Designation: D5589 – 19

Standard Test Method for Determining the Resistance of Paint Films and Related Coatings to Algal Defacement[1]

This standard is issued under the fixed designation D5589; 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 a paint or coating film to algal growth.

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 since many other factors, only a few of which are listed will affect those results.

NOTE 2—ASTM weathering standards are no longer referenced in this document, but Practices D822, D4141, D4587, D5031, and D6695 are commonly used.

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]

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

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

D6695 Practice for Xenon-Arc Exposures of Paint and Related Coatings

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 a mixture of the proper algal 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 algal growth is a common phenomenon under certain conditions. It is generally known that differences in the environment, lighting, temperature, substrate, 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.

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 ± 2°C, relative humidity of ≥85 %, and having a constant full spectrum (see Note 3) light source.

5.3 *Refrigerator.*

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

[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.

5.5 *Autoclave.*

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

5.7 *Test Substrate,* filter paper, either regular paper or glass fiber, approximately 4.2 cm (1.65 in.) in diameter, or draw-down paper (unlacquered chart paper) approximately 21.6 by 28.0 cm (8.5 by 11 in.), cut into ten strips, approximately 21.6 by 2.8-cm (8.5 by 1.1-in.).

5.8 *Tissue Grinder.*

5.9 *Atomizer or Chromatography Sprayer.*

5.10 *Sterile Glass Rods, Forceps, 250-mL Glass Erlenmeyer Flask,* and other routine microbiological equipment.

5.11 *BG-11 Medium with Trace Metals Mixture.* [3]

5.12 *Distilled Water.*

NOTE 3—Fluorescent or LED D65 bulbs, 12 hours on, 12 off. Follow manufacturers' recommendations regarding light bulb service life and when to replace them.

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 A variety of algal cultures, including wild strains isolated from paint films, may be used in this protocol. Choose strains from the following list, use field isolates or use other strains found to grow satisfactorily under the protocol conditions. It is recommended to choose at least one culture from each type. The choice of strains should be agreed upon between the parties involved in the testing.[5]

Algae	Collection/Strain
Unicellular Green	
Chlorella vulgaris	ATCC 11468
Filamentous Green	
Ulothrix gigas	ATCC 30443
Colony-forming Green	
Scenedesmus quadricauda	ATCC 11460
Filamentous Bluegreen	
Oscillatoria sp.	ATCC 29135

6.4 Cultures should be maintained separately in BG-11 broth with trace minerals. If preferred, individual cultures may be maintained on solid media prepared by dissolving 1 to 1.5 % agar in liquid medium before autoclaving.

6.4.1 Cultures should be actively growing prior to use. Use a tissue grinder to homogenize filamentous algae before preparing inoculum. Adjust each culture to approximately one million cells per millilitre in sterile water or to a light green color. Combine equal volumes of individual cultures for a mixed inoculum.

6.4.2 If preferred, harvest algae from an agar petri dish culture by pouring 10 mL of distilled water on the agar surface. Gently scrape the algae with a sterile glass rod or pipet. Pipet the suspension into a sterile 250-mL glass Erlenmeyer flask. Repeat for all the cultures by pipetting into the same flask (try to obtain approximately equal amounts of each species, and about the same total amount between runs of this test method to make correlation of data between test runs easier). Bring the mixed volume of suspension up to 100 mL with sterile water. Retain for later use as inoculum in 8.1.

NOTE 4—This procedure gives a mixed inoculum. Alternatively, each sample could be inoculated separately with individual cultures as agreed upon between the parties involved.

7. Preparation of Test Specimens

7.1 A set of coatings to be tested should contain a control paint (blank). If available, a formulation known to perform satisfactorily in this test method should also be included. A set of paper filter disks or the draw-down papers without coating may be suitable growth controls (see 5.7).

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

7.3 Coatings to be tested will be applied to the chosen test substrate (5.7) by brush coating the strips of drawdown paperboard or filter disks with each sample in duplicate. Take care to apply a thin, even coating with the same thickness for all coating samples.

7.4 After application, suspend the sample disks or strips from drying racks and allow them to dry under the manufacture's recommendations or specifications.

7.5 When any preconditioning of specimens is to be done, a minimum of 2 additional test pieces must be prepared from each coating for each type of preconditioning used. The results from the preconditioned samples may be compared with those from the unconditioned samples. All samples shall be run in triplicates unless agreed upon by all parties involved.

NOTE 5—A leaching test may be conducted as agreed upon by the parties involved.

7.6 If the drawdown strips are being used, cut them into approximately 28-mm (1.1-in.) squares. Place these specimen squares, or the coated filter disks, on the center of pre-poured BG-11 (or appropriate–see 6.4) agar plates. The plates should be prepared at least 24 h in advance, but no longer than one

[3] BG-11 medium, trace metals mix are available through Sigma-Aldrich.

[4] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, 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] Available from the following culture collections and found suitable for this test: American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852; University of Texas (UTEX), Department of Botany, The University of Texas at Austin, Austin, TX 78713-7640; Culture Collection of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, The Windermere Laboratory, Far Sawrey, Ambleside, Cumbria LA22 OLP, U.K. Grow purchased cultures in media and under incubation conditions recommended by culture collection.

week. If the plates were stored in the refrigerator, allow them to equilibrate to room temperature prior to placement of the samples.

8. Procedure

8.1 *Inoculation of the Test Specimens:*

8.1.1 Place test specimens in the center of solidified BG-11 (or appropriate) agar plates. If drawdown strips are used, first cut into 28-mm (1.1-in.) squares.

8.1.2 Transfer the mixed algal inoculum from the flask (from 6.4.2) into a sterile atomizer or chromatography sprayer.

8.1.3 Apply a thin coat of algae suspension to each specimen, making sure the surface is covered, but not over-saturating the samples. Also, be certain the amount of inoculum applied is the same between the various samples under test (this should be done by the same applicator at the same time for all samples).

8.1.4 Transfer the inoculated plates to an incubator with a constant fluorescent light source, humidity ≥85 %, and a temperature setting to maintain 25 ± 2°C.

8.2 *Incubation of Test*

8.2.1 Incubate the samples under the conditions specified in 8.1.4 with a 12 h light, 12 h dark cycle.

8.2.2 Examine weekly for growth. Growth will appear as the typical green algae-like discoloration of the coating. Other species may show different colors.

8.2.3 If there is no growth on the control after 2 weeks, the samples shall be re-inoculated.

8.2.3.1 Transfer the test pieces to fresh plates of BG-11 and spray with fresh inoculum, as described in 8.1.2.

8.2.3.2 Incubate the re-inoculated samples for an additional 4 weeks.

9. Evaluation of Results

9.1 Rate the growth on the specimen weekly for four (4) 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

9.2 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.

NOTE 6—If the viability sample shows growth and the control (blank) does not then the material under test can be described as not susceptible to algal growth under this test method.

10. Report

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

10.1.1 The date, algal species used, incubation conditions, film thickness, if applicable and sample identification.

10.1.2 The corresponding results of weekly observations, including: dates; notation of any unusual occurrences; and the rating of degree of defacement; include information of any re-inoculation.

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

10.1.4 If an ASTM test method or practice is used for preconditioning, all appropriate information as required by that test method or practice must be reported.

11. Precision and Bias

11.1 *Precision*—It is not practical to specify the precision of the procedure in this test method for measuring algal 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 effect the algal 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.

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

12. Keywords

12.1 agar plate; algae; algal resistance