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Standard Test Method for Determining the Resistance of Paint Films and Related Coatings to Fungal Defacement by Accelerated Four-Week Agar Plate Assay[1]

This standard is issued under the fixed designation D 5590; 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.

1. Scope

1.1 This test method covers an accelerated method for determining the relative resistance of two or more paints or coating films to fungal growth.

1.2 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.3 *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 822 Practice for Conducting Tests on Paint and Related Coatings and Materials Using Filtered Open-Flame Carbon-Arc Light and Water Exposure Apparatus[2]

D 3273 Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber[2]

D 3456 Practice for Determining by Exterior Exposure Tests the Susceptibility of Paint Films to Microbiological Attack[2]

D 4141 Practice for Conducting Accelerated Outdoor Exposure Tests of Coatings[2]

D 4587 Practice for Conducting Tests on Paint and Related Coatings and Materials Using a Fluorescent UV-Condensation Light- and Water-Exposure Apparatus[2]

D 5031 Practice for Conducting Tests on Paints and Related Coatings and Materials Using Enclosed Carbon-Arc Light and Water Exposure Apparatus[2]

3. Summary of Test Method

3.1 This test method outlines a procedure to (*1*) prepare a suitable specimen for testing, (*2*) inoculate the specimen with the proper fungal species, (*3*) expose the inoculated samples under the appropriate conditions for growth, and (*4*) provide a schedule and guidelines for visual growth ratings. This test method is not designed to include all the necessary procedures to maintain the proper microbiological techniques required to provide the most accurate results.

4. Significance and Use

4.1 Defacement of paint and coating films by fungal growth (mold, mildew) is a common phenomenon, and defacement by algal growth can also occur under certain conditions. It is generally known that differences in the environment, lighting, temperature, humidity, substrate pH, and other factors in addition to the coating composition affect the susceptibility of a given painted surface. This test method attempts to provide a means to comparatively evaluate different coating formulations for their relative performance under a given set of conditions. It does not imply that a coating that resists growth under these conditions will necessarily resist growth in the actual application.

NOTE 1—It is hoped that a ranking of relative performance would be similar to that ranked from outdoor exposures. However, this test method should not be used as a replacement for exterior exposure (that is, Practice D 3456) since many other factors, only a few of which are listed will affect those results.

NOTE 2—Several companies have reported reasonable correlation of results from this test with actual use when testing film-forming, pigmented coatings. Round-robin testing of this test method versus exterior exposure is planned.

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

5. Apparatus and Materials

5.1 *Balance*, capable of weighing to 0.10 g.

5.2 *Incubator*, or other device capable of maintaining a constant temperature between 25 and 30°C, relative humidity of ≤85 %.

5.3 *Refrigerator*.

5.4 *Petri Dishes*, 100 by 15 mm (3.9 by 0.6 in.).

5.5 *Autoclave*.

5.6 *Paint Brush*, coarse bristle, 12 to 19 mm (½ to ¾ in.).

5.7 *Substrate*, Filter Paper (Glass fiber, Grade 391, 4.2 cm (1.65 in.)) or draw-down paper (unlaquered chart paper 216 by

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

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[2] *Annual Book of ASTM Standards*, Vol 06.01.

280 mm (8.5 by 11 in.), cut into 10 216 by 28-mm (8.5 by 1.1-in. strips).

5.8 *DeVilbiss No. 154 Atomizer* or equivalent.

5.9 *Sterile Glass Rods, Forceps, 250-mL Glass Erlenmeyer Flasks, Test Tubes*, and other routine microbiological equipment.

5.10 *Potato Dextrose Agar (PDA) or Malt Agar.*[3]

5.11 *Nutrient-Salts Agar*.

5.12 *Nutrient-Salts Solution*, (see section 4.11 without agar).

5.13 *Counting Chamber (Hemocytometer)*.

6. Reagents and Materials

6.1 *Purity of Reagents*—Reagent grade chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[4] Other grades may be used, provided they are first ascertained to be of sufficiently high purity to permit use without decreasing the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, references to water are understood to mean distilled water or water of equal or higher purity.

6.3 PDA or Malt Agar plates can be purchased prepared, or the PDA and Malt Agar powder can be purchased and prepared according to the instructions using standard microbiological techniques and equipment.

7. Preparation of the Fungal Spore Inocula

7.1 *Fungal Cultures*—Use the following test fungi in preparing the inocula: [5,6,7]

Fungi	ATCC #[5]	MYCO #[6]
Aspergillus niger	6275	...
Penicillium funiculosum	11797	391
Aureobasidium pullulans[7]	9348	...

NOTE 3—Other organisms may be of specific interest for certain applications or geographical areas. Such other pure cultures, or isolated wild strains, may be used as agreed upon by the parties involved. These organisms were selected based on the historical data from use in Test Method D 3273.

7.2 Maintain stock cultures of these fungi separately on an appropriate medium such as potato dextrose agar plates or slants. The stock culture may be kept for not more than 4 months at approximately 3 to 10°C (37 to 50°F). Subculture individual fungi onto slants or plates 7 to 20 days at 28 to 30°C (82 to 86°F) prior to each experiment, and use these subcultures in preparing the spore suspension.

7.3 Prepare a spore suspension of each of the test fungi by pouring into one subculture of each fungus a sterile 10-mL portion of water, or of a sterile solution containing 0.05 g/L of a nontoxic wetting agent such as sodium dioctylsulfosuccinate. Swirl or gently agitate the slant or plate to loosen the spores. Carefully aspirate the water and spore suspension with a sterile pasteur pipet (trying to avoid obtaining mycelia).

7.4 Check the collected spore suspension under the microscope for mycelial contamination and make a note of the relative populations of spores versus mycelial forms.

7.5 Dilute the spores suspension with sterile nutrient salts solution such that the resultant spore suspension contains 0.8 to 1.2 by 10^6 spores/mL as determined with a counting chamber.

7.6 Repeat this operation for each organism used in the test. The *A. pullulans* spores should be maintained separately and used as a separate inoculum for a separate set of plates and samples. Blend equal volumes of the remaining organisms' resultant spore suspensions to obtain the mixed spore suspension.

7.7 The spore suspension may be prepared fresh each day or may be held in the refrigerator at 3 to 10°C (37 to 50°F) for not more than 4 days.

8. Preparation of Test Specimens

8.1 A set of coatings to be tested should preferably contain a positive and a negative growth control. That is, one that is known to support fungal growth, and one that is known to *inhibit* growth completely. A set of Whatman #2 (or equivalent) filter papers or the draw-down papers without coating may be suitable growth controls.

8.2 Make sure to handle the disks or drawdown sections with sterile tongs or tweezers.

NOTE 4—Sterilization or aseptic handling of the test material, or both, avoids bacterial or other contamination that may interfere with the test results.

8.3 Coatings to be tested will be applied to 4.2-cm (1.65-in.) glass fiber filter paper disks, or to the 28 by 216-mm (1.1 by 8.5-in.) drawdown strips. The samples are prepared for evaluation by brush coating strips of drawdown paperboard, or glass filter disks with each sample in duplicate. Take care to apply a thin, even coating, with the same thickness for all coating samples.

NOTE 5—One or both sides of the substrate (drawdown strips or filter paper) may be coated as agreed upon by the parties involved.

NOTE 6—With the drawdown strips, this can be conveniently accomplished by punching a hole in the top of the strip and suspending the strip from a drying rack with string or a twist tie. The label for each strip can be written in the top 12.7 mm (½ in.) of the strip (near the hole) and the coating applied below that 12.7-mm (½-in.) strip. Another 12.7-mm (½-in.) area can be left uncoated at the bottom of the strip to permit holding the strip while brushing. This would still leave sufficient coated area for six 28 by 28-mm (1.1 by 1.1-in.) test squares from each strip. With the filter disks, a hole can be punched near the edge of the disk.

8.4 After application, suspend the sample disks or strips from drying racks and allow them to air dry for 24 to 72 h at room temperature.

8.5 If accelerated weathering, heat aging, or other preconditioning of samples is also to be run, prepare a separate set of duplicate sample disks or strips. The results from these samples

[3] Pre-prepared plates are available from microbiological supply companies, or they may be prepared using standard microbiological equipment and techniques.

[4] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

[5] Available from American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD, 20852, and has been found suitable for this purpose.

[6] Available from Mycological Services (MYCO), Box 1056, Crawfordsville, IN, 47933, and has been found suitable for this purpose.

[7] Historically known as *Pullularia pullulans.*

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