Designation: E2274 – 16

Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants[1]

This standard is issued under the fixed designation E2274; 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 sanitizing/disinfectant laundry detergents/additives for use in traditional top-loading automatic clothes washing operations. This test method is designed predominantly to provide testing with representative vegetative bacteria but can also be designed to accommodate the testing of fungi and viruses.

NOTE 1—This test method does not evaluate sanitizing/disinfectant laundry detergent/additives for use in front-loading or top-loading, low water high efficiency automatic clothes washing operations.

1.2 This test method is intended to compliment Test Method E2406 and is to be used in the cases where this test method is determined to be the worse case scenario for product usage.

1.3 Knowledge of microbiological techniques is required for these procedures.

1.4 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.)

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

NOTE 2—In this test method, metric units are used for all applications, except for distance in which case inches are used.

1.6 Appropriate modifications to the test method may be required when the testing organisms are not specified herein.

1.7 *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

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

E1054 Test Methods for Evaluation of Inactivators of Antimicrobial Agents

E2406 Test Method for Evaluation of Laundry Sanitizers and Disinfectants for Use in High Efficiency Washing Operations

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Other Standards:*

AATCC Test Method 70 Water Repellency; Tumble Jar Dynamic Absorption Test[3]

OCSPP 810.2400 : Disinfectants and Sanitizers for Use on Fabrics and Textiles – Efficacy Data Recommendations[4]

40 CFR, Part 160 , Good Laboratory Practice Standards[5]

3. Terminology

3.1 For definitions of terms used in this test method refer to Terminology E2756.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *active antimicrobial ingredient*—a substance added to a formulation intended specifically for the inhibition or inactivation of microorganisms.

[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] Available from American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709, http://www.aatcc.org.

[4] Available from United States Environmental Protection Agency (EPA), William Jefferson Clinton Bldg., 1200 Pennsylvania Ave., NW, Washington, DC 20460, http://www.epa.gov.

[5] Available from U.S. Government Publishing Office Bookstore 710 North Capitol Street N.W. Washington, DC, http://www.gpo.gov/about/bookstore.htm

3.2.2 *antimicrobial agent(s)*—an active ingredient designed to suppress the growth or action of microorganisms.

3.2.3 *carrier count control*—procedure used to determine the initial number of microorganisms on a fabric carrier following the inoculation and drying procedure.

3.2.4 *diluent*—sterile deionized water, sterile distilled water or sterile synthetic AOAC hard water that may be used to prepare the active test formulation, vehicle control or product control for use in the test procedure.

3.2.5 *diluted product solution*—test formulation, vehicle control, or product control diluted to use concentration.

3.2.6 *numbers control*—in assessing sanitizer level performance, procedure used to determine the number of microorganisms remaining on the fabric carriers and in the wash water following the test procedure in the presence of the diluent. This may also be performed using diluent or phosphate buffer dilution water with surfactant.

3.2.7 *product control*—a formulation with or without an active ingredient(s) used for comparison to the test formulation.

3.2.8 *test formulation*—a formulation containing an antimicrobial agent(s).

3.2.9 *vehicle control*—the test formulation without the active ingredient(s) used for comparison to the test formulation.

3.2.10 *wash water*—the liquid contained in the exposure chamber previously exposed to either uninoculated fabric or fabric inoculated with the challenge microorganism.

4. Summary of Test Method

4.1 Under simulated laundry conditions, sets of inoculated fabric swatches are placed into diluted product solution and agitated. After a specified contact time, the wash water and the test fabric are individually cultured either quantitatively (sanitizer efficacy) or qualitatively (disinfectant efficacy).

NOTE 3—See appropriate regulatory guidance document for the minimum number of replicates required to meet a specific claim.

5. Significance and Use

5.1 The procedure in this test method is used to evaluate the activity of a test reagent (antimicrobial agent/active ingredient) or formulation in the reduction or complete kill of the bacterial population in fabric and wash water following a single wash. The water to fabric ratio in common top loading machines is dynamic and varies by region. The proper water to fabric ratio and temperature for the worse-case scenario should be determined prior to testing. This test method may need to modified if the worse-case scenario is determined to be in top loading high efficiancy washing machine

6. Apparatus

6.1 *Colony Counter,* any of several types may be used, for example, Quebec.

6.2 *Incubator,* any incubator that can maintain the optimum temperature, ±2°C, for growth of the challenge microorganism(s).

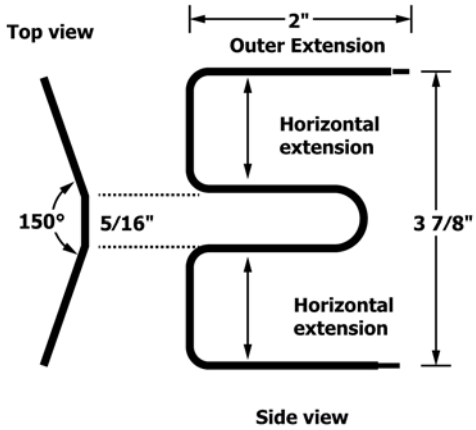

FIG. 1 Stainless Steel Spindel Schematic (top view and side view).

6.3 *Sterilizer,* any suitable steam sterilizer producing the conditions of sterility is acceptable.

6.4 *Timer (Stop-clock),* any calibrated device that can be read for minutes and seconds.

6.5 *Exposure Chamber,* container with closure that can withstand sterilization. Should be large enough to hold a single stainless steel spindle yet allow diluted product solution to completely contact the entire fabric spindle during the tumbling period.

NOTE 4—Standard lids may form a vacuum seal when steam sterilized. To avoid, prior to sterilization place a piece of paper between lid and jar.

6.6 *Stainless Steel spindles,* Spindles are fabricated from a single continuous piece of stainless steel wire, (1⁄16 in. diameter and bent to contain 3 horizontal extensions, 2 in. long connected by 2 vertical sections approximately 2 in. long.) They are shaped so that vertical sections form 150° angles, free ends of 2 outer horizontal extensions are sharpened to a point. Use as carrier for wrapping fabric ballast. See Fig. 1.

6.7 *Agitator,* tumbling device to rotate Exposure Chamber through 360° vertical orbit of 4 to 8 in. diameter at 45 to 60 rpm or comparable tumbling devices such as, launderometer or tumble jar described in AATCC Test Method 70.

6.8 *Micropipettor (and Pipet Tips),* suitable to deliver 0.01 to 0.03 mL volume.

6.9 *Forceps,* large and small, sterile.

6.10 *Safety Pins,* sterile.

6.11 *Stapler and Staples.*

6.12 *Balance,* with a platform to accommodate 15 ± 0.1 g of test fabric.

6.13 *Sterile Glass Beads,* Average size 3 to 4 mm.

6.14 *Filter Sterilization System for Media and Reagents,* a membrane or cartridge filtration system (0.22 µm pore diameter). Required for sterilizing heat-sensitive solutions.

6.15 *Membrane Filtration System for Capture of the Test Organism(s),* sterile 47 mm diameter Polyethersulfone (PES) membrane filters (0.45 µm pore diameter) and holders for such filters.

7. Reagents and Materials

7.1 *Petri Dishes,* sterile 100 × 15 mm glass and plastic. Required for performing standard plate counts and used in preparation of contaminated fabric carriers.

7.2 *Bacteriological Pipets,* sterile, various sizes.

7.3 *Test Fabric,* approximately 80 by 80 threads/in. bleached, desized, plain-weave cotton print cloth and without bluing or optical brighteners.

NOTE 5—Other test fabrics/blends may be used at the discretion of the investigator.

7.4 *Dilution Fluid,* AOAC Phosphate buffer dilution water[6] or other suitable diluent containing appropriate neutralizers for serial dilution of test samples.

7.5 *Water for Dilution of Formulations under Test:*

7.5.1 Water, sterile, deionized or distilled, equivalent to or better than Type 3, see Specification D1193.

7.5.2 AOAC Synthetic Hard Water.[6]

7.5.3 All water used for preparation of test solutions shall be sterile.

7.6 *Purity of Reagents*—reagent grade chemicals shall be used in all tests.

7.6.1 Sodium carbonate.

7.6.2 Alkaline nonionic wetting agent with HLB (hydrophilic-lipophilic balance) value of approximately 13. Prepare solution containing 0.5% nonoxynol-10 class of ethoxylated alkyl phenols, for example Tergitol NP-10 or Triton X-100 an 0.5% Na_2CO_3 .

7.7 *Neutralizing Subculture Media*—A neutralizing medium capable of supporting the growth of the test organism (for disinfection testing) following exposure to the test material in accordance with Test Methods E1054. Alternatively, the neutralizing broths may be of sufficient volume to reduce the concentration of the antimicrobials to below active levels. See 12.8.

7.8 *Challenge Microorganisms (DIS/TSS 13):*

7.8.1 *Klebsiella pneumoniae,* ATCC 4352.

7.8.2 *Staphylococcus aureus,* ATCC 6538.

7.8.3 *Pseudomonas aeruginosa,* ATCC 15442.

7.8.4 Other microorganisms, as applicable.

7.9 *Culture Media:*

7.9.1 Nutrient Agar A.[6]

7.9.2 Nutrient Agar B.[6]

7.9.3 Media suitable for identification of microorganism(s) used in the study.

7.9.4 Soybean casein digest medium or other suitable media, with or without specific neutralizers, for recovery of the challenge microorganism(s).

7.10 *Organic Soil Load*—when an organic soil load is to be incorporated in the suspension of the challenge microorganism(s), defibrinated heat-inactivated animal serum may be used or a mixture of the following stock solutions in phosphate buffer dilution water (pH 7.2) may be used (see 7.4):

7.10.1 Add 0.5 g of tryptone to 10 mL phosphate buffer.

7.10.2 Add 0.5 g of bovine serum albumin (BSA) to 10 mL of phosphate buffer.

7.10.3 Add 0.04 g of bovine mucin to 10 mL of phosphate buffer.

7.10.4 Prepare the solutions separately and sterilize by passage through a 0.22 µm pore diameter membrane filter, apportioned and stored at either 4 ± 2°C or -20 ± 2°C for no longer than 3 months.

7.10.5 To obtain a 500 µL inoculum of the challenge microorganism, add to 340 µL of the microbial suspension 25 µL, 100 µL, and 35 µL of BSA, mucin and Tryptone stock solutions, respectively.

NOTE 6—The quality of the above materials may vary among manufacturers or product lots. Therefore, preliminary screening of such items is recommended to ensure compatibility with the test microorganism(s).

NOTE 7—The investigator should confirm the appropriate organic soil usage with the appropriate regulatory agency prior to initiating testing.

8. Test Microorganisms (810,2400)

8.1 *Klebsiella pneumoniae*, ATCC 4352.

8.2 *Staphylococcus aureus*, ATCC 6538

8.3 *Pseudomonas aeruginosa*, ATCC 15442

8.4 Other microorganisms, as applicable.

9. Preparation of Test Microorganisms

9.1 Subculture microorganism(s) on Nutrient Agar A through at least one daily transfer, incubating at 35 ± 2°C.

9.2 On the day prior to testing, wash the slant and transfer the cells into French square bottles containing 20 mL of solidified Nutrient Agar B. Incubate 18 to 24 h at 35 ± 2°C, agar side down.

9.3 Remove growth from the French square bottles using three-mL dilution fluid and five sterile glass beads to suspend growth. The cultures will be standardized to yield approximately 10^8 colony forming units (CFU) per mL of *S. aureus* and 10^9 CFU/mL of *K. pneumoniae* and *P. aeruginosa*.

NOTE 8—The initial inoculum concentration for the and other challenge microorganisms may vary and should be determined from carrier and wash water numbers control recovery (see Section 12).

9.4 A soil load may be added to each inoculum (see 7.10)

10. Fabric and Spindle Preparation

10.1 Scour test fabric by boiling approximately 300 g of material for 1 h in 3 L of distilled or deionized water containing 1.5-g sodium carbonate and 1.5-g nonionic wetting agent. Rinse fabric, first in boiling water and then in cold water, until all visual traces of wetting agent are removed (that is, foaming). Remove as much water as possible from fabric.

10.2 Air dry for at least 24 h at ambient room temperature ensuring the material is completely dry.

10.3 Cut scoured dry fabric into strips 2 in. (5 cm) wide and weighing 15 ± 0.1 g each. For cotton fabrics, pierce one end of the 15-g test fabric strip and secure onto the outer horizontal extension of a stainless steel spindle. Wind the strip around the three horizontal extensions with sufficient tension to obtain 12

[6] *Official Methods of Analysis of the AOAC International* , AOAC, Washington, DC, Chapter 6: Disinfectants.

but not 13 laps while using the entire 15 ± 0.1 g of fabric. Staples, a pin, or autoclaveable fabric tag may be used to secure the fabric. Fabric wrapped spindles may be sterilized in individual exposure chambers. Alternatively, fabric wrapped spindles may be sterilized separately from exposure chambers. Ensure fabric on spindles and exposure chambers are dry prior to testing.

NOTE 9—Fabric may be purchased in pre-cut strips and then scoured.

10.4 Fabric carriers of approximately 1 by 1.5 in. will be cut from the remaining scoured fabric. Nontoxic permanent marker may be used to place a mark on the edge of each carrier. Alternatively, attach a pin to the short side of each carrier. Place fabric carriers in glass petri dishes and sterilize. Ensure dryness of fabric prior to testing.

10.5 For each challenge microorganism, prepare at least 3 fabric carriers and 1 fabric wrapped spindle for each active test formulation/product and control/numbers control.

11. Preparation of Test Sample

11.1 Prepare a sufficient volume of diluted active test formulation and product control (at least 1 L) according to manufacturer instructions, using diluent pre-equilibrated to test temperature, as necessary.

NOTE 10— Fabric to wash-water ratios based on usage patterns must be considered in this step (see EPA 810.2400.)

NOTE 11—When appropriate use AOAC hard water in preparation of test product (see 7.5.2).

11.2 Using diluent at test temperature, prepare test product dilution no more than 3 h prior to use and maintain solution at test temperature. Some active ingredients may require preparation and usage in less than 3 h.

12. Procedure

12.1 Inoculate three sterile fabric carriers (in a single sterile Petri dish) with 0.030 mL of prepared inoculum per carrier. Disperse the inoculum over an approximate 1- by 1.5 in. area of each carrier, avoiding the marker, staple, or safety pin. Dry the carriers in a 35 ± 2°C incubator until the carriers are visibly dry, but not longer than 30 min. Use swatches (carriers) within one hour of drying.

12.2 Using sterile forceps, aseptically place three dried inoculated carriers in an upright position between the sixth and seventh folds of a single wrapped spindle. secure individual swatches by tucking them deeply into the preformed "pockets." Do not allow the inoculated carriers to overlap. The marker, staple, safety pins, or autoclavable fabric tag will allow for easy removal at the end of the procedure.

NOTE 12—Weight of the wetted fabric strip should keep the contaminated swatches intact.

12.3 To simulate top load washing machines, aseptically place the spindle into the sterile exposure chamber.

12.4 Add prepared test sample (see Section 11).

12.5 Firmly close exposure chamber.

NOTE 13—Additional steps may be needed to prevent leaking from exposure chamber (that is, seal with parafilm).

12.6 Place the exposure chamber into the agitator for the specified exposure period (pre- and post-agitation times can be specified in the study protocol separately).

12.7 Using large, sterile forceps or sterile gloves, remove spindle from exposure chamber, wring the solution and aseptically remove each fabric carrier to a separate wide mouth tube containing 10 mL neutralizing broth.

12.8 Add concentrated neutralizing broth to wash water and mix well. Alternatively, 0.5 ml wash water may be added to 9.5 mL of neutralizer –10 mL of neutralizer.

NOTE 14—The specific neutralizer and concentrations should be determined prior to testing. For addition to wash water, concentration should be increased in order to reduce the amount of neutralizer added to wash water. Otherwise, the volume of neutralizer needed may overflow the exposure chamber.

12.9 Addition of fabric carrier to neutralizing broth and concentrated neutralizing broth to wash water completes the exposure time.

12.10 All tubes containing fabric carriers will be mixed on a Vortex-type mixer for approximately ten seconds. Alternatively, other methods such as a foot-arc technique or sonication may be used to extract surviving microorganisms from fabric swatches.

12.10.1 If necessary in disinfection testing (see Practices E1054), after 30 to 60 min from original subculture, transfer carrier to a second tube containing neutralizing broth and mix thoroughly.

NOTE 15—More than one technical person is needed to meet exposure time requirements.

12.11 *Determination of Disinfectant Efficacy:*

12.11.1 Filter entire volume of wash water containing neutralizing broth and plate filter on appropriate agar containing neutralizers when the entire volume of wash water is being tested.

12.11.2 Incubate plates and tubes containing carriers for 48 ± 2 h at 35 ± 2°C.

12.11.3 Results are reported as growth (+) or no growth (–). Positive results should be confirmed by Gram stain and streaking onto an appropriate growth medium for identification or other appropriate identification method.

12.12 *Determination of Sanitizing Efficacy:*

12.12.1 Serially dilute the neutralizing broth. Plate 1.0 mL in duplicate of the 10^0 (neutralizer) and 0.1 mL in duplicate of the 10^0 through 10^{-3} dilutions (or 1.0 mL of 10^{-1} through 10^{-4} dilutions) in or on agar containing neutralizers as needed. Incubate plates at 35 ± 2°C for 48 ± 2 h. To determine survivors, count colonies and record as CFU/plate. Average duplicate plates and multiply by the dilution factor to arrive at CFU/carrier. This average count should be converted into $\log_{10}$.

12.12.2 Serially dilute the mL neutralized wash water to 10^{-2} using dilution fluid containing neutralizers. Plate 1.0 mL in duplicate of the 10^0 (neutralizer) and 0.1 mL in duplicate of the 10^0 through 10^{-3} dilutions (or 1.0 mL of 10^{-1} through 10^{-4} dilutions) in or on agar containing neutralizers as needed. Filter remaining neutralizing broth/wash water combination and plate filter on appropriate agar containing neutralizers as needed.

Incubate plates at 35 ± 2°C for 48 ± 2h. To determine survivors, count colonies and record as CFU/plate. Average duplicate plates and multiply by the dilution factor to arrive at CFU/carrier. This average count should be converted into $\log_{10}$.

12.12.3 Results are reported as percent reduction of number of organisms on fabric carriers and in wash water as compared to numbers control.

13. Numbers Control

13.1 In place of the test formulation, use a diluent, or diluent-containing surfactant, and follow steps 12.1 – 12.9 above. Ensure that carriers used in this control were treated in the same manner as the test carriers.

13.1.1 Serially dilute the neutralizing broth containing a single carrier to 10^{-4} and plate duplicate 1.0 mL or 0.1 mL aliquots of all dilutions in or on an appropriate agar. Incubate plates for 48 ± 2 h at 35 ± 2°C. To determine survivors, count colonies and record as CFU/plate. Average duplicate plates and multiply by the dilution factor to arrive at CFU/carrier. This average count should be converted into $\log_{10}$. A minimum average of 1.0×10^4 CFU/carrier must be recovered for a valid test.

13.1.2 Serially dilute the neutralizing broth/wash water combination to 10^{-4} and plate duplicate 1.0 mL or 0.1 mL aliquots of all dilutions in or on an appropriate agar. Incubate plates at 35 ± 2°C for 48 ± 2 h. To determine survivors, count colonies and record as CFU/plate. Average duplicate plates and multiply by the dilution factor to arrive at CFU/mL. This average count should be converted into $\log_{10}$. A minimum average of 1.0×10^4 CFU/mL must be recovered fo a valid test.

14. Neutralizer Efficacy Control

14.1 *Disinfection testing*—To represent worst-case neutralization, simulate the neutralization of the wash water procedure by neutralizing an identical aliquot of test substance (for example, 0.5 mL) into an identical aliquot of neutralizer (for example, 9.5-10 mL). Add an aliquot (for example, 0.1 mL) of test organism to each tube targeting an addition of 10-100 CFU. Multiple organism dilutions may be utilized to target this range. In duplicate, plate identical aliquots added to the tubes onto agar plates as a numbers control. Incubate 48 ± 2h at 35±2°C. The acceptance criterion is growth in the broth with ≤100 CFU.

14.2 *Sanitizer testing*—To represent worst-case neutralization, simulate the neutralization of the wash water procedure by neutralizing an identical aliquot of test substance (for example, 0.5 mL) into an identical aliquot of neutralizer (for example, 9.5-10 mL). Add a 1.0 mL aliquot of diluted test organism to each tube to target an addition of 1000-10 000 CFU yielding 10-100 CFU per 0.1 mL. Multiple organism dilutions may be used to target this range. Mix and hold for at least as long as the anticipated dwell time (for example, 30 min). Mix again and plate 0.1 mL aliquots in duplicate in or on an appropriate agar. As a numbers control, repeat this procedure using simply 10 mL of untreated neutralizer. Incubate plates at 35 ± 2°C for 48 ±2h. The acceptance criteria is growth in the neutralization test with 1 $\log_{10}$ of the numbers control.

NOTE 16—This control should be conducted prior to testing. At the discretion of the investigator, this control may be performed again on the test date.

14.3 CFU recovered should be within a Log_{10} of 0.20 of the confirmed CFU for the neutralizer to be considered effective. (see Practices E1054

15. Carrier Count Control

15.1 Repeat step 12.1 for each challenge microorganism.

NOTE 17—Carrier Count Control should be performed on swatches dried and held for the same amount of time as swatches used for the test procedure.

15.2 Place each carrier into a tube containing 10 mL diluent utilized for step 13.1.

15.3 Mix each tube on a Vortex-type mixer for ten seconds. Other methods such as a foot-arc technique or sonication may be used to extract microorganisms from fabric swatches.

15.4 Perform serial ten-fold dilutions to the 10^{-5} dilution and plate duplicate aliquots of all dilutions in or on the appropriate agar. Incubate plates at 35 ± 2°C for 48 ± 2h.

NOTE 18—The control is optional and should be performed at the discretion of the investigator. Various microorganisms respond differently to the inoculum preparation, carrier inoculation and drying procedures. This procedure may serve as a troubleshooting tool if a microorganism does not perform as expected in the numbers control and test procedure.

16. Calculation

16.1 Percent reduction is calculated as follows:

$$\%\text{reduction} = (a - b)/a \times 100 \quad (1)$$

where:

a = average number of organisms surviving in the fabric carriers of the numbers control, or the wash water of the numbers control.

b = average of the number of organisms surviving in the fabric carriers or wash water.

17. Report

17.1 For sanitizer efficacy, report the percent reduction for all microorganisms and product lots tested.

17.2 For disinfectant efficacy, report the number of carriers showing growth of the test microorganism out of the total number of carriers. Also report the number of wash water samples showing growth of the test microorganism out of the total number of samples.

17.3 Also report the following information:

17.3.1 Test agent identity under test.

17.3.2 Chemical class of product(s) under test.

17.3.3 Concentration(s) and class of active ingredient(s) tested.

17.3.4 Water employed to dilute product (if AOAC hard water employed, report hardness levels).

17.3.5 Whether or not soil was employed.

17.3.6 Test microorganisms.

17.3.7 Media and reagents employed.

17.3.8 Neutralizing broth and neutralizer concentration employed.

17.3.9 Growth/no growth determination from neutralizer efficacy control.

TABLE 1 Precision Statistics for Testing per E2477

Product Concentration	Average[A] $\bar{X}$	Repeatability Standard Deviation $S\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation s_R	Repeatability Limit r	Reproducibility Limit R
A	4.88	0.52	0.49	0.67	1.37	1.88
B	4.43	1.02	0.65	1.17	1.82	3.28
C	2.65	1.57	0.74	1.70	2.07	4.76

[A] The average of the laboratories' calculated averages.

17.3.10 Number of microorganisms surviving on each of the three test carriers.

17.3.11 Number of microorganisms surviving on each of the three numbers control carriers.

17.3.12 Number of microorganisms surviving in the test wash water.

17.3.13 Number of microorganisms surviving in the numbers control wash water.

17.3.14 If applicable, number of microorganisms surviving on each of the carrier count controls.

17.3.15 Statement that the test was done in accordance with this test method.

18. Precision and Bias[7]

18.1 The precision of this test method is based on an interlaboratory study (ILS) of E2274, conducted in 2007. Five separate laboratories participated in this study. Each of the labs reported out reduction in microbial viability results from three independent by-day replicate tests for three laundry detergent treatments (blinded) that differed in the concentration of microbicide active. Testing was conducted with a single challenge organism, *Klebsiella pneumoniae* (ATCC4352). Because of inconsistencies in determining baseline populations of the challenge organism, data produced by one of the five laboratories were not used for calculating the precision of the method. Every "test result" reported represents an individual determination, and only the contaminated cloth swatches were assayed for bacteria. Except for the use of data from only four laboratories, Practice E691 was followed for the design and analysis of the data; the details are given in ASTM Research Report No. E35–1003.

18.1.1 *Repeatability limit (r)*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the "*r*" value for that material; "*r*" is the interval representing the critical difference between two test results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory.

18.1.1.1 Repeatability limits are listed in Table 1.

18.1.2 *Reproducibility limit (R)*—Two test results shall be judged not equivalent if they differ by more than the "*R*" value for that material; "*R*" is the interval representing the critical difference between two test results for the same material, obtained by different operators using different equipment in different laboratories.

18.1.2.1 Reproducibility limits are listed in Table 1.

18.1.3 The above terms (repeatability limit and reproducibility limit) are used as specified in Practice E177.

18.1.4 Any judgment in accordance with statements 18.1.1 and 18.1.2 would normally have an approximate 95 % probability of being correct; however, the precision statistics obtained in this ILS must not be treated as exact mathematical quantities applicable to all circumstances and uses. The limited number of laboratories reporting results provides a less than sufficient estimate of the overall repeatability and reproducibility limits when considering use and application of this test method. Providing more reliable estimates of the method would require participation of additional labs and execution of testing with the other requisite surrogate organism, *Staphylococcus aureus* (ATCC6538). Consider the repeatability limit and the reproducibility limit as general guides, and the associated probability of 95 % as only a rough indicator of what can be expected.

18.2 *Interpretation of Precision Statistics*—For testing of Treatment A, data from 95 % of studies conducted per ASTM E2477 will not vary in absolute mean values more than 1.37 $\log_{10}$ within laboratories, nor more than 1.88 $\log_{10}$ between laboratories; for Treatment B, no more than 1.82 $\log_{10}$ within laboratories, nor more than 3.28 $\log_{10}$ between laboratories; and for Treatment C, no more than 2.07 $\log_{10}$ within laboratories, nor more than 4.76 $\log_{10}$ between laboratories.

18.3 *Bias*—At the time of the study, there was no accepted reference material suitable for determining the bias for this test method; therefore, no statement on bias is being made.

18.4 The precision statement was determined through statistical examination of all valid results from testing performed in four laboratories, using *Klebsiella pneumoniae* to challenge a heavy duty granule (HDG) detergent provided by Procter & Gamble Co., and containing as an active three different concentrations of sodium dichloroisocyanurate (NaDCC). These three test formulations were described as the following:

Concentration A: AATCC HDG detergent + 10 % NaDCC
Concentration B: AATCC HDG detergent + 5 % NaDCC
Concentration C: AATCC HDG detergent + 2.5 % NaDCC

18.4.1 To judge the equivalency of two test results, it is recommended that the repeatability and/or reproducibility statistics associated with to choose the concentration closest in characteristics to the test concentration be selected.

19. Keywords

19.1 launderometer; laundry additives; laundry disinfectant; laundry sanitizer; Petrocci and Clarke; tumble jar

[7] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR: RR:E35-1003.

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(2) Official Methods of Analysis of AOAC International, AOAC, Washington, D.C., Chapter 6: Disinfectants, 17th ed., 2000.

(3) 40 CFR Part 160 Good Laboratory Practice Standards (Code of Federal Regulations).

(4) T-1-215 Canadian Pest Management Regulatory Standards, Trade Memorandum, Oct. 1980.