



Standard Test Method for Evaluating the Performance of Antimicrobials in or on Polymeric Solids Against Staining by *Streptover-* *ticillium reticulum* (A Pink Stain Organism)¹

This standard is issued under the fixed designation E 1428; 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 used for determining the performance of antimicrobial agents used in or on synthetic polymeric solids against staining by the actinomycete *Streptover-ticillium reticulum*. This organism has been chosen as an indicator organism, although other organisms have been known to cause undesirable staining in polymeric solids.

1.2 This test method is not suitable for evaluating dark-pigmented test samples.

1.3 A knowledge of microbiological techniques is recommended for these procedures.

1.4 *This standard does not purport to address all of the safety problems, 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. Summary of Test Method

2.1 Test specimens are placed on an agar surface inoculated with *Streptover-ticillium reticulum* and incubated. After incubation, test specimens are rated visually by percentage of sample area stained.

3. Significance and Use

3.1 This test method provides a technique for evaluating antimicrobials in or on polymeric solids against staining by *Streptover-ticillium reticulum*, and should assist in the prediction of performance of treated articles under actual field conditions.

3.2 Conditioning of the specimens, such as exposure to leaching, weathering, and heat treatment, may have significant effects on performance of antimicrobials against staining. Determination of these effects is not included in this test method.

4. Interferences

4.1 An interference may be caused by contamination of

plates and agar by unwanted organisms that settle from the environment.

4.2 Dark pigments mask observation of the pink stain.

5. Apparatus

5.1 *Petri dishes*, 100 mm diameter.²

5.2 *Cotton swabs*, sterile.

5.3 *Incubator*—Incubating equipment for this test method shall maintain a temperature of $29 \pm 1^\circ\text{C}$.

5.4 *Autoclave*.

5.5 *Sterilizer*, ethylene oxide (optional).

6. Reagents and Materials

6.1 *Yeast Malt Extract Agar (ISP Medium 2)*³—Prepare this medium according to manufacturer's directions.

6.2 *Inoculum—Streptover-ticillium reticulum* ATCC 25607.⁴ Maintain stock cultures on yeast malt extract agar. The stock may be kept for not more than 12 months at approximately 3 to 10°C . Subcultures, incubated at $29 \pm 1^\circ\text{C}$ for 7 to 14 days, shall be used for inoculation.

7. Test Specimens

7.1 From each test unit,⁵ cut duplicate 0.75 in. diameter discs. If the test unit is of different construction on each side, two specimens of each, two face up and two face down, shall be tested.

7.2 A test unit containing no biocide should be included as a positive stain control.

7.3 A test unit known to inhibit staining by *Streptover-ticillium reticulum* should be included as a negative staining control.

7.4 Heavily-soiled specimens should be rinsed vigorously in water.

7.5 If sterilization of test specimens is considered necessary, ethylene oxide is the sterilant of choice. Ethylene oxide residue

¹ This test method is under the jurisdiction of ASTM Committee E-35 on Pesticides and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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² Presterilized and disposable plastic petri dishes are available from most laboratory supply houses.

³ Bacto-Yeast Malt Extract Agar, Stock No. 0770-01, available from Difco Labs, P.O. Box 1058A, Detroit, MI 48232, has been found suitable for this purpose.

⁴ American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110.

⁵ A test unit is a solid in the form of plastic sheets, films, coated fabrics or similar polymeric materials.