Designation: E1428 – 15a

Standard Test Method for Evaluating the Performance of Antimicrobials in or on Polymeric Solids Against Staining by *Streptomyce species* (A Pink Stain Organism)[1]

This standard is issued under the fixed designation E1428; 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.

INTRODUCTION

When certain bacteria and mold species grow on the surface of flexible or "plasticized" polymers, metabolites such as pigments in the case of certain bacteria and melanin (dark stains from fungal growth) cause undesirable stains on the polymer surface. Theses stains may persist even after the surface growth is removed. This test method is used for determining the performance of antimicrobial agents used in or on synthetic polymeric solids against pink-staining by the actinomycete, *Streptomyces species*. This organism has been chosen as an indicator organism, although other organisms have been known to cause undesirable staining in polymeric solids.

1. Scope

1.1 This test method is intended to assess susceptibility of flat two dimensional vinyl films and other solid polymer products as well as products that may directly contact vinyl to pink-staining by the actinomycete bacteria *Streptomyces species*. This test method may not be suitable for highly textured or porous substrates.

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 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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]

D3273 Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber

D3274 Test Method for Evaluating Degree of Surface Disfigurement of Paint Films by Fungal or Algal Growth, or Soil and Dirt Accumulation

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

3. Terminology

3.1 For definitions of terms used in this standard refer to Terminology E2756.

3.2 *Definitions:*

3.2.1 *microbially induced staining*—undesirable pigmentation or disfiguration of an object due to surface colonization by certain microorganisms.

3.2.1.1 *Discussion*—Both bacteria and fungi produce metabolic pigments that can result in surface stains on susceptible objects.

[1] This test method is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agentsand 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.

3.2.2 *Pink stain organism, n*—refers to a staining phenomena caused by a metabolic pigment produced by actinomycete bacteria specifically, *Streptomyces species* ATCC 25607 (deposited as *Streptoverticillium reticulum*).[3]

4. Summary of Test Method

4.1 Test specimens are placed on an agar surface inoculated with *Streptoverticillium species* and incubated. After incubation, test specimens are rated visually by percentage of sample area stained.

5. Significance and Use

5.1 Methods such as D3273 Standard Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber and D3274 Standard Test Method for Evaluating the Degree of Surface Disfigurement of Paint Films by Fungal or Algal Growth or Soil or Dirt Accumulation provide means for assessing mold and algal staining on paints.

5.2 This test method provides a technique for evaluating antimicrobials in or on polymeric solids against staining by *Streptomyces species*, bacteria and should assist in the prediction of performance of treated articles under actual field conditions.

5.3 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.

6. Interferences

6.1 An interference may be caused by contamination of plates and agar by unwanted organisms that settle from the environment.

6.2 Dark pigments mask observation of the pink stain.

6.3 If contaminants from the test specimen overgrow the Actinomycete inoculum and block the production of pink pigment or direct specimen contact, the test is to be declared invalid and steps to address contaminants taken.

7. Apparatus

7.1 *Petri dishes,* 100 mm diameter.

NOTE 1—Presterilized and disposable plastic petri dishes are available from most laboratory supply houses.

7.2 *Cotton swabs,* sterile.

7.3 *Incubator*—Incubating equipment for this test method shall maintain a temperature of 29 ± 1°C.

7.4 *Autoclave.*

7.5 *Sterilizer,* ethylene oxide (optional).

8. Reagents and Materials

8.1 *ISP Medium 2 (Yeast Malt Extract Agar)* [3]—Prepare this medium according to manufacturer's directions. Sabouraud Dextrose agar prepared per label directions may also be used (initiates robust vegetative growth and pigment production).

8.2 *Inoculum Streptomyces species*— ATCC 25607 (deposited as *Streptoverticillium reticulum*).[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 ± 1°C for 7 to 14 days, shall be used for inoculation.

9. Test Specimens

9.1 From each test unit (Note 2), 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.

NOTE 2—A test unit is a solid in the form of plastic sheets, films, coated fabrics or similar polymeric materials.

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

9.3 A test unit known to inhibit staining by *Streptomyces species* should be included as a negative staining control.

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

9.5 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.

10. Procedure

10.1 *Inoculation*—Pour sufficient yeast malt extract agar into suitable sterile dishes (see 7.1) 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.

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

10.3 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.

10.4 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 5/8 in. sterile stainless steel nuts) may be placed on top of specimens to maintain good contact.

[3] The sole source of supply of ISP Medium 2(Yeast Malt Extract Agar), Catalog No. 2770-010 known to the committee at this time is BD, 1 Becton Drive Franklin Lakes, NJ 07417 or www.bd.com. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[4] American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110. Note current ATCC nomenclature revisions lists this organism as *Streptomyces species* instead of *Streptoverticllium reticulum*. Colony pigmentation should be monitored to ensure pink pigment is present.

10.5 *Incubation*—Cover the inoculated test plates containing the specimens and incubate at 29 ± 1°C for 14 days.

NOTE 3—Covered petri dishes containing yeast malt extract agar are considered to have the desired humidity.

11. Interpretation of Results

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

11.2 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.

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

11.3 *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.

12. Report

12.1 Report the visual rating of stain in accordance with 11.2.

13. Precision and Bias

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

14. Keywords

14.1 antimicrobial efficacy; pink stain; plastics; polymeric solids; *Streptomyces species*