Designation: ~~E1428 – 99 (Reapproved 2009)~~ E1428 – 15

Standard Test Method for Evaluating the Performance of Antimicrobials in or on Polymeric Solids Against Staining by ~~*Streptoverticillium reticulum*~~*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 ~~used for determining the performance of antimicrobial agents used in or on synthetic polymeric solids against staining~~ intended to assess susceptibility of vinyl and other solid polyer products as well as products that may directly contact vinyl to pink-staining by the actinomycete ~~Streptoverticillium reticulum.~~bacteria ~~*This*~~ *Streptomyces species*.~~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 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:*

[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.

3.2.1 *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.

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* ~~*reticulum*~~*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 ~~*Streptoverticillium reticulum,*~~*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.

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 ~~*Yeast Malt Extract Agar (ISP Medium 2)*~~*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—Streptoverticillium reticulum*~~ *Inoculum Streptomyces species*— ATCC ~~25607.~~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.

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[3] The sole source of supply of ~~Bacto-Yeast~~ ISP Medium 2(Yeast Malt Extract ~~Agar, Stock~~Agar), Catalog No. ~~0770-01~~2770-010 known to the committee at this time is ~~Difco Labs, P.O. Box 1058A, Detroit, MI 28232.~~ 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.