



Designation: **E2274–09 E2274 – 16**

Standard Test Method for Evaluation of Laundry Sanitizers and Disinfectants¹

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~~, front-loading or top-loading, low water volume ~~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 Documents/Standards:*

[AATCC Test Method ~~70-1997~~70 Water Repellency; Tumble Jar Dynamic Absorption Test](#)³

[~~DIS/FSS-13~~OCSP 810.2400 Laundry Additives—Disinfection and Sanitization, U.S. Environmental Protection Agency, Office of Pesticide Programs, April 1980; Disinfectants and Sanitizers for Use on Fabrics and Textiles – Efficacy Data Recommendations](#)⁴

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

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 Nov. 1, 2009 April 15, 2016. Published December 2009 May 2016. Originally approved in 2003. Last previous edition approved in 2003 as E2247–03: E2247 – 09. DOI: 10.1520/E2274-09:10.1520/E2274-16.

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-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. Terminology

3.1 For definitions of terms used in this test method refer to Terminology [E2756](#).

3.2 ~~Definitions:~~ *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.

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.

~~3.1.6 *neutralization*—a process that results in quenching the antimicrobial activity of a test formulation. This may be achieved by dilution of the test formulation(s) to reduce the concentration of the antimicrobials, or through the use of chemical agents, called neutralizers, to suppress antibacterial activity.~~

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 be modified if the worse-case scenario is determined to be in top loading high efficiency 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, $\pm 2^{\circ}\text{C}$, for growth of the challenge microorganism(s).

6.3 *Sterilizer*, any suitable steam sterilizer producing the conditions of ~~sterility~~ 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, ($\frac{1}{16}$ 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 [70-1997:70](#).

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

6.9 *Forceps*, large and small, sterile.

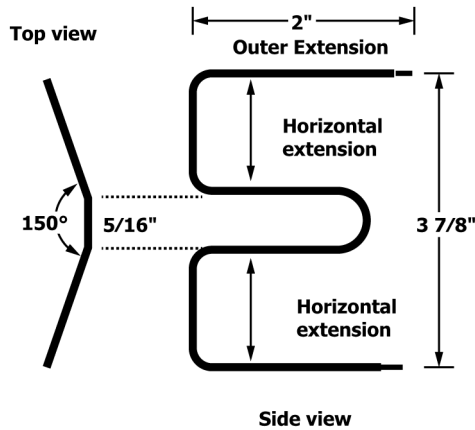


FIG. 1 Stainless Steel Spindel Schematic (top view and side view).

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 by 15 mm \times 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 water⁶ 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 Broths*—Subculture Media—~~growth media appropriate for the challenge microorganism containing chemical agents to suppress antibacterial activity. 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 H-812.8.~~

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.

⁶ *Official Methods of Analysis of the AOAC International*, AOAC, Washington, DC, 16th ed., Chapter 6: ~~Disinfectants~~, 1995—Disinfectants.

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 10⁸ colony forming units (CFU) per mL of *S. aureus* and 10⁹ 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 but not 13 laps while using the entire 15 ± 0.1 g of fabric. ~~Staples or a pin~~ 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 ~~dryness of fabric on spindles and exposure chambers are dry~~ dryness of 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.