Designation: D5590 – 00 (Reapproved2010)

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 D5590; 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:*[2]

D822 Practice for Filtered Open-Flame Carbon-Arc Exposures of Paint and Related Coatings

D3273 Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber

D3456 Practice for Determining by Exterior Exposure Tests the Susceptibility of Paint Films to Microbiological Attack

D4141 Practice for Conducting Black Box and Solar Concentrating Exposures of Coatings

D4587 Practice for Fluorescent UV-Condensation Exposures of Paint and Related Coatings

D5031 Practice for Enclosed Carbon-Arc Exposure Tests of Paint and Related Coatings

G21 Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi

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 D3456) 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 %.

[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 D01.28Biodeterioration.

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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.

5.3 *Refrigerator,* or other device capable of maintaining a temperature of 4 ± 2°C.

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

5.5 *Autoclave,* capable of producing 103 kPa (15 psi) of steam pressure at 121°C and maintaining it for a minimum of 15 min. An autoclave is not necessary if pre-prepared media plates are used.

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 drawdown paper (unlaquered chart paper 216 by 280 mm (8.5 by 11 in.), cut into ten 216 by 28-mm (8.5 by 1.1-in. strips).

5.8 *Atomizer or Chromatography Sprayer.*

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.* (see Practice G21, 6.3.)

5.12 *Nutrient-Salts Solution,* (see 5.11 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:[5,6,7,8]

Fungi	ATCC #[5]	MYCO #[7]
Aspergillus niger	6275	...
Penicillium funiculosum	11797	391
Aureobasidium pullulans [5]	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 D3273.

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 10^4 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.

[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. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[5] The sole source of supply of *Aspergillus niger and Aureobasidium pullulans* strains known to the committee at this time is the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD, 20852.

[6] If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

[7] The sole source of supply of *Penicillium funiculosum* strain known to the committee at this time is the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD, 20852.

[8] Historically known as *Pullularia pullulans*.