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.

Current edition approved May 15, 2014. Published June 2014. DOI: 10.1520/E2966–14

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

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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.

9.1.3 Adjust cell density to 1-5 × 10^9 CFU/mL in sterile phosphate-buffered saline. This may be based on hemacytometer, McFarland standards, spectrophotometer or historical culture counts, or a combination thereof. Note that concentration by centrifugation may be required to achieve the above titer.

9.1.4 Add 5 % horse or fetal bovine serum to mimic soil load (if one-step "cleaner and sanitizer" claim is to be made)

9.2 *Mold:*

9.2.1 Grow *Aspergillus brasiliensis* for 7-14 d at 30±2°C on potato dextrose or Sabaroud dextrose agar (modified) until mature conidia are present. Multiple plates of mature *Aspergillus* growth may be required along with "pooled conidia suspensions from these plates" to achieve the specified spore densities.

9.2.2 Harvest mature conidia using phosphate-buffered saline and gently scraping the surface with a bent glass rod.

9.2.3 Filter hyphal fragments through sterile funnels fitted with sterile glass wool or sterile gauze.

9.2.4 Standardize condia solution in phosphate buffered saline to 1-5 × 10^9 CFU/ml using a hemocytometer.

10. Procedure

10.1 *Carpet Preparation:*

10.1.1 Use a utility knife or mechanical die to cut the carpet into 20 cm by 30 cm samples. Two of these carpet samples should be prepared for each test substance and carpet type tested, one carpet piece for evaluating the test substance lot and one 20 cm by 30 cm carpet piece for the scrubbed and unscrubbed control solution.

10.1.2 Use a utility knife or mechanical die to cut 5 cm by 5 cm square carriers (two rows with three carrier squares per row) within the 20 cm by 30 cm rectangular piece of carpet. Space the cuts leaving approximately 10 cm between the centers of each carrier square. The test substance is evaluated on 6 carriers from a single 20 cm by 30 cm carpet piece. From a separate 20 by 30 cm carpet section, three 5 cm by 5 cm carriers are used as scrubbed controls and three 5 cm by 5 cm carriers are used as un-scrubbed controls for the inert solution.

10.1.3 Wrap the carpet in aluminum foil, autoclave for 15 min, and then air dry. Make sure the foil seam is at the top of the carpet for access to the samples.

10.1.4 After autoclaving, apply the adhesive tabs to the backside of the carpet to secure the smaller square carpet carriers within the larger rectangular carpet. Use a marking pen to mark the center of each small carpet carrier (carpet pile side).

10.1.5 Only carpets with no antimicrobial activity should be used. Test Method E2471 (seeded agar overlay test) may be used to document no inherent inhibitory activity.

10.2 *Inoculation of Carpet:*

10.2.1 Place 0.1 mL of the standardized bacterial innoculum onto the center (previously marked) of each 5 cm by 5 cm carpet square.

10.2.2 Allow inoculated carpet to dry for 1 h ± 2 min in a 35-37 ±2°C incubator with foil cover loosely in place.

10.2.3 Inoculate the control solution carpet sample in the same manner as described in 10.2.1.

10.2.4 Determine "0-hr" cell density by performing serial dilution and plating of the inoculum.

10.3 *Application of Sanitizer:*

10.3.1 Calculate the intended application rate of the carpet sanitizer per square meter and extrapolate the amount of material to be applied to a 600 cm^2 test piece of carpet.

10.3.2 Use the spray device unit to apply the sanitizer uniformly to the inoculated carpet. Alternately, glass chromatography spayers may be used to apply the product in a manner consistent with intended application and dosing rate.

10.3.3 Dip the surgical hand brush into fresh sanitizer and scrub the first inoculated square of carpet with a clockwise motion for 30 s making 30 clockwise and 30 counter-clockwise passes with moderate pressure (slight bend of brush bristles). Repeat this process until all six inoculated samples have been scrubbed. Report the actual total sanitizer volume applied to the carpet by calculating the liquid holding capacity of the scrub brush. This can be accomplished by dipping and tapping the brush (five replicates) into a weigh boat and calculating based on mass of the liquid.

10.3.4 Allow the carpet with applied sanitizer to remain at room temperature for 60 min.

10.4 *Control Carpet:*

10.4.1 The following steps can be performed during the 60-min contact period for the sanitizer-treated carpet.

10.4.2 Apply the inert control solution (sterile phosphate-buffered saline) to three of the six inoculated squares on the control carpet. Use half the volume calculated for the treated samples. Scrub three of the six inoculated control carrier samples as described in 10.3.3 but with brush dipped into the inert control solution. Do not scrub three of the carriers designated as "unscrubbed population controls".

10.4.3 Allow the scrubbed and unscrubbed control carriers to stand at room temperature for 60 ±1 min.

10.5 *Recovery of Viable Cells from Carpet:*

10.5.1 After the 60±1 min contact period, use sterile forceps or hemostats or both to lift the small carpet carrier samples from the larger carpet field.

10.5.2 Transfer each carpet carrier sample to separate extraction bottles containing 100 ml of neutralizing broth.

10.5.3 Place the bottles with neutralizing solution and carpet into a sonic bath for 1 min ± 10 s. The fluid level in the sonic bath should equal the fluid level in the recovery bottle when immersed.

10.5.4 After sonication, pat bottles dry with paper towels and mount them onto a wrist-action shaker. Shake the bottles at maximum setting for 1 min ± 10 seconds. Alternately, manually shake the bottles for the same time period.

10.5.5 Perform serial dilutions from each neutralizing solution bottle. Plate each serial dilution in duplicate (pour plates or spread plates for bacteria, spread plates for mold) and incubate as described in sections 9.1.2 or 9.2.1.

11. Controls

11.1 *Sterility controls*—Incubate alongside the test as least one tube or plate of each type of broth or agar media used in the study. Where soil load was used, add 1 ml of soil to 20 ml

of growth media and incubate alongside the test. Where a neutralization solution was used add 1 mL it to 20 mL of growth media and incubate alongside the test. These sterility controls should not demonstrate growth.

11.2 *Neutralization Control*—To confirm neutralization of the test substance, conduct the test using sterile carpet carriers. Use sufficient carriers to evaluate each lot of test substance and each test organism. After the contact time has elapsed, transfer the carriers to the neutralizer/growth media as in the test. Inoculate the neutralizer carrier tubes with <100 CFU/ml media. To ensure the target level of bacteria/media volume is achieved, it may be necessary to test several dilutions of a test organism. Mix the tubes, plate 1 mL aliquots in duplicate from each tube, and incubate alongside the test. Plate 1 mL of each inocula level to confirm the inoculation density of each organism or dilution of organism evaluated, or both. Neutralization has been achieved where the neutralized test substance plate counts fall within 0.5–1 log of the inoculum density.

12. Calculation

12.1 Use the following formula to determine the log reduction of the microbial population from the sanitized carpet when compared to the set of scrubbed control carpet samples.

12.1.1 *Log Reduction Formula:*

12.1.1.1 Determine log ($x * 10^a$) of control samples.

12.1.1.2 Determine log ($x * 10^a$) of treated samples

12.1.1.3 Determine geometric mean of control samples:

(1) Log values of control samples: $b_1, b_2, b_3, \ldots b_n$.

(2) Mean = $(b_1 + b_2 + b_3 \ldots b_n)/n$.

12.1.1.4 Determine geometric mean of treated samples:

(1) Log values of treated samples: $c_1, c_2, c_3, \ldots c_n$.

(2) Mean = $(c_1 + c_2 + c_3 + \ldots c_n)/n$.

$$\text{Log reduction} = \text{mean of the control samples} - \text{mean of the treated samples} \quad (1)$$

where:

x = Value of samples,
a = Exponent value,
b = Log value of control samples,
c = Log value of treated samples, and
n = Number of log values in set.

13. Report

13.1 Report the intended application rate of the carpet sanitizer (mL/m^2).

13.2 Report the volume (sprayed plus brush volume) and application method of sanitizer applied.

13.3 Report the presence or absence of organic load in the inoculum.

13.4 Report the log reduction of the sanitized versus scrubbed control carpet.

14. Precision and Bias

14.1 *Precision*—The repeatability standard deviation from a single operator has been determined to be 0.14 (0.0943 for 95 % confidence limit) for scrubbed control carpet samples, 0.22 (0.178 for 95 % confidence limit) for unscrubbed control carpet samples, and 0.23 (0.152 for 95 % confidence limit) for the sanitizer treated carpet samples. An interlaboratory study of this test method is being planned and a complete precision statement is expected to be available on or before Dec. 31, 2015.

14.2 *Bias*—No information can be presented on the bias of the procedure in Test Method E2966 for measuring the bacterial log reduction of the sanitized versus control carpet because no material having an accepted reference value is available.

15. Keywords

15.1 biological decontamination of carpet; carpet; cleaning; sanitizers

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