Designation: D5590 – 17 (Reapproved 2021)

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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *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]

D1005 Test Method for Measurement of Dry-Film Thickness of Organic Coatings Using Micrometers

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

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

D4708 Practice for Preparation of Uniform Free Films of Organic Coatings

D6132 Test Method for Nondestructive Measurement of Dry Film Thickness of Applied Organic Coatings Using an Ultrasonic Coating Thickness Gage

D6695 Practice for Xenon-Arc Exposures 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. The method is not intended to simulate or replace indoor or outdoor exposure of paint films or related coatings.

NOTE 1—It is hoped that a ranking of relative performance would be similar to that ranked from outdoor exposures. Paint designated for service in exterior conditions should be pre-conditioned by laboratory accelerated weathering prior to exposure to fungi. All pre-conditioning must be detailed in the final report. This test method however, 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.

[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.

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,* 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* (as found in Practice G21).

5.11.1 Prepare this medium by dissolving in 1 L of water the designated amounts of the following reagents:

Monopotassium phosphate (KH_2PO_4)	0.7 g
Mangnesium sulfate ($MgSO_4 \cdot 7H_2O$)	0.7 g
Ammonium nitrate (NH_4NO_3)	1.0 g
Sodium chloride (NaCL)	0.005 g
Ferrous sulfate ($FeSO_4 \cdot 7H_2O$)	0.002 g
Zinc sulfate ($ZnSO_4 \cdot 7H_2O$)	0.002 g
Manganese sulfate ($MnSO_4 \cdot H_2O$)	0.001 g
Agar	15.0 g
Dipotassium phosphate (K_2HPO_4)	0.7 g

5.11.2 Sterilize the test medium by autoclaving at 121°C (250°F) for 20 min. Adjust the pH of the medium so that after sterilization the pH is between 6.0 and 6.5.

5.12 *Nutrient-Salts Solution,* (see 5.11 without agar).

5.13 *Counting Chamber (Hemocytometer).*

5.14 *Glass Wool.*

5.15 *Laboratory Accelerated Weathering and Leaching Equipment,* if used.

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]	NRRL[6]
Aspergillus niger	6275	334
Penicillium pinophilum[7]	11797	3647
Aureobasidium pullulans[8]	9348	62100

NOTE 2—These organisms were selected based on the historical data from use in Test Method D3273. 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.

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 Filter the shaken or ground suspension through a thin layer of sterile glass wool in a glass funnel into a sterile flask in order to remove mycelial fragments.

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

[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] American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852, is the current known source of the fungal strains used in this testing.

[6] NRRL strains without a Certificate of Analysis are available from the USDA Agricultural Research Service.

[7] Historically known as *Penicillium funiculosum*. It has also been re-identified as *Talaromyces pinophilus* by DNA barcode sequencing.

[8] Historically known as *Pullularia pullulans*.

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 mixed 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 four days. The individual spore suspensions may be held in the refrigerator at 3 to 10°C (37 to 50°F) for not more than fourteen days.

8. Preparation of Test Specimens

8.1 A set of coatings to be tested shall 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 drawdown papers without coating may be suitable growth controls serving as a viability control for the fungal spores. A sterile agar plate can be used as one of the controls.

8.2 Handle the disks or drawdown sections with sterile tongs or tweezers.

8.3 Coatings to be tested shall 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 triplicate. Films can also be prepared as instructed in Practice D4708. Take care to apply a thin, even coating, with the same thickness for all coating samples, as specified by the paint manufacturer, unless parties decide otherwise. Coating thickness can be recorded by spread rate (for example, grams/square cm) or confirmed with Test Method D1005 or Test Method D6132, or both, as agreed between parties and reported in the final report.

NOTE 3—One or both sides of the substrate (drawdown strips or filter paper) may be coated as agreed upon by the parties involved and should be noted in the report.

NOTE 4—Coating thickness can be confirmed with Test Method D1005 or Test Method D6132, or both, and may be reported in the final report if performed. Alternatively, coating thickness can be recorded by spread rate (for example, grams/square cm).

8.4 After application, allow to dry or cure at conditions agreed upon by interested parties.

8.5 If accelerated weathering, heat aging, or other preconditioning of samples is also to be run, prepare a separate set of triplicate sample disks or strips. The results from these samples may be compared with those from the unweathered or unconditioned samples.

NOTE 5—There are a variety of methods that could be used to simulate, in an accelerated manner, effects of weathering (sunlight or rain, or both) on the sample. Recommended conditioning of specimens by artificial weathering may be done per one of the following practices: Test Cycle 2 in Practice D4587 or Test Cycle 6 for Practice D6695. Accelerated weathering conditions must be specified in the report and agreed upon by interested parties.

NOTE 6—A leaching test may be conducted as agreed upon by interested parties.

8.6 If the drawdown strips are being used, cut them into roughly 28-mm (1.1-in.) squares. Place these specimen squares, or the coated filter disks, on the center of pre-poured agar plates. If the plates were stored in the refrigerator, allow them to equilibrate to room temperature prior to placement of the samples.

8.6.1 This test may be conducted on a nutritive agar plates (either PDA or Malt Agar) alone. However, if all samples fail completely on the nutritive agar plates, additional information could be obtained by repeating the samples' testing using nutrient salts agar plates (without a carbon source in the plates, growth and test conditions are less severe). This additional testing may be run simultaneously if agreed upon between the parties involved.

9. Procedure

9.1 *Inoculation of the Test Specimens:*

9.1.1 The *A. niger* and *P. funiculosum* may be tested together on the same plates. The *A. pullulans* must be tested separately to ensure its survival.

9.1.2 Combine an equal portion of the *A. niger* and *P. funiculosum* spore suspensions.

9.1.3 Run a count of the spores using a counting chamber to confirm the inoculum count for each test (see 7.5).

9.1.4 Apply a thin coat of fungal suspension to each specimen using a sterile atomizer or pipet, making sure the entire surface of the sample and the agar plate are covered, but not to oversaturate the samples. Alternately, a separate sterile cotton swab may be used to apply and evenly spread the inoculum over the surface of each test sample and the agar plate. Be certain that the amounts of inoculum used are the same between each of the various samples under test. This should be done using the same method by the same applicator at the same time for all samples.

9.1.5 Incubate all plates at 28 ± 2°C under ≥85% relative humidity for four weeks, checking and recording temperature and humidity before performing the weekly readings.

10. Evaluation of Results

10.1 Rate the growth weekly for four weeks according to the following:

Observed Growth on Specimens	Rating
None	0
Traces of growth (<10 %)	1
Light growth (10–30 %)	2
Moderate growth (>30–60 %)	3
Heavy growth (>60 % to complete coverage)	4

10.2 Notations should be made for "zones of inhibition" of growth on the surrounding agar if present in addition to a "0" growth rating on the sample.

11. Report

11.1 Report the following information or as otherwise agreed upon between the parties involved in the testing:

11.1.1 The date, fungal species used, incubation conditions, spread rate or coating thickness if performed, sample identification and nutritive or non-nutritive media.

11.1.2 The corresponding results of weekly observations, including: dates; notation of any unusual occurrences; and the rating of degree of defacement for each replicate.

11.1.3 Complete description of exposure cycle, time of exposure, and device(s) utilized for any preconditioning of