Designation: D 5589 – 97 (Reapproved 2002)

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 D 5589; 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.

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 Filtered Open-Flame Carbon-Arc Exposures of Paint and Related Coatings[2]

D 4141 Practice for Conducting Black Box and Solar Concentrating Exposures of Coatings[2]

D 4587 Practice for Fluorescent UV-Condensation Exposures of Paint and Related Coatings[2]

D 5031 Practice for Enclosed Carbon-Arc Exposure Tests of Paint and Related Coatings[2]

G 53 Practice for Operating Light- and Water-Exposure Apparatus (Fluorescent UV-Condensation Type) for Exposure of Nonmetallic Materials[3]

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 (see Note 1).

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 fluorescent light source.

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 *Test Substrate*, filter paper, either regular paper or glass fiber, 4.2 cm (1.65 in.) in diameter, or drawdown paper (unlaquered chart paper) 21.6 by 28.0 cm (8.5 by 11 in.), cut into ten 21.6 by 2.8-cm (8.5 by 1.1-in.) strips may be used.

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.

[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 Biodeterioration.

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

[3] *Annual Book of ASTM Standards*, Vol 14.02.

5.11 *Allen's Medium* [4] or *Bold's Basal Medium*[5] ingredients (see 6.3).

5.12 *Distilled Water*.

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.[6] 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 *Allen's Medium*—Prepare liquid medium by dissolving in 1000 mL of water the following reagents in the designated amounts:

Reagent	Amount, g/1000 mL
$NaNO_3$	1.5
K_2HPO_4	0.039
$MgSO_4 \cdot 7H_2O$	0.075
$CaCl_2 \cdot 2H_2O$	0.027
Na_2CO_3	0.020
$Na_2SiO_3 \cdot 9H_2O$	0.058
Citric acid	0.006
EDTA[A]	0.006
Allen's trace element solution	1.0 mL[B]
Distilled water	to 1000 mL
Ferric citrate (see Note 2)	0.006 (see Note 2)

[A] Ethlenediaminetetraacetate.

[B] *Allen's Trace-Element Solution:*

Dissolve in 500 mL of distilled water:

Reagent	Amount, g
H_3BO_3	2.86
$MnCl_2 \cdot 4H_2O$	1.81
$ZnSO_4 \cdot 7H_2O$	0.222
$Na_2MoO_4 \cdot 2H_2O$	0.391
$CuSO_4 \cdot 5H_2O$	0.079
$Co(NO_3)_2 \cdot 6H_2O$	0.0494

NOTE 2—The ferric citrate must be autoclaved separately. The ferric citrate should be added after the medium has cooled from being autoclaved.

6.3.1 Adjust the pH of the medium to 7.8 using 1.0 *M* NaOH/1.0 M HCl and autoclave at 121°C (without ferric citrate added) to 45 to 50°C before aseptically adding the ferric citrate (see Note 2).

6.3.2 *Allen's Agar*—Prepare by dissolving 15 g of agar in 1000 mL Allen's Medium before autoclaving. Cool to 45 to 50°C before aseptically adding the ferric citrate. After mixing, pour the media into petri dishes.

6.4 *Bold's Basal Medium*—Prepare ten individual stock solutions in distilled water as indicated:

Stock Solutions	g/400 mL
1. $NaNo_3$	10.0
2. $MgSO_4 \cdot 7H_2O$	3.0
3. NaCl	1.0
4. K_2HPO_4	3.0
5. KH_2PO_4	7.0
6. $CaCl_2 \cdot 2H_2O$	1.0
Trace Element Solutions:	g/L
7. $ZnSO_4 \cdot 7H_2O$	8.82
$MnCl_2 \cdot 4H_2O$	1.44
MoO_3	0.71
$CuSO_4 \cdot 5H_2O$	1.57
$Co(NO_3)_2 \cdot 6H_2O$	0.49
Distilled Water	to 1 L
Autoclave to dissolve.	
8. H_3BO_3	11.42
9. EDTA–KOH solution:	
EDTA	50.0
KOH	31.0
10. $FeSO_4 \cdot 7H_2O$	4.98
H_2SO_4(concentrate)	1.0 mL

6.4.1 Combine 10 mL each of Stock Solutions 1 through 6 with 1 mL each of Stock Solutions 7 through 10 in 936 mL distilled water. Autoclave at 121°C.

6.5 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.

Algae	Collection/Strain[A]
Unicellular Green	
Chlorella sp.	ATCC 7516
Chlorella vulgaris	ATCC 11468
Filamentous Green	
Ulothrix gigas	ATCC 30443
Trentepohlia aurea	UTEX 429
Trentepohlia odorata	CCAP 483/4
Colony-forming Green	
Scenedesmus quadricauda	ATCC 11460
Filamentous Bluegreen	
Oscillatoria sp.	ATCC 29135
Calothrix sp.	ATCC 27914

[A] 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.

6.6 Cultures should be maintained separately in liquid media recommended by the culture supplier. Allen's Medium (6.3) is commonly used for bluegreen and other algae. The recipe for Bold's Basal Medium, which supports the growth of a wide range of algae is given in 6.4. If preferred, individual

[4] Bold, H. C., Wynne, M. J., "Introduction to the Algae," *Prentiss-Hall*, Englewood Cliffs, NJ, 1978, pp. 574–5.

[5] Kirsop B. E., and Snell J. J. S., "*Maintenance of Microorganisms*," *Academic Press*, Harcourt Brace Jovanovich, Orlando, FL, 1984, p. 158.

[6] *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.

cultures may be maintained on solid media prepared by dissolving 1 to 1.5 % agar in liquid medium before autoclaving.

6.6.1 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.

6.6.2 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 3—The previous 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 without algicide (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.

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

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.

NOTE 5—One or both sides of the substrate (drawdown strips or filter paper) may be coated as agreed upon between 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 strip. Another 12.7-mm 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.

7.4 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.

7.5 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 algae growth) is outlined in Note 8. Conditioning of specimens by artificial weathering may be done according to one of the following practices: D 822, D 4141, D 4587, or D 5031.

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 4-L (1-gal) containers of distilled water with a flow rate such that there are six changes in 24 h (or other flow rate as agreed upon between the parties involved). Note differences in the integrity of the coatings after this leaching. The test panels are then air dried for 24 h under the same conditions as the unleached samples, as in 7.4.

7.6 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 Allen's (or appropriate–see 6.6) agar plates. The plates should be prepared at least 24 h in advance, but no longer than one 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 Allen's (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 6.6.2) 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 oversaturating 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.

NOTE 9—If the capability is available, a cycle of 14-h light and 10-h darkness can improve the growth of the algae.

8.1.5 Incubate the samples under the specified conditions just stated and examine weekly for growth. Growth will appear as the typical green algae-like discoloration of the coating. Other species may show different colors.

9. Evaluation of Results

9.1 Rate the growth on the specimen weekly for three 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 10—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 algae-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.

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. 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).

NOTE 11—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.

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, and some means of 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,

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 is used for preconditioning, all appropriate information as required by that test method 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