

Standard Practice for Determining Algal Resistance of Plastic Films¹

This standard is issued under the fixed designation G 29; 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 practice covers the determination of the susceptibility of plastic films to the attachment and proliferation of surface-growing algae.

1.2 The values in SI units are to be regarded as the standard. The inch-pound units 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. Summary of Practice

2.1 In this practice, test strips of plastic film are suspended in glass jars maintained at room temperature. The test strips are exposed to fluorescent light and in direct contact with a standardized inoculum of the filamentous blue-green alga *Oscillatoria* in culture medium. The sample test jars are reinoculated with fresh alga every second or third day. A control using untreated plastic film is used as a basis of comparison. The inoculum is prepared with the help of a propagation apparatus made from a small fish tank. The test is terminated at the end of two weeks, or whenever the test film shows dense algal growth.

3. Significance and Use

3.1 Bodies of water, such as swimming pools, artificial ponds, and irrigation ditches often are lined with plastic films. Algae tend to grow in such bodies of water under the proper atmospheric conditions, and they can produce slimy and unsightly deposits on the film. The method described herein is useful in evaluating the degree and permanency of protection against surface growth of algae afforded by various additives incorporated in the film.

4. Apparatus

4.1 Propagation Tank² (see Fig. 1):

4.1.1 A small fish tank (10 gal) is used to contain a simulated cooling tower, made of redwood slats, over which culture medium is recirculated through a plastic tube with holes punched in the bottom. This design was developed in order to provide ideal conditions for propagation of the algae that serve as inocula for each test. Three redwood slats are joined by two supports in such a way that water cascades over each slat in turn from a distributor tube above the upper slat. A small, fully immersed recirculating pump³ rests on the bottom of the tank and operates continuously to deliver the tank contents to the distributor tube. The light required for algal propagation is provided by a 100-W bulb placed 300 mm (12 in.) away from the redwood slats. A timing device turns the light on for 12 h each day.

4.1.2 The propagation tank, that is used as the permanent source of inoculum, is filled to approximately one-third capacity with the culture medium.⁴ Heavy growth of *Oscillatoria* rapidly develops on the redwood tower and, at different phases, this growth appears light green, dark green, or black.

4.2 Test Chambers (see Fig. 2):

4.2.1 One-litre (1-qt) wide-mouth glass jars, 170 mm ($6\frac{3}{4}$ in.) high by 76 mm (3 in.) in inside diameter serve as test chambers wherein water containing an inoculum of the algal organisms and strips of the plastic film are maintained in contact.

4.2.2 The jars in 4.2.1 are placed in a 38-L (10-gal) fish tank, 508 by 267 by 305 mm (20 by $10\frac{1}{2}$ by 12 in.) high, that is illuminated by four 20-W "cool white" fluorescent bulbs, arranged two on each long side of the tank, at the level of the growing algae in the jars. The lamps are mounted on a bracket that holds the outer surface of the bulbs 25 mm (1 in.) from the wall of the tank. The tank is filled with water to within 25 mm of the top of the exposure jars in order to create uniform temperature conditions for all jars.

4.3 *Homogenizer*—Any suitable commercial homogenizer for preparing the algal inocula.

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 $^{^{2}\,\}mathrm{The}$ redwood tower should be soaked in water before use, until the water is colorless. Water is changed daily.

³ Model 1 MA manufactured by the Little Giant Corp., 3800 N. Tulsa Ave., Oklahoma City, OK 73107, has been found suitable for this purpose.

⁴ Culture medium in the propagation tank is discarded, and the tank is refilled with fresh medium at monthly intervals.



FIG. 1 Propagation Tank



FIG. 2 Test Chambers

5. Reagents and Materials

5.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall 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 it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

5.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean distilled water or water of equal purity.

5.3 *Culture Medium for Propagation Apparatus*—Prepare this medium by dissolving in 15 L of water the designated amounts of the following reagents:

Arnon's trace metal solution (see Note 1)	15 mL
Ethylenediamine tetraacetic acid disodium salt	0.04 g
Ferric ammonium citrate	0.85 g
Magnesium sulfate (MgSO ₄ ·7H ₂ O)	10.0 g
Potassium acid phosphate (K ₂ HPO ₄)	12.3 g
Potassium nitrate (KNO ₃)	12.1 g
Sodium citrate	2.0 g
Sulfuric acid, 10 %	10 mL

NOTE 1—Prepare this solution by combining the following reagents in the amounts designated in 1 L of water:

Boric acid	2.86 g
Copper sulfate (CuSO ₄ ·5H ₂ O)	0.079 g
Manganese chloride (MnCl ₂ ·4H ₂ O)	1.18 g
Molybdic oxide (MoO ₃)	0.018 g
Zinc sulfate $(ZnSO_4-7H_2O)$	0.22 g

5.3.1 Adjust to 7.2 to 7.5 pH range if necessary. The culture medium need not be sterilized.

5.4 *Culture Medium for Test Chambers*— Dilute 50 mL of the culture medium in 5.3 with 950 mL of tap water and place this medium in the test chambers.

6. Test Specimens

6.1 Select at random from the sample sufficient film to prepare the required number of test specimens 25 by 65 mm (1 by $2\frac{1}{2}$ in.).

6.2 When possible, use untreated film, similar in all other respects to the treated film, for testing in the same manner as the test specimens, in order to verify the viability of the test organism. If this untreated viability control material fails to show any abundant growth of the test organisms, consider the test inconclusive and repeat it.

7. Procedure

7.1 Attachment of Specimens—Samples are suspended in each test chamber by means of plastic⁶ clips hooked over a plastic or metal rod extending over the test chambers. Additional means of suspension may be used as long as no biologically active materials (for example, adhesives, cements) come in contact with the test specimens during submersion.

7.2 Inoculation of Test Chambers—Remove an area of growth about 6 mm ($\frac{1}{4}$ in.) in thickness and 6.5 cm² (1 in.²) in area from a redwood slat and place in 500 mL of test chamber culture medium (see 5.4). Homogenize until no discrete large particles can be seen. Then add 100 mL of this inoculum to each test chamber containing either a test specimen or an untreated control, and fill the jar with the culture medium.

7.3 *Incubation*—Store the inoculated test chambers under light exposure for 12 h, then under darkness for 12 h, continually repeating this cycle. Every second or third day

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.

⁶ Chromatography clips (Chromclips[®], VWR Scientific).



remove each test chamber, place in a sink, and reinoculate with 100 mL the fully homogenized algae. Introduce the inoculum into the bottom of each test chamber, allowing the same volume of old chamber water to flow over the top of the chamber. This is done in order to simulate the addition of new water and the continuous reinfection which occurs under natural conditions. At the end of two weeks, an untreated plastic film will usually develop a uniform dense coating of algal growth.

7.4 *Termination of Test*—At the end of 2 weeks, or whenever untreated films show dense algal growth, remove the films from the test chambers and place them upon white filter paper surfaces for examination.

8. Interpretation of Results

8.1 Describe the extent of growth upon each surface as follows:

None	0
Traces of growth (less than 10 %)	1
Light growth (10 to 30 %)	2
Medium growth (30 to 60 %)	3
Heavy growth (60 % to complete coverage)	4

9. Precision and Bias

9.1 A precision and bias statement cannot be made for this practice at this time.

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