



Designation: E3364 – 22

Standard Test Method for Evaluating the Performance of Antimicrobials in or on Polymeric Porous and Nonporous Materials Against Staining by *Streptomyces* species (A Pink Stain Organism)¹

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INTRODUCTION

When certain bacteria and mold species grow on the surface of non-rigid, 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. These stains may persist even after the surface growth is removed. This test method is used to determine the performance of antimicrobial agents used in or on synthetic polymeric porous and non-porous materials against 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 porous and non-porous materials.

1. Scope

1.1 This test method is intended to assess susceptibility of polymer materials, as well as products that may directly contact the treated polymer, to staining by the Actinomycete *Streptomyces* species.

1.2 This test method is also suitable for evaluating dark-pigmented test samples since the bacterial growth inhibition can be assessed.

1.3 Familiarity with microbiological techniques is required. This test method should not be used by persons without at least basic microbiological training.

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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recom-*

mendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

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

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E1428 Test Method for Evaluating the Performance of Antimicrobials in or on Polymeric Solids Against Staining by *Streptomyces* species (A Pink Stain Organism)

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this method, refer to Terminology **E2756**.

3.2 *Definitions of Terms Specific to This Standard:*

¹ This test method is under the jurisdiction of ASTM Committee **E35** on Pesticides, Antimicrobials, 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.

3.2.1 *microbially induced staining, n*—undesirable pigmentation or disfiguration of an object due to surface colonization by certain microorganisms. Both bacteria and fungi produce metabolic pigments that can result in surface stains on susceptible objects.

3.2.2 *pink stain organism, n*—an Actinomycete such as *Streptomyces* species ATCC 25607 (deposited as *Streptovorticillium reticulum*) that is capable of metabolically producing a light rose to orange-colored discoloring pigment.

4. Summary of Test Method

4.1 Test specimens are challenged with slurry inoculated with *Streptomyces* species and incubated. After incubation, test specimens are visually rated for microbial growth, cleaned, and 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. The Test Method E1428 Evaluating the Performance of Antimicrobials in or on Polymeric Solids Against Staining by *Streptomyces* species (A Pink Stain Organism) is used for solid polymeric materials, but is not appropriate for all antimicrobial technologies.

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

6. Interferences

6.1 An interference may be caused by contamination of plates and agar by unwanted organisms that settle on samples from the environment. Sample bioburden may out-grow the *Streptomyces* sp. inoculum, subsequently blocking direct contact with the test specimen or preventing production of pink pigments.

6.2 Darkly pigmented samples may mask observation of the pink stain.

6.3 Biocide performance may be affected if material is conditioned. Examples of conditioning include, but are not limited to: exposure to leaching, weathering, heat treatment, or addition of fire or flame retardant.

7. Apparatus

7.1 *Petri dishes*, Height and diameter appropriate to sample thickness and diameter.

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

7.2 *Cotton swabs*, sterile.

7.3 *Incubator*—capable of maintaining a temperature of $29^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

7.4 *Autoclave*.

7.5 *Sterilizer*, suitable for the substrate to be tested, that is, 70 % isopropanol (optional).

7.6 *Parafilm*, sealable plastic bags or equivalent to prevent moisture loss.

7.7 *Spreader*, sterile.

7.8 *Waterbath*, capable of maintaining water at $47^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

7.9 *Pipette*, capable of pipetting 2.0 mL.

8. Reagents and Materials

8.1 A modified ISP-2 agar is used in performing the testing. Prepare the medium used for testing according to the following directions and maintain at 45°C to keep it molten.

8.1.1 Dissolve in 1 L of water the designated amount of the following:

Yeast extract	4g
Malt extract	10g
Dextrose	4g
Agar	4g

8.2 Phosphate buffered saline (PBS) prepared according to manufacturer's instructions.

The most common composition of PBS (1X)		
Salt	Concentration (mmol/L)	Concentration (g/L)
NaCl	137	8.0
KCl	2.7	0.2
Na ₂ HPO ₄	10	1.42
KH ₂ PO ₄	1.8	0.24

8.3 Cycloheximide of 95 % purity as fungal antibiotic for slurry media (optional).

8.4 *Inoculum Species*—*Streptomyces* species ATCC 25607 (deposited as *Streptovorticillium reticulum*)— Maintain stock cultures on yeast malt extract agar. The stock should be subcultured every three months and stored at approximately 3°C to 10°C in plastic bags or covered with Parafilm.

9. Test Specimens

9.1 From each test unit (Note 2), cut triplicate 5.0 cm. diameter discs or 5.0 cm. squares. If the test unit is of different construction on each side triplicate specimens of each side, three face up and three face down, shall be tested.

NOTE 2—A test unit may be in the form of foam, vinyl, porous films, coated fabrics, or any other polymeric material, as well as any product that may directly contact them.

9.2 A test unit containing no biocide (Blank) should be included as a positive stain control. If a blank test unit is unavailable, the inoculum slurry plate should be considered as positive control and viability of the test at the same time.

9.3 Heavily soiled specimens should not be used. For stain comparison, a non-inoculated test unit should be kept until the end of the experiment.

9.4 If sanitization of test specimens is considered necessary, samples can be sanitized by spraying with 70 % alcohol and allowing to air dry thoroughly.

10. Procedure

10.1 *Slurry inoculum*: