Designation: D5590 – 00 (Reapproved 2010)ε1

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.

ε[1] NOTE—An editorial change was made in 7.5 in November 2012.

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.

[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.

Current edition approved June 1, 2010. Published July 2010. Originally approved in 1994. Last previous edition approved in 2005 as D5590 – 00 (2005). DOI: 10.1520/D5590-00R10E01.

[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.

Copyright © ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959. United States

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.* (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.

[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.

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 10^6 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.

[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*.

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 drawdown 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 may be compared with those from the unweathered or unconditioned samples.

NOTE 7—There are a variety of methods that could be used to simulate accelerated effects of weathering (sunlight or rain, or both) on the samples. For example, a leach test that is frequently used to simulate the effects of rainwater (an important factor for fungal growth) is outlined in Note 8. Conditioning of specimens by artificial weathering may be done according to one of the following practices: D822, D4141, D4587 or D5031.

NOTE 8—A leaching test may be conducted as follows: One of the replicate sets is leached with distilled water for 24 h, then allowed to air dry. The coated substrate can be leached by suspension for 24 h in 1-gal (4-L) containers of distilled water with a flow rate such that there are six (6) changes in 24 h (or other flow rate as agreed upon by the parties involved). Differences in the integrity of the coatings after this leaching should be noted. The test panels are then air dried for 24 h under the same conditions as the unleached samples (see 7.4).

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) 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 surface is 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. 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°C under 85 to 90 % relative humidity for 4 weeks.

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

NOTE 9—These ratings are for microbial growth, not coating performance, so as not to be confused with exterior evaluations that run from 10 to 0. The lower growth ratings should correspond to longer time periods of fungus-free surface under actual use conditions between the samples compared in a given test (if the samples are leached/weathered). Comparisons of actual ratings between samples tested at different times (not together in the same test) should be avoided since changes in inocula, substrate or other conditions could affect the growth rating. Comparisons of relative rankings of performance between samples tested at different times should be valid.

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. Such zones can be designated by a Z prefix with a number following it. The number would correspond to the average width in millimetres of the zone around the sample. A large zone of inhibition indicates good biocidal effectiveness against the test organism(s), but it also suggests that the biocide is rapidly migrating out of the coating (high potential for leaching). Leached samples showing a significant decrease in efficacy (increase in growth rating or decrease in zone of inhibition) versus the corresponding unleached sample indicate that the biocide is leaching from the coating to some extent. This may indicate the potential for diminished exterior performance.

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, and some means of sample identification,

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

11.1.3 Complete description of exposure cycle, time of exposure, and device(s) utilized for any preconditioning of specimens. If an ASTM method is used for preconditioning, all appropriate information as required by that method must be reported.

12. Precision and Bias

12.1 *Precision*—It is not practical to specify the precision of the procedure in this test method for measuring fungal resistance of a coating because the actual rating numbers for samples tested at different times or in different laboratories will be affected by changes in inoculum strength, substrate, or other conditions that affect the fungal growth. In addition, differences in the perception and experience of the individual determining the growth ratings may effect the actual rating numbers assigned. Comparisons may be made between samples tested at the same time using the same inoculum with a given laboratory. A relative ranking in order of the performance ratings (that is, good, better, best) should remain the same between samples tested at different times or in different laboratories. Comparisons of the actual rating numbers between samples tested at different times or in different laboratories should be avoided.

12.2 *Bias*—No information can be presented on the bias of the procedure in this test method for measuring fungal resistance of a coating because materials having acceptable reference values are not available.

13. Keywords

13.1 agar plate assay; fungal resistance; fungi; mildew; mold

ASTM International takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, at the address shown below.

This standard is copyrighted by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States. Individual reprints (single or multiple copies) of this standard may be obtained by contacting ASTM at the above address or at 610-832-9585 (phone), 610-832-9555 (fax), or service@astm.org (e-mail); or through the ASTM website (www.astm.org). Permission rights to photocopy the standard may also be secured from the ASTM website (www.astm.org/COPYRIGHT/).