviving on each of the five test squares.

14.2.9 Number of organisms surviving on each of the three control squares.

14.2.10 Statement that the test was done in accordance with Test Method E 1153.

14.2.11 Initial number of organisms/millilitre in inoculum.

15. Precision and Bias

15.1 A collaborative study with glass surfaces involving ten laboratories was conducted with this procedure prior to incorporation of the humidity chamber for drying the inoculum on the test squares. This study showed the procedure to have poor intralaboratory as well as interlaboratory reproducibility of test results. The problem was traced to inconsistent drying of the inoculum on the test squares. Following incorporation of a constant humidity chamber for drying the inoculum on the test squares prior to exposure to the solutions under test, excellent intralaboratory reproducibility was obtained. However, currently there is insufficient interlaboratory data available to provide a sound basis for a statistical analysis of the interlaboratory test reproducibility, but such an analysis will be carried out as soon as sufficient data has been accumulated.

16. Keywords

16.1 efficacy; glass; glazed ceramic tile; *Klebsiella pneumoniae*; neutralizer; non-food contact surface; plastic; sanitizer; *Staphylococcus aureus*; steel