Designation: D5589 – 09 (Reapproved 2013)

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.

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

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 (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.*

[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.

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

5.11 *Allen's Medium* [3] or *Bold's Basal Medium*[4] 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.[5] 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
$MgSO_4·7H_2O$	0.075
$CaCl_2·2H_2O$	0.027
Na_2CO_3	0.020
$Na_2SiO_3·9H_2O$	0.058
Citric acid	0.006
EDTA[A]	0.006
Allen's trace element solution	1.0 mL[B]
Distilled water	to 1000 m
Ferric citrate (see Note 2)	0.006 (see Note 2)

[A] Ethylenediaminetetraacetic acid, disodium salt

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

Dissolve in 500 mL of distilled water:

Reagent	Amount, g
H_3BO_3	2.86
$MnCl_2·4H_2O$	1.81
$ZnSO_4·7H_2O$	0.222
$Na_2MoO_4·2H_2O$	0.391
$CuSO_4·5H_2O$	0.079
$Co(NO_3)_2·6H_2O$	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. $MgSO_4·7H_2O$	3.0
3. NaCl	1.0
4. K_2HPO_4	3.0
5. KH_2PO_4	7.0
6. $CaCl_2·2H_2O$	1.0
Trace Element Solutions:	g/L
7. $ZnSO_4·7H_2O$	8.82
$MnCl_2·4H_2O$	1.44
MoO_3	0.71
$CuSO_4·5H_2O$	1.57
$Co(NO_3)_2·6H_2O$	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. $FeSO_4·7H_2O$	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

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

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

[5] *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.