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Designation: ~~E1153 – 14ε1~~ E1153 – 22

Standard Test Method for Efficacy of Sanitizers Recommended for 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—Section 13.3.2 was editorially corrected in June 2020.~~

1. ~~Scope~~ 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*~~*Klebsiella 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

E1054 Practices for Evaluation of Inactivators of Antimicrobial Agents

E2274 Test Method for Evaluation of Laundry Sanitizers and Disinfectants

[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] 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.

***A Summary of Changes section appears at the end of this standard**

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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.

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.

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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~~100 mL volumetric flask. This balance should be sensitive to 0.01 g.

5.2 *Nonporous Test Surfaces,* pre-cleaned.

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

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~~18 mm to ~~20~~20 mm by 150 mm and ~~25~~25 mm 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~~100 mL and 1000 mL, sterile.

5.8 *Petri dishes,* recommended sizes: ~~50~~50 mm 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~~100 mL 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~~125 mm chamber depth from inside plate to cover flange, glass.

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

5.17 *Sterilizer,* steam sterilizer and hot air oven ~~(≥180~~(≥180 °C ± ~~2°C~~2 °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~~2 °C to ~~8°C.~~8 °C.

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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~~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 ($CaCO_3$) 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.

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

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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 ~~22~~ °C to ~~8°C~~8 °C prior to use. The solution must be analyzed for hardness on the day of use.

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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))]

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[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.
~~(*a*) Method 955.11 Section A. (a).~~
~~(*b*) Method 955.11 Section A. (c).~~
~~(*c*) Method 960.09 Section Sections D and E.~~

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~~25 °C (approximately 86.5 % glycerin in distilled water will provide this refractive index). This will provide a constant ~~40~~40 % to 41 % relative humidity at ~~35~~35 °C to ~~39°C~~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~~35 °C to ~~39°C~~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~~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.~~≥180 °C.

7.2.3 After sterilization, place each square into separate ~~50~~50 mm by 9 mm or ~~100~~100 mm 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*~~*Klebsiella 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~~35 °C to ~~39°C~~39 °C for *K. pneumoniae* and *S. aureus* or ~~25~~25 °C to ~~32°C~~32 °C for ~~*E.*~~*K. aerogenes*, then refrigerate at approximately ~~2~~2 °C to ~~8°C~~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.*~~*K. 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~~24 h ± ~~2h~~2 h at ~~35~~35 °C to ~~39°C~~39 °C for *K. pneumoniae* and *S. aureus* or ~~25~~25 °C to ~~32°C~~32 °C for ~~*E.*~~*K. 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.

[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.

9.2 *Inocula for Testing Sanitizers for Use on Pre-cleaned Surfaces*—Thoroughly mix ~~48~~48 h 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*—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 of decanting and add bovine serum (for example, 19 mL of a ~~48~~48 h to 54 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.

10. Preparation of Test Solutions

10.1 Prepare the sanitizer in accordance with the manufacturer's recommended dilution. Dilutions for the test may be made in sterile distilled/deionized water or in AOAC formula synthetic hard water of any hardness desired (see 6.2).

10.2 For each organism to be tested prepare 100 mL aliquots of the test solution, or other appropriate volumes needed to execute the assay.

11. Preparation of Neutralizer Solutions

11.1 A suitable neutralizer must be used in testing. Data should be developed to show adequate neutralization can be achieved by the selected neutralizer. Refer to Test Methods E1054 for the Evaluation of Inactivators of Antimicrobial Agents. The following provides examples of neutralizer solutions that may be considered:

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11.2 *Quarternary Ammonia and Phenolic Solutions:*

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

11.2.2 *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.~~121 °C.

ASTM E1153-22
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11.2.3 *Neutralizer Stock*—Mix 40.0 g Azolectin,[8] 280 mL polysorbate 80, and 1.25 mL phosphate stock solution buffer (see 11.2.1). Adjust to pH 7.2 with 1*N* NaOH. Dilute to 1 L with distilled/deionized water. Dispense in suitable portions and sterilize for 20 min at ~~121°C.~~121 °C.

11.2.4 *Neutralizer Solution*—Mix 62.5 mL of neutralizer stock (see 11.2.3), 6.25 mL of phosphate buffer stock solution (see 11.2.1), and 381.25 mL of distilled/deionized water. Dispense 20 mL portions into 25 by 150 mm culture tubes and sterilize for 20 min at ~~121°C.~~121 °C.

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

11.4 *Other Sanitizing Agents*—Use appropriate neutralizers (see Practices E1054).

11.5 Other neutralizers may be used where appropriate.

12. Procedures

12.1 *Inoculation of Test Squares:*

12.1.1 Inoculate each sterile glass or other nonporous surface (see 7.2.3) squares with ~~0.01~~0.01 mL to 0.03 mL of ~~48~~48 h to 54 h culture. Spread the inoculum to within approximately 3 mm of the edges of the nonporous square. Prepare appropriate number of test squares, depending upon the test parameters.

12.1.2 Number each plate used in the order in which the squares are inoculated, as necessary. Place all plates containing the inoculated squares in the ~~35~~35 °C to ~~39°C~~39 °C constant humidity desiccator or chamber. Allow the squares to remain at this temperature and at an appropriate humidity for exactly ~~20~~20 min. to 40 min. until visibly dry. (**Warning—**When using a desiccator, be very careful to remove the desiccator lid only long enough to place the plates on the porcelain floor plate, and set their lids ajar and replace the desiccator lid.)

12.2 *Inoculum Count:*

12.2.1 Plate the appropriate dilutions of ~~*E.*~~*K. aerogenes*,*K. pneumoniae* or *S. aureus*, or a combination thereof, inoculum using nutrient agar or tryptic soy agar with or without ~~5%~~5 % sheep's blood. (If alternative agar is used, recovery should be confirmed using population control titers.) Incubate the organisms for ~~48~~48 h ± 4 h at ~~35~~35 °C to ~~39°C~~39 °C for *K. pneumoniae* and *S. aureus*. or ~~25~~25 °C to ~~32°C~~32 °C for ~~*E.*~~*K. aerogenes*. Count the colonies to determine the number of organisms per mL of culture present at the start of the test. Cultures used for further testing may be kept at approximately ~~2~~2 °C to ~~8°C~~8 °C for no more than 8 h.

12.2.2 Report inoculum count for the test organisms.

12.3 *Sanitizer or Cleaner-Sanitizer Treatment of Inoculated Test Squares:*

12.3.1 Transfer five inoculated and dried squares to five sterile 2 oz (60 mL) ointment jars using sterile forceps. Be sure to resterilize the forceps between each transfer if forceps are re-used. (Dip in ~~70~~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.

12.3.2 At zero time on the timer, cover inoculated square No. 1 (the first one inoculated) with exactly 5 mL of the sanitizing 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.

12.3.3 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 or vortex mix the jar for a similar amount of time (for example, approximately ~~10-15~~10 s-15 s) 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.

NOTE 1—The timing and sequence of these treatment steps may be modified provided the actual exposure time is monitored and maintained for each test carrier.

12.3.4 Within 30 min after the addition of the neutralizer to the sanitizing test solution or cleaner-sanitizing test solution, plate in duplicate ~~1.0~~1.0 mL and 0.1 mL of the neutralizer solution from each of the five jars using standard spread plate or pour plate techniques. Alternatively, the aliquots may be appropriately passed through individual filter units and the filters plated onto the agar if neutralization is a concern. Use nutrient agar or tryptic soy agar with or without ~~5%~~5 % sheep's blood. (If alternative agar is used, recovery should be confirmed using population control titers.) Incubate for 48 h ±4 h, ~~K. pneumoniae and S. aureus~~at ~~35~~35 °C to ~~39°C~~39 °C for *K. pneumoniae* and *S. aureus* or ~~25~~25 °C to ~~32°C~~32 °C for ~~*E. aerogenes.*~~*K. aerogenes* Count the number of colonies on the plates.

12.4 *Inoculation of Control Squares*—Allow the refrigerated cultures to come to ambient temperature, if refrigerated. Prepare three glass squares (or other surface types used in testing) for each organism type as in 12.1.1 and 12.1.2.

12.5 *Treatment of Inoculated Control Squares:*

12.5.1 Proceed as in 12.3.1.

12.5.2 Proceed as in 12.3.2, use 5 mL of sterile diluent (for example, distilled/deionized water or 0.85-0.9% saline) in place of test solutions.

12.5.3 Exactly 5 min after treating control square No. 1 with diluent, cover with 20 mL of the appropriate neutralizer solution used. Rotate the jar vigorously on an even plane for approximately 50 rotations or vortex mix the jar for a similar amount of time (for example, approximately 10-15 s) to suspend the surviving organisms in the neutralizer solution. In like manner, add 20 mL of the