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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 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, low water volume automatic clothes washing operations.

1.2 Knowledge of microbiological techniques is required for these procedures.

1.3 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 method, metric units are used for all applications, except for distance in which case inches are used.

1.4 *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

2.2 *Other Documents:*

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

DIS/TSS 13 Laundry Additives—Disinfection and Sanitization, U.S. Environmental Protection Agency, Office of Pesticide Programs, April 1980[4]

3. Terminology

3.1 *Definitions:*

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

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

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

3.1.4 *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.

3.1.5 *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.

3.1.7 *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.

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

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

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[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides 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), Ariel Rios Bldg., 1200 Pennsylvania Ave., NW, Washington, DC 20460, http://www.epa.gov.

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

3.1.11 *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.

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.

6.4 *Timer (Stop-clock)*, any 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 70-1997.

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*, 3 to 4 mm.

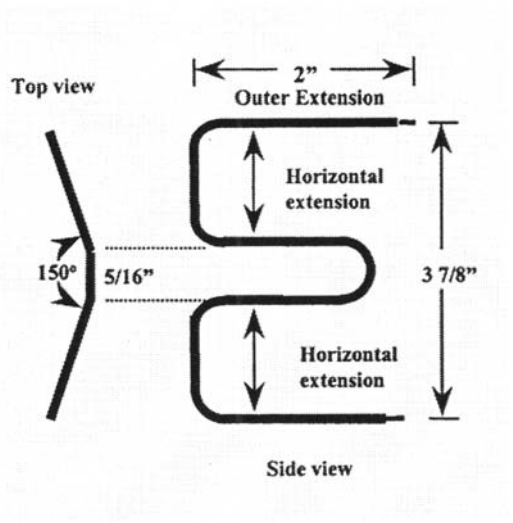

FIG. 1 Stainless Steel Spindel Schematic (top view and side view).

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 filters (0.45 µm pore diameter) and holders for such filters.

7. Reagents and Materials

7.1 *Petri Dishes*, sterile 100 by 15 mm. 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[5] 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.[5]

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.

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