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Standard Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi¹

This standard is issued under the fixed designation G 21; 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.

This standard has been approved for use by agencies of the Department of Defense. Consult the DoD Index of Specifications and Standards for the specific year of issue which has been adopted by the Department of Defense.

1. Scope

1.1 This practice covers determination of the effect of fungi on the properties of synthetic polymeric materials in the form of molded and fabricated articles, tubes, rods, sheets, and film materials. Changes in optical, mechanical, and electrical properties may be determined by the applicable ASTM methods.

1.2 The values stated in SI units are to be regarded as the standard.

2. Referenced Document

2.1 ASTM Standard:

D618 Methods of Conditioning Plastics and Electrical Insulating Materials for Testing²

3. Summary of Practice

3.1 The procedure described herein consists of (1) selection of suitable specimens for determination of pertinent properties, (2) inoculation of the specimens with suitable organisms, (3) exposure of inoculated specimens under conditions favorable to growth, (4) examination and rating for visual growth, and (5) removal of the specimens and observations or testing, either before cleaning or after cleaning and reconditioning.

NOTE 1—Since the procedure involves handling and working with fungi, it is recommended that personnel trained in microbiology perform the portion of the procedure involving handling of organisms and inoculated specimens.

4. Significance and Use

4.1 The resin portion of these materials is usually fungus-resistant in that it does not serve as a carbon source for the growth of fungi. It is generally the other components, such as plasticizers, cellulose, lubricants, stabilizers, and colorants, that are responsible for fungus attack on plastic materials. It is important to establish the resistance to microbial attack under conditions favorable for such attack, namely, a temperature of 2 to 38°C (35 to 100°F) and a relative humidity of 60 to 100 %.

4.2 The effects to be expected are as follows:

4.2.1 Surface attack, discoloration, loss of transmission (optical).

4.2.2 Removal of susceptible plasticizers, modifiers, and lubricants, resulting in increased modulus (stiffness), changes in weight, dimensions, and other physical properties, and deterioration of electrical properties such as insulation resistance, dielectric constant, power factor, and dielectric strength.

4.3 Often the changes in electrical properties are due principally to surface growth and its associated moisture and to pH changes caused by excreted metabolic products. Other effects include preferential growths caused by nonuniform dispersion of plasticizers, lubricants, and other processing additives. Attack on these materials often leaves ionized conducting paths. Pronounced physical changes are observed on products in film form or as coatings, where the ratio of surface to volume is high, and where nutrient materials such as plasticizers and lubricants continue to diffuse to the surface as they are utilized by the organisms.

4.4 Since attack by organisms involves a large element of chance due to local accelerations and inhibitions, the order of reproducibility may be rather low. To assure that estimates of behavior are not too optimistic, the greatest observed degree of deterioration should be reported.

4.5 Conditioning of the specimens, such as exposure to leaching, weathering, heat treatment, etc., may have significant effects on the resistance to fungi. Determination of these effects is not covered in this practice.

5. Apparatus

5.1 *Glassware*—Glass vessels are suitable for holding specimens when laid flat. Depending on the size of the specimens, the following are suggested:

5.1.1 For specimens up to 75 mm (3 in.) in diameter, 150-mm (6-in.) covered Petri dishes.

5.1.2 For 75 mm (3 in.) and larger specimens, such as tensile and stiffness strips, large Petri dishes, trays of borosilicate glass, or baking dishes up to 400 by 500 mm (16 by 20 in.) in size, covered with squares of window glass.

5.2 *Incubator*—Incubating equipment for all test methods shall maintain a temperature of 28 to 30°C (82.4 to 86°C) and a relative humidity not less than 85 %. Automatic recording of wet- and dry-bulb temperature is recommended.

6. Reagents and Materials

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended

¹ This practice is under the jurisdiction of ASTM Committee G-3 on Durability of Nonmetallic Materials and is the direct responsibility of Subcommittee G03.04 on Biological Deterioration.

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² Annual Book of ASTM Standards, Vol 08.01.

that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specification are available.³ 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.

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

6.3 *Nutrient-Salts Agar*⁴—Prepare this medium by dissolving in 1 L of water the designated amounts of the following reagents:

Potassium dihydrogen orthophosphate (KH ₂ PO ₄)	0.7 g
Magnesium sulfate (MgSO ₄ ·7H ₂ O)	0.7 g
Ammonium nitrate (NH ₄ NO ₃)	1.0 g
Sodium chloride (NaCl)	0.005 g
Ferrous sulfate (FeSO ₄ ·7H ₂ O)	0.002 g
Zinc sulfate (ZnSO ₄ ·7H ₂ O)	0.002 g
Manganous sulfate (MnSO ₄ ·H ₂ O)	0.001 g
Agar	15.0 g
Potassium monohydrogen orthophosphate (K ₂ HPO ₄)	0.7 g

Sterilize the test medium by autoclaving at 121°C (250°F) for 20 min. Adjust the pH of the medium by the addition of 0.01 N NaOH solution so that after sterilization the pH is between 6.0 and 6.5. Prepare sufficient medium for the required tests.

6.4 *Mixed Fungus Spore Suspension*:

NOTE 2—Since a number of other organisms may be of specific interest for certain final assemblies or components, such other pure cultures of organisms may be used if agreed upon by the purchaser and the manufacturer of the plastic. Reference (1) illustrates such a choice.

6.4.1 Use the following test fungi in preparing the cultures:

Fungi	ATCC No. ^a	MYCO No. ^b
<i>Aspergillus niger</i>	9642	386
<i>Penicillium pinophilum</i> ^c	11797	391
<i>Chaetomium globosum</i>	6205	459
<i>Glocladium virens</i>	9645	365
<i>Aureobasidium pullulans</i>	15233	279c

^a American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852.

^b Mycological Services, Box 1056, Crawfordsville, IN 47933.

^c Historically known as *funiculosm*.

Maintain cultures of these fungi separately on an appropriate medium such as potato dextrose agar. The stock cultures may be kept for not more than 4 months at approximately 3 to 10°C (37 to 50°F). Use subcultures incubated at 28 to 30°C (82 to 86°F) for 7 to 20 days in preparing the spore suspension.

6.4.2 Prepare a spore suspension of each of the five 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 dioctyl sulfosuccinate. Use a sterile platinum or nichrome inoculating wire to scrape gently the surface growth from the culture of the test organism.

³ "Reagent Chemicals, American Chemical Society Specifications," Am. Chem. Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, NY, and the "United States Pharmacopeia."

⁴ Agar and nutrient-salts agar are obtainable from biological laboratory supply sources.

6.4.3 Pour the spore charge into a sterile 125-mL glass-stoppered Erlenmeyer flask containing 45 mL of sterile water and 10 to 15 solid glass beads, 5 mm in diameter. Shake the flask vigorously to liberate the spores from the fruiting bodies and to break the spore clumps.

6.4.4 Filter the shaken suspension through a thin layer of sterile glass wool in a glass funnel into a sterile flask in order to remove mycelial fragments.

6.4.5 Centrifuge the filtered spore suspension aseptically, and discard the supernatant liquid. Resuspend the residue in 50 mL of sterile water and centrifuge.

6.4.6 Wash the spores obtained from each of the fungi in this manner three times. Dilute the final washed residue with sterile nutrient-salts solution (Note 3) in such a manner that the resultant spore suspension shall contain 1 000 000 ± 200 000 spores/mL as determined with a counting chamber.

6.4.7 Repeat this operation for each organism used in the test and blend equal volumes of the resultant spore suspension to obtain the final mixed spore suspension.

NOTE 3—Nutrient salts solution is identical with the composition for nutrient salts agar given in 6.3 except that the agar is omitted.

6.4.8 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.

7. Viability Control

7.1 With each daily group of tests place each of three pieces of sterilized filter paper, 25 mm (1 in.) square, on hardened nutrient-salts agar in separate Petri dishes. Inoculate these with the spore suspension by spraying the suspension from a sterilized atomizer⁵ so that the entire surface is moistened with the spore suspension. Incubate these at 28 to 30°C (82 to 86°F) at a relative humidity not less than 85 % and examine them after 14 days' incubation. There shall be copious growth on all three of the filter paper control specimens. Absence of such growth requires repetition of the test.

8. Test Specimens

8.1 The simplest specimen may be a 50 by 50-mm (2 by 2-in.) piece, a 50-mm (2-in.) diameter piece, or a piece (rod or tubing) at least 76 mm (3 in.) long cut from the material to be tested. Completely fabricated parts or sections cut from fabricated parts may be used as test specimens. On such specimens, observation of effect is limited to appearance, density of growth, optical reflection or transmission, or manual evaluation of change in physical properties such as stiffness.

8.2 Film-forming materials such as coatings may be tested in the form of films at least 50 by 25 mm (2 by 1 in.) in size. Such films may be prepared by casting on glass and stripping after cure, or by impregnating (completely covering) filter paper or ignited glass fabric.

8.3 For visual evaluation, three specimens shall be inoculated. If the specimen is different on two sides, three specimens of each, face up and face down, shall be tested.

⁵ DeVilbiss No. 163 atomizer or equivalent has been found satisfactory for this purpose.