Designation: D 2020 – 92 (Reapproved 2003)

An American National Standard

Standard Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard[1]

This standard is issued under the fixed designation D 2020; 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 These test methods cover the qualitative determination of mildew (fungus) resistance of paper and paperboard, particularly those types which have been given a fungus resistant treatment.

1.2 The two test methods appear in the following order:

	Sections
Method A—Direct Inoculation, Pure Culture, Nonsterile Specimen	6 to 12
Method B—Soil Burial	13 to 17

1.3 The direct inoculation, pure culture, nonsterile specimen method is applicable to paper products that are expected to be used or stored in a damp, warm atmosphere, but out of contact with damp soil and is the preferred method. The soil burial method may be useful, and is recommended, for papers, with or without fungus resistant treatment, which may be in contact with damp soil for long periods of time.

1.4 *This standard does not purport to address all of the safety problems, 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]

D 585 Practice for Sampling and Accepting a Single Lot of Paper, Paperboard, Fiberboard, or Related Product

D 828 Test Method for Tensile Breaking Strength of Paper and Paperboard

D 1193 Specification for Reagent Water

D 1968 Terminology Relating to Paper

3. Terminology

3.1 *Definitions*—Definitions shall be in accordance with Terminology D 1968 and the *Dictionary of Paper*.[3]

4. Significance and Use

4.1 Paper products used or stored in damp warm atmospheres or in contact with damp soil are subject to attack by fungus and other microorganisms. These test methods cover procedures for evaluating the degree of resistance to attack and for evaluating the degree and permanency of protection to attack by paper treatments.

5. Sampling

5.1 Obtain a sample of the paper to be evaluated in accordance with Practice D 585.

METHOD A—DIRECT INOCULATION, PURE CULTURE, NONSTERILE SPECIMEN

6. Apparatus

6.1 *Autoclave*, capable of being operated at a pressure of 15 psi (103 kPa) steam pressure and an exhaust temperature of 121°C (250°F), for sterilization of media.

6.2 *Bottles for Inoculum*—Several 250-mL, narrow-mouthed, square-sectional glass bottles fitted with screw caps are used for water blanks. Standard milk-dilution bottles scribed at the 99-mL level are recommended. The blanks are prepared by adding to each bottle approximately 13 mm of glass beads, 100 mL of water, and three or four drops of suitable wetting agent. The water blanks are then ready for sterilizations.

6.3 *Plugs*, nonabsorbent cotton or other suitable closures.

6.4 *Flaming Equipment*—Depending upon the circumstances, an alcohol lamp or a Bunsen burner may be used to flame the inoculating needle and the mouths of sterile containers.

6.5 *Containers*—Erlenmayer flasks, 250 mL, or bottles are convenient containers for sterile media.

6.6 *Oven*, capable of maintaining a temperature of 165°C.

6.7 *Incubator*, capable of maintaining a temperature of 28 ± 1°C to provide proper incubation of the inoculum and for the inoculated specimens.

6.8 *Inoculating Needle*—A standard inoculating needle fitted with either 22- or 24-gage Nichrome wire.

6.9 *Test Tubes*—18 by 150-mm rimless bacteriological test tubes are used for growing the test organisms.

6.10 *Petri Dishes*, disposable, 100 by 15 mm are recommended.

[1] These test methods are under the jurisdiction of ASTM Committee D06 on Paper and Paper Products and are the direct responsibility of Subcommittee D06.92 on Test Methods.

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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.

[3] Formerly published by American Paper & Pulp Assoc. (API), New York, NY (4th ed., 1980).

6.11 *Pipets*—Graduated 5- or 10 mL Mohr pipets, with the tips cut off and fire-polished to give an opening about 3 mm in diameter at the delivery end, are best for pipetting the spore-mycelial inoculum and for measuring the volume of culture medium added to each Petri dish. The Kolmer is also a satisfactory type of pipet. Presterilized disposable pipets may be used.

6.12 *Scissors*—A satisfactory type has approximately 100 mm cutting edges.

6.13 *Colony Counter*—Either of the standard bacterial colony counters or a large magnifying lens is helpful but not required for the examination of fungus growth on the test specimen.

7. Reagents and Materials

7.1 *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.[4] 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.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean freshly boiled and cooled reagent water, Type I or II, as described in Specification D 1193.

7.3 *Test Organisms*—The fungi recommended as standard organisms for this test are: *Chaetomium globosum*[3] (A.T.C.C.-6205), *Aspergillus terreus*[3] (QM-82J), and *Aspergillus niger*[3] (BL-89). Other species of fungi may be included in the test which have been isolated from paper products that have failed under certain use conditions. These additional test organisms may require the addition of dextrose to the nutrient salt agar before they will grow on the test specimens.

7.4 *Nutrient Media*—Two kinds are needed, as follows:

7.4.1 *Potato-Dextrose Agar*—This medium is used to maintain the test organisms in stock and to grow the test organism for the inoculum. This standard medium can be obtained from many biological supply houses.

7.4.2 *Mineral-Salt Agar*—This medium is used in the test to provide a portion of the nutrient requirements of the test organisms. The composition is as follows:

Ammonium nitrate (NH_4NO_3)	3.0 g
Dipotassium phosphate (K_2HPO_4)	1.4 g
Potassium chloride (KCl)	0.25 g
Magnesium sulfate ($MgSO_4$ $7H_2O$)	0.25 g
Agar	10.0 g
Tap water	1000.0 mL

7.4.3 After the ingredients are dissolved, the hot mixture is dispensed into appropriate containers. For the medium described in 7.4.1, approximately 10 mL of the mixture are dispensed into each test tube, and then the tube is plugged with cotton. For the medium described in 7.4.2, the mixture may be dispensed into either Erlenmeyer flasks or bottles and plugged with cotton, or the bottles may be capped with suitable screw caps. The culture media are then ready to be sterilized.

7.4.3.1 The mineral-salt agar may be modified by adding 0.5 % of dextrose, provided this sugar is necessary to fulfill the nutritive requirements of the test organisms. The use of this modified mineral-salt agar must be stated in the report.

7.5 *Preparation of Inoculum*:

7.5.1 *Spore-Mycelial Method*:

7.5.1.1 Inoculate two potato-dextrose agar slants with each organism to be used in the test and incubate for 14 days at 28°C. At the end of this incubation period, check to see that each agar slant is covered with mycelium and fruiting bodies of the test organism.

7.5.1.2 To each agar slant, add 5 mL of sterile water and suspend the surface growth in the liquid by gently scraping the surface of the slant with a sterile inoculating needle. Pool the suspension from each set of two slants in the water blank from which the water was removed. Shake the spore-mycelial suspension thoroughly to break the spore and mycelial clumps. Repeat this procedure for each organism used in the test.

7.5.2 *Spore-Cloud Method*:

7.5.2.1 If desired, this method of preparing the inoculum may be substituted for the spore-mycelial method. Thoroughly mix 30 g of wheat brand with an equal weight of 0.1*N* HCl and sterilize in a loosely capped 947 mL (1-qt) Mason jar for 30 min at 103 kPa (15 psi). Inoculate the cooled sterilized bran by intimate mixing with 10 mL of a spore-mycelial suspension of the test organism which has been grown on a potato-dextrose agar slant. Prepare the suspension by washing the surface of the slant with 10 mL of sterile water. If necessary, loosen the spores and mycelium with a sterile inoculating needle.

7.5.2.2 To secure optimum surface, spread out the inoculated bran in the Mason jar, which is set on its side, and incubate in this position at 28°C. After about 48 h of incubation, the fungus will have grown rapidly through the bran. Then, using aseptic techniques, break the mat which has formed into small clumps with a sterile knife or spatula and incubate until the fungus has sporulated throughout the bran. After sporulation, spread out the bran in the closed container and allow to dry. Then stopper the dried bran tightly and store at room temperature. It is recommended that the inoculated bran be prepared every 6 months.

8. Viability Control

8.1 When possible, use untreated material similar in all other respects to the treated material, for testing in the same manner as the test specimen to verify the viability of test organisms. When such material is not available, use material known to support the growth of the particular organism, such as filter paper. If this untreated material fails to show any abundant growth of the test organisms, consider the test inconclusive and repeat it.

9. Test Specimens

9.1 Select at random from the sample test specimens by cutting under aseptic conditions (preferably under a laminar-flow hood), 50 mm squares at random from the sample, the

[4] "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, D.C. 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, N.Y., and the "United States Pharmacopeia."