



Mildew, Mixed Fungal Resistance - Mixed Culture Method

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 antifungal effectiveness of plastics, coated fabrics and other polymeric materials.

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 Organisms:

- *Aspergillus brasiliensis* Varga et al., strain designation SN 26 (Australian Mycol. Panel series 26, CBS 246.65, DSM 63263, IFO 6342, IMI 91855, NRRL 3536, NRRL A-5243, QM 386), American Type Culture Collection (ATCC) ATCC 9642 available from <https://www.atcc.org/products/all/9642.aspx>
- *Aspergillus flavus* Link, strain designation SN 3 (Aust. Mycol. Panel 3, CBS 131.61, DSM 1959, Harvard 997, IFO 6343, IMI 91856, NRRL A-3537, NRRL A-5244, QM 380, RIB 1403), ATCC 9643 available from <https://www.atcc.org/products/all/9643.aspx>
- *Talaromyces pinophilus* (Hedgcock) Samson et al., strain designation SN 41 (Australian Mycol. Panel 41, CBS 170.60, IFO 6345, IMI 87160, NRRL 3503, NRRL A-5245, QM 391), ATCC 9644 available from <https://www.atcc.org/products/all/9644.aspx>
- *Chaetomium globosum* Kunze: Fries, strain designation QM 459 (1042.4, CBS 148.51, CBS 161.52, CEB 1218.1, CEB 1218.2, CECT 2701, DSM 1962, IFO 6347, IMI 45550, MUCL 1984, NRRL 1870, UPSC 3159, VTT D-81079), ATCC 6205 available from <https://www.atcc.org/products/all/6205.aspx>

3 Resources

3.1 Facilities. Materials test laboratory certified to use the Test Organisms (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 9642, ATCC 9643, ATCC 9644, and ATCC 6205 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 Sterile Platinum or Nichrome Inoculating Loop.

3.2.4 Sterile Glass Wool.

3.2.5 Sterile Funnel.

3.2.6 Sterile Bottle. Volume 100 mL.

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

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

3.2.9 Atomizer. DeVilbiss Model 15-RD, or equivalent, available at <https://www.drivemedical.com/us/en/products/respiratory/aerosol-therapy-%26-accessories/aerosol-therapy/model-15-rd-atomizer/p/3092-1>

3.2.10 Culture Medium. The following dissolved in 1 L of sterile distilled water without adjusting the pH:

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|--|--------|
| • Ammonium Nitrate (NH_4NO_3) | 3.0 g |
| • Potassium Dihydrogen Orthophosphate (KH_2PO_4) | 2.0 g |
| • Magnesium Sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) | 0.5 g |
| • Potassium Chloride (KCl) | 0.5 g |
| • Dextrose | 1.0 g |
| • Agar | 15.0 g |

All components except the dextrose heated to boiling and completely dissolved before adding the dextrose, sterilized in autoclave at 100 kPa ($121\text{ }^{\circ}\text{C}$) pressure for 15 minutes, covering the bottom of a 100 mm petri dish (15 mL to 20 mL) and cooled to room temperature.

3.2.11 Test Organisms.

- *Aspergillus brasiliensis* Varga et al., strain designation SN 26 (Australian Mycol. Panel series 26, CBS 246.65, DSM 63263, IFO 6342, IMI 91855, NRRL 3536, NRRL A-5243, QM 386), American Type Culture Collection (ATCC) ATCC 9642 available from <https://www.atcc.org/products/all/9642.aspx>
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- *Chaetomium globosum* Kunze: Fries, strain designation QM 459 (1042.4, CBS 148.51, CBS 161.52, CEB 1218.1, CEB 1218.2, CECT 2701, DSM 1962, IFO 6347, IMI 45550, MUCL 1984, NRRL 1870, UPSC 3159, VTT D-81079), ATCC 6205 available from <https://www.atcc.org/products/all/6205.aspx>

All Stock Cultures, except for *Chaetomium globosum*, should be maintained separately on appropriate medium such as potato dextrose agar, or on the test medium with 0.05% yeast extract added. *Chaetomium globosum* should be maintained on a sterile strip of craft paper placed on the test medium without dextrose. Stock Cultures are stored in refrigerators at $3\text{ }^{\circ}\text{C}$ to $7\text{ }^{\circ}\text{C}$ and transfers made at six-month intervals. Stock Subcultures, incubated at $28\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ for 7 days to 14 days, should be used in preparing the Spore Suspension.

A Spore Suspension is prepared by pouring into one subculture of each organism, 10 mL of sterile distilled water containing 0.05 mL/L of non-toxic wetting agent such as Sodium Dioctyl Sulfosuccinate ($\text{C}_{20}\text{H}_{37}\text{NaO}_7\text{S}$). A sterile platinum or nichrome inoculating loop is used to gently scrape the surface growth from the agar medium.

Spore mixtures are poured into a sterile jar containing ten to fifteen 5 mm diameter solid glass beads, and shaken vigorously to break up the spore lumps. The shaken Spore Suspension is filtered through a thin layer of sterile glass wool in a sterile funnel into a sterile bottle. Distilled water is added to make up a volume of 100 mL.