Designation: E2966 – 14

Standard Test Method for Quantitative Assessment of Sanitizing Solutions for Carpet[1]

This standard is issued under the fixed designation E2966; 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 designed to evaluate quantitatively the antibacterial and antifungal activity of solutions for sanitizing carpets.

1.2 Efficacy is reported as the log reduction in viable bacteria and fungi.

1.3 The bacteria used in the test are *Staphylococcus aureus*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*. The mold used is *Aspergillus brasiliensis*.

1.4 Knowledge of microbiological techniques is required for this test method.

1.5 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *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]

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2471 Test Method for Using Seeded-Agar for the Screening Assessment of Antimicrobial Activity In Carpets

3. Terminology

3.1 *Definitions:*

3.1.1 *carpet cleaner/sanitizer, n*—solution that cleans embedded soil from the carpet fiber and reduces biocontaminant levels on carpet when applied at the recommended dilution and contact time specified on the product label.

3.1.2 *carpet sanitizer, n*—chemical solution that reduces biocontaminant levels on carpet when applied at the recommended dilution and contact time specified on the product label.

3.1.3 *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.

3.1.3.1 *Discussion*—The US EPA regulates sanitizers used on porous and non-porous surfaces. EPA 810 Guidelines provide a description of each category, the required test method, test conditions, and performance criteria. EPA 810.2400 (f) describes requirements for testing carpet sanitizers.[3]

3.1.3.2 *Discussion*—In the context of this method effective sanitization reduces the number of microorganisms to levels considered safe as determined by public health codes or regulations.

3.1.4 *vacuum extraction unit, n*—machine for deep cleaning carpet that delivers a spray of cleaning solution, provides brush agitation of the pile, and recovers soil and cleaning solution under vacuum.

4. Summary of Test Method

4.1 In this test method, the efficacy of solutions intended to have a sanitizing effect on carpet are quantitatively evaluated. Carpet sample coupons are cut from a larger field of carpet and re-embedded within the field. The carpet coupons are inoculated with microorganisms followed by a drying period. After the inoculum-drying period, the sanitizing solution is applied to the carpet coupons followed by scrubbing. Additional carpet coupons are spray treated and scrubbed with a inert solution. After the chemical contact period, carpet coupons are aseptically removed and placed into neutralizing broth. Each neutralizing broth with carpet coupon is placed into a ultrasonic bath for 1 min followed by 1 min of wrist action shaking. Serial dilutions are performed on each sample followed by plating (pour or spread plates or other standard for enumerating viable cells). Log reduction of the viable cell counts recovered from "scrubbed-controls" versus viable cell counts recovered from the sanitizer-treated carpets are recorded.

[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.

[3] http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series810.htm

5. Significance and Use

5.1 Carpet, when exposed to the environment or foot traffic, accumulates soil and biocontaminants during its in-service life. While routine vacuuming may effectively remove dry particulate soils, it has a limited effect on removing or killing accumulated and embedded biocontaminants. In this test method, steps are described to assess test substances for the ability to sanitize carpet.

5.2 This test method compares an inert control solution to a sanitizing test solution for the ability to reduce viable bacteria and fungi inoculated onto carpet samples.

5.3 This test method provides for efficient recovery of surviving bacteria from inoculated carpets.

6. Apparatus

6.1 For broadloom-type carpets (typically a 3.7-m roll carpet), a 2 cm thick plywood cut (40 by 40 cm) with the same dimension tempered hardboard attached is used to mount the carpet. Brass brads are used to secure the carpet sample to the tempered hardboard.

6.2 For carpet tile with a dimensionally stable backing, no mounting board is required.

6.3 Cutting the carpet into samples can be accomplished with a traditional carpet knife or the use of mechanical cutting dies and a hydraulic press. One mechanical cutting die, 20 cm by 30 cm, and another mechanical cutting die, 5 cm by 5 cm, are used.

6.4 One-sided adhesive tabs are used to temporarily secure the precut 5-cm by 5-cm carpet carriers "in plane" with the remaining carpet during the scrubbing procedure. Alternately, the 90 corners of the 20 cm by 30 cm section may be nailed or tacked to the mounting board.

6.5 *Spray Device*—A spray unit is used to atomize the carpet sanitizer.

NOTE 1—An atomizer may also be used. Aerosol formulated spray products may be directly sprayed onto the carpet.

6.6 Scrub Brushes, surgical hand brush.

6.7 Extraction Bottles, wide-mouth round 500-mL polypropylene bottles with screw caps.

NOTE 2—For the procedure, each bottle will contain 100 mL of sterile neutralizer broth.

6.8 Ultrasonic Bath.

6.9 Wrist-action-shaker.

7. Reagents and Media

7.1 *Sanitizer Solutions:*

7.1.1 Test a single lot of the candidate carpet sanitizer. Consult the appropriate regulatory 101 guidelines for lot replication requirements for registration purposes (for example, US EPA 810 102 Guidelines).

7.1.2 If the product is to be used as a "One-step cleaner-sanitizer," a 5% soil load (for example, animal sera) may be added to the inoculum.

7.1.3 Recommended application rates (volume per unit area, that is, ml/m^2) are extrapolated and reported from the proposed carpet application rate to the sample size used for this test method.

7.2 *Carpet Specifications:*

7.2.1 Two carpet types should be tested. For example, carpet with nylon face fiber or polypropylene face fiber may be used for this test. If the candidate product is to be used on wool carpet, a wool carpet sample shall be included in the test.

7.2.2 The test report should indicate: face fiber composition, weight of the pile fiber (kg/m), pile density, and pile height.

7.3 *Media:*

7.3.1 Phosphate-buffered saline.

7.3.2 Nutrient agar.

7.3.3 Nutrient broth.

7.3.4 Potato dextrose agar or Saubaroud's agar (Emmons Modified).

7.3.5 Appropriate neutralization media / technique must be selected that allows for immediate neutralization at the completion of the contact time. For example double strength neutralizer broth (Letheen broth + 0.7-g lecithin and 5-g polysorbate 80 per litre) may be used for quaternary actives. Sodium thioglycolate 0.1 %, and 0.01 % iso-octyl-phenoxy-poly ethoxyethanol, are suggested for heavy metal and halogen-based actives. Neutralizer efficacy can be confirmed using Test Method E1054. EPA 810.2000 also outlines neutralization requirements.

7.3.6 Neutralizing agar (Letheen agar) or Dey-Engley neutralizing agar.

7.3.7 Sterile deionized water.

8. Microorganisms

8.1 *Staphylococcus aureus* ATCC 6538.

8.2 *Enterobacter aerogenes* ATCC 13048.

8.3 *Pseudomonas aeruginosa* ATCC 15442.

8.4 *Aspergillus brasiliensis* ATCC 9642 or ATCC 16404.

8.5 Maintain bacterial stocks on nutrient agar slants or as frozen stocks. Bacterial stocks should be purchased from the supplier every 18 months. Stock cultures should be transferred monthly. Transfers from lyophilized stocks are limited to six before replacement is required.

8.6 Maintain mold on potato dextrose agar or as suspensions of conidia. Mold stocks should be purchased from the supplier every 18 months. Stock cultures should be transferred monthly. Transfers from lyophilized stocks are limited to six before replacement is required.

9. Inoculum Preparation

9.1 *Bacteria:*

9.1.1 Transfer one 4 mm loop scraping of *Staphylococcus*, *Enterobacter* and *Pseudomonas* bacteria from stock slants to separate 9.0 ml tubes of sterile nutrient broth. Additional test organisms may require different media or growth conditions.

9.1.2 Grow broth cultures overnight (18-24 h) at 37°±2°C. Incubate *Enterobacter aerogenes* at 25-30°±2°C.