



Designation: D 3273 – 9400

Standard Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber¹

This standard is issued under the fixed designation D 3273; 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.

This standard has been approved for use by agencies of the Department of Defense.

1. Scope

1.1 This test method describes a small environmental chamber and the conditions of operation to evaluate reproducibly in a 4-week period the relative resistance of paint films to surface mold fungi, mildew growth in a severe interior environment. The

¹ This test method is under the jurisdiction of ASTM Committee D-1 D01 on Paint and Related Coatings, Materials, and Applications and is the direct responsibility of Subcommittee D01.28 on Biodeterioration.

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apparatus is designed so it can be easily built or obtained² by any interested party and will duplicate results obtained in a large tropical chamber.

1.2 This test method can be used to evaluate the comparative resistance of interior coating to accelerated mildew growth. Performance at a certain rating (in accordance with Test Method D 3274) does not imply any specific period of time for a fungal free coating. However, a better rated coating nearly always performs better in actual end use.

NOTE 1—This test method is intended for the accelerated evaluation of an interior coatings' resistance to fungal defacement. Use of this test method for evaluating exterior coatings' performance has not been validated, nor have the limitations for such use been determined. Should this test method be used for the testing of an exterior coating system, a precautionary statement regarding interpretation of results as being outside of the scope of this test method must be included. Any accelerated weathering (leaching, weathering machine exposure, etc.) should be reported and should also bear reference to the fact that it is beyond the current scope of this test method.

1.3 Temperature and humidity must be effectively controlled within the relatively narrow limits specified in order for the chamber to function reproducibly during the short test period. Severity and rate of mold growth on a film is a function of the moisture content of both the film and the substrate. A relative humidity of 95 to 98 % at a temperature of $90 \pm 2^\circ\text{F}$ ($32.5 \pm 1^\circ\text{C}$) ($90 \pm 1^\circ\text{C}$) (2°F) is necessary for test panels to develop rapidly and maintain an adequate moisture level to support mold growth.

1.4 The values stated in ~~inch-pound~~ SI units are to be regarded as the standard. The values given in parentheses are for information only.

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:

D 3274 Test Method for Evaluating Degree of Surface Disfigurement of Paint Films by Microbial (Fungal or Algal) Growth or Soil and Dirt Accumulation³

3. Significance and Use

3.1 An accelerated test for determining the resistance of interior coatings to mold growth is useful in estimating the performance of coatings designed for use in interior environments that promote mold growth and in evaluating compounds that may inhibit such growth and the aggregate levels for their use (see also Note 1).

3.2 This test method should preferably be used by persons who have had basic microbiological training.

4. Apparatus

4.1 *Environmental Chamber*, capable of maintaining a relative humidity of 95 to 98 % at a temperature of $90 \pm 2^\circ\text{F}$ ($32.5 \pm 1^\circ\text{C}$) ($90 \pm 1^\circ\text{C}$) (2°F) while providing a continuous inoculation of the surface of exposed panels with mold spores. The chamber should be kept in a room controlled to $70 \pm 2^\circ\text{F}$ ($21 \pm 1^\circ\text{C}$) ($70 \pm 2^\circ\text{F}$) ($21 \pm 1^\circ\text{C}$) so that heat loss from the cabinet is insignificant and that 95 to 98 % relative humidity is readily obtained at the test temperature. Alternatively the cabinet must be insulated with suitable materials to minimize heat loss.

4.2 *Cabinet*, suitable to accommodate the desired number of test panels [typically at least twenty-five $3 \frac{1}{2}$ by $4 \frac{1}{2}$ in. (75 by 100 mm) (3 by $4 \frac{1}{2}$ in.) panels] under these test conditions can be constructed as follows (Fig. 1):

4.2.1 *Tank*, polypropylene or polyethylene, with an offset shoulder at the top rim is used as the chamber.⁴ The minimum recommended tank size is $18 \frac{1}{2}$ by $18 \frac{1}{2}$ by 24 in. (46 by 46 by 61 cm) (18 by 18 by 24 in.). A pitched top with straight sides should be constructed out of acrylic plastic so moisture condensation will run down the sides and be recirculated instead of dripping onto the panels.

4.2.2 *Heating Coil*,⁵ installed in the bottom of the chamber by water tight connections through the end wall. The heater should be sized to allow reasonable recovery time and uniform heating of the water when the chamber is opened and closed to place or inspect samples.⁶ It is so placed that it is immersed when there are $2 \frac{1}{2}$ to 3 in. (50 to 75 mm) (2 to 3 in.) of water in the bottom

² Additional specifications for construction of a chamber that has been found suitable for this method may be obtained from New Jersey Industrial Controls, P.O. Box 601, Rockaway, NJ 07866.

³ Annual Book of ASTM Standards, Vol 06.01.

⁴ Dynalab Corp., Rochester, NY, and Cole Parmer Instrument Co., Chicago, IL have tanks

⁴ Tanks of this type available in dimensions approximating $27 \frac{1}{2}$ by 46 by 46 cm (27 by 18 and 18 in.) (686 by 457 and 457 mm) high.) are available from laboratory supply companies. Nalgene tanks have been found suitable.

⁵ Omega Engineering, Inc. makes

⁵ The sole source of supply of a 0.315 -in. ($7/8$ -mm) (0.315 -in.) diameter inconel sheathed heater, Model STRI, which has been found suitable for STRI (STRI-1248/120), known to the committee at this time is Omega Engineering, Inc., One Omega Drive, Stamford, CT 06907, 800-848-4286. If you are aware of alternative suppliers, please provide this information to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible committee, which you may attend.

⁶ For an $18 \frac{1}{2}$ by $18 \frac{1}{2}$ by 24 in. (46 by 46 by 61 cm) (18 by 18 by 24 in.) tank, a 250-watt heater is recommended. For a $24 \frac{1}{2}$ by $24 \frac{1}{2}$ by 36 in. (61 by 61 by 91 mm) (24 by 24 by 36 in.) tank, an 800-watt heater is recommended.

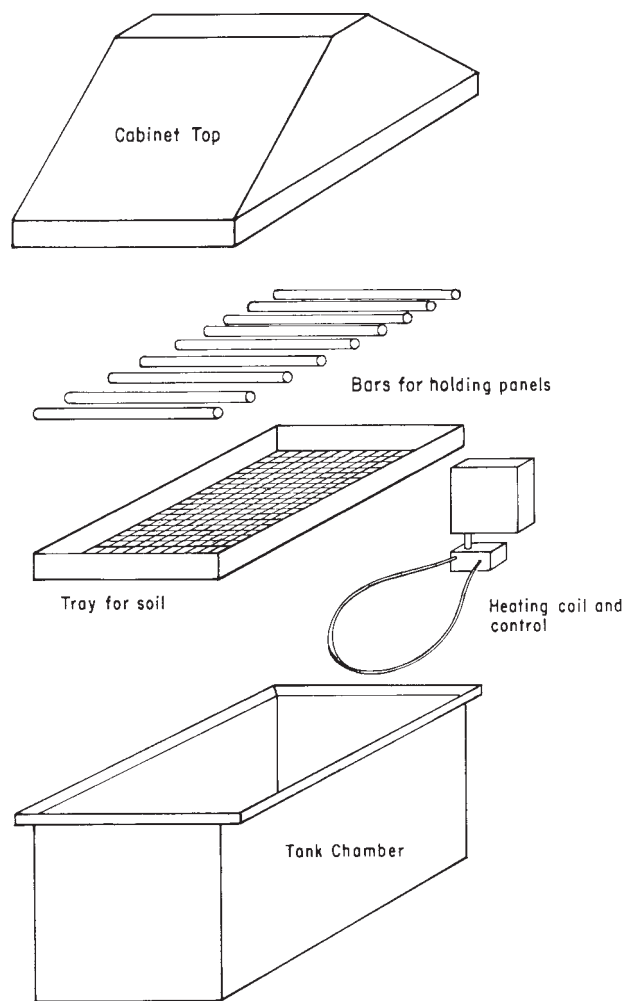


FIG. 1 Environmental Cabinet Assembly

of the chamber. The temperature in the chamber should be monitored and controlled by placing a suitable thermocouple or RTD⁷ in an area near the test panels. The temperature can be displayed and controlled by a solid state proportional controller.⁸

4.2.3 *Tray*, stainless steel or plastic, approximately ~~1 in. (25 mm)~~ 25 mm (1 in.) smaller than the inside dimensions of the chamber and ~~1 25 to 3 in. (25 to 75 mm)~~ 25 mm (1 to 3 in.) deep with a noncorrodible metal⁹ mesh bottom should be supported ~~1 in. (25 mm)~~ 25 mm (1 in.) above the water level and centered in the chamber. One layer of fine plastic or fiberglass screen may be placed over the metal mesh, if needed for holding soil.

NOTE 12—It has been found that eliminating the plastic screen helps improve water vapor transfer into soil, and maintain active fungal cultures.

4.2.4 *Series of Wood, Glass, or Fiberglass Reinforced Plastic Bars*, suspended across the width of the chamber at a height and spacing that allows the use of test panels ~~75 by 100 mm (3 by 4 in. (75 by 100 mm); in.)~~, hung vertically, with approximately ~~3 in. (75 mm)~~ 75-mm (3-in.) clearance above the inoculated soil with a suitable method of fastening. Screw eyes are used with the wooden panels while a wire frame or a large clip is used with the gypsum board panels. Other support systems may be utilized.

NOTE 23—Other angles of exposure may be used but may alter the rate and severity of mold growth.

4.3 *Psychrometer*, for measuring relative humidity in the test area.

5. Reagents and Materials

5.1 *Soil*—A good quality greenhouse-grade potting soil, suitable for plant propagation, containing 25 % peat moss. pH of the soil should fall from 5.5 to 7.6. Do not allow soil to become compacted.

5.2 *Cultures*:

⁷ A grounded $\frac{1}{16}$ (1.5 mm) or $\frac{3}{32}$ -in. (2.4 mm) 1.5 mm ($\frac{1}{16}$) or 2.4 mm ($\frac{3}{32}$ -in.) “J” type stainless thermocouple gives good response for this application.

⁸ A Eurotherm Model 91 controlling the heater via solid state relay has demonstrated that it can be calibrated and provide calibratable, accurate, and reliable performance.

⁹ 150-mesh 316 stainless screen gives a high percentage of open area and will not allow dirt to contaminate the water.

5.2.1 *Aureobasidium pullulans*,¹⁰ ATCC 9348

5.2.2 *Aspergillus niger*,¹⁰ ATCC 6275

5.2.3 *Penicillium*,¹⁰ Sp. 12667 or ATCC 9849

5.3 Test Panels:

5.3.1 *Ponderosa Pine (Pinus ponderosa Laws) Sapwood Panels*, 12.7 mm (½ in. (12.7 mm) in.) thick, 3 75 by 4 in. (75 by 100 mm (3 by 4 in.)), free of excessive resins, knots, growth rings or other abnormalities, surfaced smooth on four sides. Wood shall be kiln dried after sawing to avoid infestation of wood-rotting fungi, and any wood showing evidence of such infestation shall be eliminated as test material. Wood shall be weighed after conditioning at room temperature in a dry room to 15 % moisture content. Calculated weight shall fall between 6.0 365 and 7.0 g/in. 425 kg/m³ (365 (6.0 and 425 kg/m 7.0 g/in.³)). Panels containing heartwood areas should not be used as they will inhibit mold growth under test conditions.

5.3.2 *Gypsum Board Panels*, 12.7 mm (½ in. (12.7 mm)) thick, 3 75 by 4 in. (75 by 100 mm (3 by 4 in.)).

5.3.3 *Other Substrates such as Drawdown Paper, Tongue Depressors, Glass, etc.*, may be used as agreed upon by the parties involved. However, when comparing the relative performance of various coatings, the substrates must be the same in order for the results to be meaningful. Also, when using substrates that are not themselves susceptible to attack (like glass), another type of positive growth control must be used rather than the uncoated panel as specified in 7.2.

6. Preparation of Apparatus

6.1 Place greenhouse soil in the tray in the cabinet and add water to the tank chamber to the desired depth. Allow the cabinet to equilibrate for 24 h before inoculating the soil with the specified mold suspensions.

6.2 Prepare mold slants of all three cultures and age 10 to 14 days or purchase prepared mold slants⁸ of all three cultures. Prepare mold suspensions from each type of mold slant by the following procedure: Add one drop of 25 % nonionic surfactant¹¹ solution to 95 to 100 mL sterile deionized or distilled water and shake. Pipet 5 mL of this solution onto the mold slant. Scrub the surface of the slant with a sterile cotton swab to remove as much spore and mycelial growth as possible without digging up the surface of the agar. Pour the water from the scrubbed slant back into the surfactant-sterile water mixture for dilution. Shake gently for 15 to 20 min to break up clumps of mold. Use a pipet to distribute the mold suspensions evenly over the surface of the greenhouse soil in the tray in the cabinet.

6.3 Allow two weeks of continuous operation for the mold to sporulate and equilibrate with the environment before starting a test. It should not be necessary to recontaminate continually the chamber of panels after sufficient microorganism growth has built up in the soil, if the chamber is maintained in continuous operation.

6.4 Viability of the mold growth in the cabinet can be checked by placing several malt agar or potato dextrose agar plates,¹² open and face up, at several locations on the panel support rods. After 1 h, cover plates and place in incubator at 32.5 ± 1°C (90 ± 2°F (32.5 ± 1°C) 2°F) for 3 days. If an incubator is not available, leave the covered plates in the cabinet. Mold growth should be medium-heavy to heavy and cover the complete surface of the agar plate.

7. Procedure

7.1 *Preparation of Test Panels*—Wear disposable plastic gloves or utilize other techniques when handling panels to avoid fingerprints. Prepare triplicate panels by applying two coats of the material under test to both faces and to all edges of the panels at a spreading rate of approximately 450 ft 11 m²/gal (11 m/L (450 ft²/L)/gal) per coat or as specified by the coating manufacturer, allowing 1 day between coats unless otherwise specified. Duplicates may be run instead of triplicates, if agreed upon by parties involved. Condition the panels at 73.5 23 ± 3.5°F (23 2°C (73.5 ± 2°C) 3.5°F) and 50 ± 5 % relative humidity for 4 days after application of the last coat before placing in the test chamber for start of environmental exposure.

7.2 *Exposure*—Hang the panels vertically with the bottom approximately 3 in. (75 mm) above the surface of the inoculated soil and with sufficient spacing to allow free circulation of air and to prevent contact between panels or with wall surfaces. Place replicate panels randomly in the cabinet. Include uncoated control panels, or panels coated with a material known to fail under the test condition if the substrate is not susceptible to mildew growth, in all tests. If the cabinet is operating properly, unpainted panels should develop a 4 to 6 mold growth rating within 2 to 3 weeks. If this growth is not obtained, the cabinet conditions are not satisfactory or there is some interfering treatment on the panel.

7.3 *Rating*—Rate the panels for mold growth each week for 4 weeks on a 0 to 10 rating scale using photographic standards (Test Method D 3274). The 50 and 100 magnification photographic standards for types of fungal or dirt disfigurement (Test Method D 3274) should be used to confirm the presence of mold as the cause of discoloration.

8. Report

8.1 Report the results at the end of the 4-week exposure giving the mean and range of the three panels. The result from any panel

¹⁰ Suitable cultures can be obtained from American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852; P.O. Box 1549, Manassas, VA 20108. Cultures can be maintained on malt agar or potato dextrose agar. Prepared slants can be obtained from Difco Laboratories, Inc., Detroit, MI 48232; Baltimore Biological Laboratories, Baltimore, MD 21218 or from equivalent sources; microbiological supply companies.

¹¹ Triton X100; Rohm & Haas, Igepal CA-630, G.A.F., or equivalent.

¹² Octyl phenol ethoxylates, 9–10 mole EO, have been found suitable.

¹² Prepared agar plates can be obtained are available from Difco Laboratories, Inc., Detroit, MI 48232; Baltimore Biological Laboratories, Baltimore, MD 21218, or from equivalent sources; microbiological supply companies.

that differs by more than 2 rating units from either of the others can be considered manifestly faulty and discarded and the mean of the remaining two panels reported. If all panels in a set differ by more than 2 units in their ratings, discard all results and repeat the test.

9. Precision and Bias

9.1 On the basis of an interlaboratory study of this test method in which one operator, in each of seven laboratories, rated resistance to mold growth of interior paints at seven levels of resistance by visual comparison with photographic standards, the standard deviations within and between laboratories were found to be:

| Standard Deviations | |
|----------------------|-----------|
| Within laboratories | 0.4 units |
| Between laboratories | 0.7 units |

Based on these deviations the following criteria should be used to judge the precision of results at a 95 % confidence level:

9.1.1 *Repeatability*—Two ratings, each the mean of a set, obtained by a single operator should be considered suspect if they differ by more than 1 unit.

9.1.2 *Reproducibility*—Two ratings, each the mean of a set, obtained in different laboratories should be considered suspect if they differ by more than 2 units.

9.2 *Bias*—No bias has been determined for this test method.

10. Keywords

10.1 environmental chamber; mold; surface interior coatings

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