



Designation: E 1428 – 99

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.

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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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.

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.

7.3 A test unit known to inhibit staining by *Streptovorticillium 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 may affect test outcome. Proper aeration after sterilization is necessary.

8. Procedure

8.1 *Inoculation*—Pour sufficient yeast malt extract agar into suitable sterile dishes (see 5.1) to provide a solidified agar layer from 5 to 8 mm in depth. After the agar has solidified, moisten the agar surface by streaking with a cotton swab dipped in sterile water.

8.2 Add 3 mL of sterile saline or phosphate buffered saline to the stock culture plate and use the premoistened swab to harvest the *Streptovorticillium* cells.

8.3 Streak the cell-laden swab on the agar surface by streaking in a manner which will ensure total coverage by the organism. This swabbing technique typically provides sufficient growth to yield measurable pink stain on positive stain controls.

8.4 Use sterile forceps to place the sterile test specimens, two to a plate, on the agar surface. There shall be good contact established between the test specimen and the inoculated agar surface. Should the specimens tend to curl, weights (such as $\frac{5}{8}$ in. sterile stainless steel nuts) may be placed on top of specimens to maintain good contact.

8.5 *Incubation*—Cover the inoculated test plates containing the specimens and incubate at $29 \pm 1^\circ\text{C}$ for 14 days.

NOTE 1—Covered petri dishes containing yeast malt extract agar are considered to have the desired humidity.

9. Interpretation of Results

9.1 Observe the degree of staining on the samples by comparing with the inoculated, untreated control.

9.2 If staining is not seen on the surface of the specimen, use sterile forceps to lift and turn the specimen over and inspect the underside for stain. The degree of staining is determined by the amount of sample surface stained rather than the intensity of the color. Any possible sample discoloration that is not pink in color should be disregarded when determining the degree of stain. However, pigmented samples that show a color change associated with pink (that is, a blue pigmented sample changing to purple) should be rated according to degree of stain. Rate the degree of stain in accordance with Table 1.

9.3 *Leachability of Antimicrobials*—A “zone of inhibition” may be observed where no organisms grow on the agar adjacent to the specimen. This indicates that the antimicrobial may be leaching from the specimen.

10. Report

10.1 Report the visual rating of stain in accordance with 9.1.

11. Precision and Bias

11.1 A precision and bias statement cannot be made for this test method at this time.

12. Keywords

12.1 antimicrobial efficacy; pink stain; plastics; polymeric solids; *Streptovorticillium reticulum*

TABLE 1 Degree of Stain Rating

Observed Stain on Specimens	Rating
No Stain	0
Trace of Stain (less than 10 % coverage)	1
Slight Stain (10 to 30 % coverage)	2
Moderate Stain (30 to 50 % coverage)	3
Heavy Stain (50 % to complete coverage)	4

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