



Mildew Resistance - Pink Stain for Plastics

1 Introduction

Note: Nothing in this standard supercedes applicable laws and regulations.

Note: In the event of conflict between the English and domestic language, the English language shall take precedence.

1.1 Purpose. This standard describes a procedure used to evaluate the resistance of plastics to growth of a microorganism known to cause pink staining in vinyl and other polymer systems.

1.2 Applicability. Integral colored exterior grade, typically biocide treated, Polyvinyl Chloride (PVC) trim.

2 References

Note: Only the latest approved standards are applicable unless otherwise specified.

2.1 External Standards/Specifications.

None

2.2 GM Standards/Specifications.

None

2.3 Additional References.

- Test Organism. *Streptomyces* sp. Waksman and Henrici, deposited as *Streptoverticillium Reticulum* (Waksman and Curtis) Baldacci, strain designation NRRL B-1484 (CIP 103969, R. Benedict T-1), American Type Culture Collection (ATCC) ATCC 25607 available from <https://www.atcc.org/products/all/25607.aspx>.
- Culture Medium. Bennet's Agar, available from VWR Part of Avantor, Catalog Number 61001-244 <https://us.vwr.com/store/product/22292362/hivegtm-bennets-agar-himedia>.

3 Resources

3.1 Facilities. Materials test laboratory certified to use the Test Organism (Biosafety Level 1). The laboratory should have controlled temperature and humidity and an air and purification system in the sample preparation area to prevent contamination of the samples.

Note: ATCC 25607 Biosafety Level 1 is based on United States Public Health Service Guidelines. It is the responsibility of the user of this standard to ensure that their facilities comply with biosafety regulations for their own country.

3.1.1 Calibration. The test facilities and equipment shall be in good working order and shall have a valid calibration label.

3.1.2 Alternatives. Alternative test facilities and equipment may also be used. However, all measuring variables as specified in this standard shall be determined correctly with respect to their physical definition.

3.2 Equipment.

3.2.1 Petri Dishes. 100 mm.

3.2.2 Sterile Cotton Tipped Swabs.

3.2.3 Incubator. Capable of maintaining $29\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

3.2.4 Autoclave. Capable of maintaining a pressure of 100 kPa at a temperature of $121\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$.

3.2.5 Culture Medium. The following dissolved in 1 L of sterile distilled water:

- Yeast Extract 1.0 g
- Beef Extract 1.0 g
- N-Z Amine 2.0 g
- Dextrose 10.0 g
- Bacto Agar 18.0 g

with the pH adjusted to 4.3 with One Normal (1 N) Sodium Hydroxide (NaOH), sterilized in autoclave at 100 kPa (121 °C) pressure for 15 minutes, covering the bottom of a 100 mm petri dish (15 mL to 20 mL) and cooled to room temperature.

3.2.6 Test Organism. *Streptomyces* sp. Waksman and Henrici, deposited as *Streptoverticillium Reticulum* (Waksman and Curtis) Baldacci, strain designation NRRL B-1484 (CIP 103969, R. Benedict T-1), American Type Culture Collection (ATCC) ATCC 25607 available from <https://www.atcc.org/products/all/25607.aspx>.

As a Stock Culture, maintain the Test Organism on Bennet's Agar, available from VWR Part of Avantor Catalog Number 61001-244 <https://us.vwr.com/store/product/22292362/hivegtm-bennets-agar-himedia>. The Stock Culture may be kept for not more than four months in a refrigerator at 3 °C to 10 °C.

Stock Subcultures, incubated at 29 °C ± 1 °C for 7 days to 14 days, shall be used for inoculations. The Stock Subcultures may be held in a refrigerator at 3 °C to 10 °C for not more than one month.

3.3 Test Vehicle/Test Piece. One 19 mm diameter disc from each biocide treated PVC sample to be tested. For coated fabrics, two discs cut from each biocide treated PVC covered cloth sample to be tested. Several 19 mm diameter untreated, no biocide, specimens to serve as a control. Plasticizer flexible PVC film containing no biocide is susceptible to pink staining and may also be used as a control.

3.4 Test Time.

Calendar time: 14 d to 16 d

Test time: 80 h to 96 h

Coordination time: 8 h to 16 h

3.5 Test Required Information. Not applicable.

3.6 Personnel/Skills. Familiarity with the equipment and training/certification to use the Test Organism.

4 Procedure

4.1 Preparation.

4.1.1 Prepare the Test Specimens. See 3.3.

4.1.2 Prepare the Culture Medium and enough petri dishes to accommodate the number of test samples to be tested. See 3.2.5.

4.1.3 Prepare the Stock Culture and Stock Subcultures. See 3.2.6.

4.2 Conditions. The laboratory should have an air purification system in the sample preparation area to prevent contamination of the samples.

4.2.1 Environmental Conditions. The laboratory should have controlled temperature and humidity and an air and purification system in the sample preparation area to prevent contamination of the samples.

4.2.2 Test Conditions. Deviations from the requirements of this standard shall have been agreed upon. Such requirements shall be specified on component drawings, test certificates, reports, etc.

4.3 Instructions.

4.3.1 Inoculation. Moisten the solidified Culture Medium surface in the petri dish by streaking with a sterile cotton tipped swab dipped in sterile distilled water. Then streak the swab on the Stock Subculture and inoculate the Culture Medium surface by streaking in a crosshatch pattern.

4.3.2 Test Specimen Placement. Place the biocide treated test specimen on the inoculated Culture Medium. For coated fabrics, place two biocide treated test specimens, one with the plastic side and the other with the fabric side in contact with the Culture Medium. It is important to establish good contact between the sample