Designation: E2406 – 16

Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants for Use in High Efficiency Washing Operations[1]

This standard is issued under the fixed designation E2406; 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 front loading high efficiency (HE) automatic clothes washing operations that typically utilize very low wash water volumes. This test method is designed to provide testing with representative vegetative bacteria but can also be designed to accommodate the testing of fungi and viruses.

1.2 This test method is intended to compliment Test Method E2274 and is to be used in the cases where this test method is determined to be the worse case scenario for product usage.

NOTE 1—Test Method E2274 is the recommended method to evaluate sanitizing/disinfectant laundry detergent/additives for use in traditional high wash water volume automatic clothes washing operations.

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

E2274 Test Method for Evaluation of Laundry Sanitizers and Disinfectants

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

2.2 *Other Standards:*

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

OSCPP 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.

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

Current edition approved April 15, 2016. Published May 2016. Originally approved in 2004. Last previous edition approved in 2009 as E2406 – 09. DOI: 10.1520/E2406-16.

[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), Ariel Rios 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

3.2.3 *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 low volumes of 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 make a specific claim.

5. Significance and Use

5.1 The procedure in this test method is used to evaluate the effectiveness of a test reagent (antimicrobial agent/active ingredient) or formulation to reduce or completely kill bacterial populations on contaminated fabrics and in wash water following a single wash under simulated low wash volume conditions. The water to fabric ratio in common front loading machines is dynamic and varies by region and machine used. The proper water to fabric ratio and temperature for the worse-case scenario for product use should be determined and documented prior to testing.

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).

6.3 *Sterilizer*—Any suitable steam sterilizer producing the conditions of sterility, is accetable.

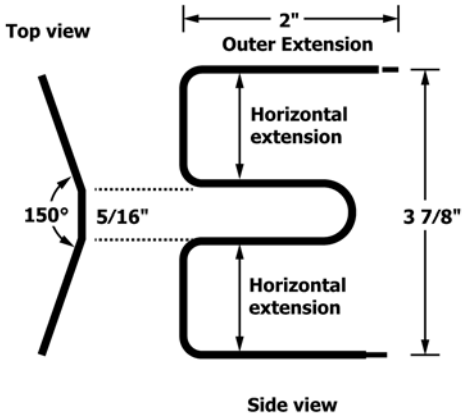

FIG. 1 Stainless Steel Spindle Schematic (Top View and Side View)

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. Dimension and volume capacity should be consistent for use in Test Method E2274.

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° angle where the free ends of the 2 outer horizontal extensions are sharpened to a point. This will be used as scaffolding for initial wrapping of fabric ballast. See Fig. 1.

6.7 *Agitator*—Tumbling device intended to rotate Exposure Chamber through 360° vertical orbit of 4 to 8 in. diameter at 45 to 60 rpm or a comparable tumbling devices such as Launder-ometer or Tumble Jar described in AATCC 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 membrane Polyethersulfone (PES) 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 and 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 step 12.8.

7.8 *Culture Media:*

7.8.1 Nutrient Agar A[6].

7.8.2 Nutrient Agar B[6].

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

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

7.9 *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).

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

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

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

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

7.9.5 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. 15442, or 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 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 these 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 7.9).

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 that 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 but not 13 laps while using the entire 15 ± 0.1 g of fabric. Staples, a pin, or autoclavable fabric tag may be used to secure the fabric strip end. Apply additional staples to the 6th and 7th folds along one horizontal side of the fabric bundle to create "pockets" that will secure individual fabric swatches during tumbling. 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.

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