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

Designation: D3273 – 21

Standard Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber[1]

This standard is issued under the fixed designation D3273; 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 U.S. Department of Defense.

1. Scope

1.1 This test method describes the use of an environmental chamber and operating conditions to evaluate the relative resistance of interior coatings to surface fungal growth in a severe interior environment during a 4-week period.

1.2 This test method can be used to evaluate the comparative resistance of interior coatings to accelerated mold growth. Performance at a certain rating 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. If this test method is to 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 in the test report. 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 >93 % at a temperature of 32.5 ± 1 °C (90 ± 2 °F) is necessary to initiate and maintain mold growth and for test panels to develop rapidly and maintain an adequate moisture level to support mold growth.

1.4 The values stated in 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, 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 Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

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

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

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 >93 % at a temperature of 32.5 ± 1 °C (90 ± 2 °F) while providing a continuous inoculation of the surface of the exposed test panels with mold spores. The chamber could be a stand-alone unit[3] that maintains the specified temperature and humidity and can accommodate the sample holding tank (Fig. 1) or an environmental room that fits one or multiple sample holding tanks.

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

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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] An example of this equipment is Model Hastest HST-800B-LJS.

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FIG. 1 Sample Holding Tank with Test Samples

Alternatively, a self-contained environmental cabinet that generates the prescribed humidity and temperature conditions can be constructed as described by Weathering Direct / New Jersey Industrial Controls, LLC (Rockaway, NJ) at https://www.weathering-direct.com/D3273.html. The self-contained environmental cabinet can generate the prescribed temperature and humidity as per this method and can also hold the test panels (Fig. 2(A) and Fig. 2(B)). The self-contained environmental cabinet should be kept in a room controlled to no less than 21 °C (75 °F) so that heat loss from the cabinet is insignificant and >93 % relative humidity is readily obtained at the test temperature. Alternatively, the cabinet must be insulated with suitable materials to minimize heat loss.

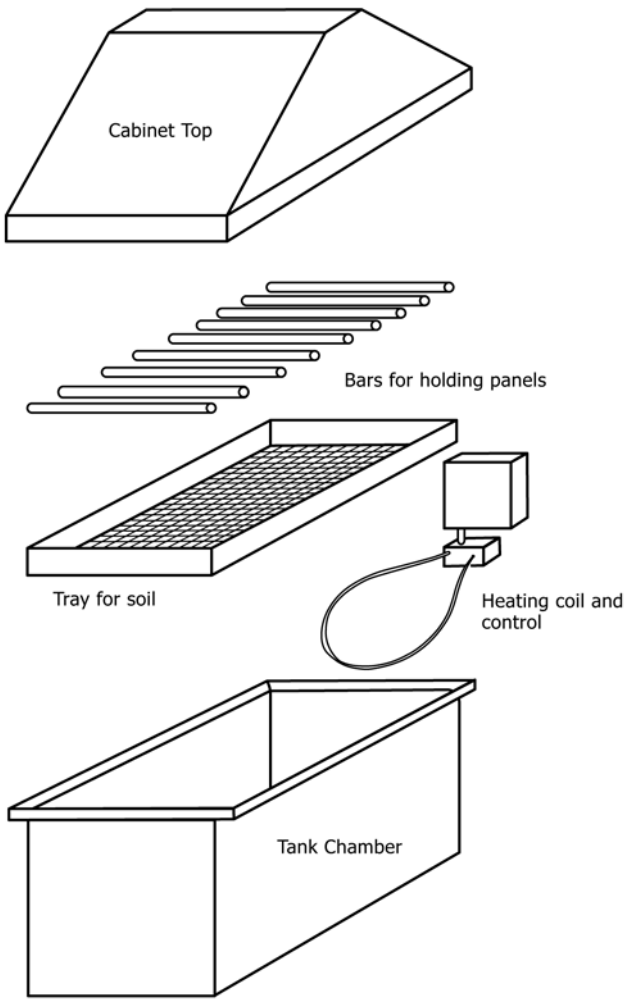

FIG. 2 (A) Environmental Cabinet Assembly

FIG. 2 (B) Environmental Cabinet Example *(continued)*

4.2 *Sample Holding Tank* used to hold the test panels and the inoculating soil tray (Fig. 1). The tank can be made of polypropylene, polyethylene, acrylic, or glass, with an offset shoulder at the top rim or holes for suspending rods. The minimum recommended tank size is 46 × 46 × 61 cm (18 × 18 × 24 in.). This typically holds a minimum of twenty-five 75 by 100 mm (3 by 4-in.) panels.

4.3 *Soil Tray,* stainless steel, aluminum or plastic, approximately 25 mm (1 in.) smaller than the inside dimensions of the sample holding tank and 25 to 75 mm (1 to 3 in.) deep with a non-corrodible metal[4] mesh bottom. If using a self-contained environmental cabinet, the tray should be supported 25 mm (1 in. ± ¼ 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 2—Eliminating the plastic screen helps improve water vapor transfer into the soil, helping maintain active fungal cultures.

4.4 *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.), hung vertically, with approximately 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, plastic cable ties, or a large clip is used with the gypsum board panels. Other support systems may be utilized.

NOTE 3—Other angles of exposure may be used but may alter the rate

[4] 150-mesh 316 stainless screen gives a high percentage of open area and will not allow dirt to contaminate the water.

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and severity of mold growth. This change of positioning must be included in the final report.

5. Reagents and Materials

5.1 *Soil*—A good quality greenhouse-grade potting soil, suitable for plant propagation, containing 25 % peat moss. The pH range of the soil should fall from 5.5 to 7.0. Do not allow soil to become compacted. Additional peat moss can be added to lower the pH into the required range.

5.2 *Cultures:*

5.2.1 *Aureobasidium pullulans,*[5] ATCC 9348.

5.2.2 *Aspergillus niger,*[5] ATCC 6275.

5.2.3 *Penicillium polonicum,*[5] ATCC 12667 or *Penicillium citrinum*,[5] ATCC 9849.

5.3 *Test Panels:*

5.3.1 *Softwood Sapwood, such as Ponderosa Pine (Pinus ponderosa Laws) Sapwood Panels,* approximately 13 mm (½ in.) thick, 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 infestation such as blue stain 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 365 and 425 kg/m^3 (6.0 and 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,* 13 to 25 mm (½ to 1 in.) thick, 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. When using substrates that are not themselves susceptible to attack (like glass), use another type of positive growth control rather than the uncoated panel as specified in 7.2.

5.4 The water used in the tank chamber is required to be DI or equivalent.

6. Preparation of Apparatus

6.1 Follow the manufacturer's guidelines for operating the environmental chamber. If using a self-contained environmental cabinet, add water to the tank to the specified depth. Place greenhouse soil in the tray of the sample holding tank and allow to equilibrate for 24 h before inoculating the soil with the specified mold suspensions.

6.2 Prepare fungal plates or slants of all three cultures and incubate 10 to 14 days. Prepare suspensions from each fungus by the following procedure: Add one drop of 25 % nonionic surfactant[6] solution to 95 to 100 mL sterile deionized or distilled water and gently mix. Dispense 5 to 10 mL of this solution onto each of the fungus cultures. Scrub the surface of the slant with a sterile cotton swab or sterile glass rod 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. If necessary, shake gently to break up clumps of spores. Distribute the fungal suspensions evenly over the surface of the greenhouse soil in the tray in the cabinet.

6.3 Before starting a test, verify the readiness of the soil by having untreated controls achieve a rating of 4 to 6 within 2 to 3 weeks of being placed in the chamber. It should not be necessary to continually re-inoculate the chamber soil after sufficient microorganism growth has been established. If the chamber is maintained in continuous operation, a tray of soil can produce mold spores for many months but should be replaced with a fresh inoculated soil twice per year.

6.4 Viability of the mold growth in the cabinet can also be checked by placing several malt agar or potato dextrose agar plates,[7] 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) for 5 to 7 days. If an incubator is not available, leave the covered plates in the cabinet. Conformational fungus growth must be that of test organisms, 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 or equivalent 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 11 m^2/L (450 ft^2/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 23 ± 2 °C (73.5 ± 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. Test pieces may also be prepared by the customer and submitted to the laboratory for testing.

7.2 *Exposure*—Hang the panels vertically in the sample holding tank 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 a panel.

[5] Cultures can be obtained from American Type Culture Collection, P.O. Box 1549, Manassas, VA 20108. Cultures can be maintained on malt agar or potato dextrose agar. Prepared slants can be obtained from microbiological supply companies.

[6] Octyl phenol ethoxylates, 9–10 mole EO, have been found suitable.

[7] Prepared agar plates are available from microbiological supply companies.

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