



Designation: D4300 – 01 (Reapproved 2021)^{ε1}

Standard Test Methods for Ability of Adhesive Films to Support or Resist the Growth of Fungi¹

This standard is issued under the fixed designation D4300; 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 U.S. Department of Defense.

^{ε1} NOTE—Editorial changes were made to 8.1 and 8.2 in April 2021.

1. Scope

1.1 These test methods test the ability of adhesive films to inhibit or support the growth of selected fungal species growing on agar plates by providing means of testing the films on two agar substrates, one which promotes microbial growth, and one which does not.

1.2 These test methods are not appropriate for all adhesives. The activity of certain biocides may not be demonstrated by these test methods as a result of irreversible reaction with some of the medium constituents.

NOTE 1—As an example, quaternary ammonium compounds are inactivated by agar.

1.3 A test method is included for use with low-viscosity adhesives along with an alternative method for use with mastic-type adhesives. Also, a method approved by the government is given.

1.4 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.5 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* These test methods are designed to be used by persons trained in correct microbiological techniques. Specific precautionary statements are given in Section 7 and in 14.3.2.

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recom-*

mendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced Documents

2.1 *ASTM Standards:*²

D907 Terminology of Adhesives

D1286 Method of Test for Effect of Mold Contamination on Permanence of Adhesives Preparations and Adhesives Bonds (Withdrawn 1983)³

G21 Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi

2.2 *TAPPI Method:*⁴

T487 Fungus Resistance for Paper and Paperboard

3. Terminology

3.1 *Definitions*—Many terms in this test method are defined in Terminology D907.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *adhesive preparation, n*—the adhesive as packaged for distribution, storage, and use.

3.2.2 *adhesive film, n*—the small portion of the adhesive preparation, as prepared for use by the consumer, either with additives or as received, which is cast on a substrate, cured 24 h, and represents the glue line.

3.2.2.1 *Discussion*—For purposes of these test methods the *adhesive film* is the thin layer of adhesive spread on either the 21-mm fiberglass disk as described in 14.2, or the adhesive layer 3 mm thick which is cast on the tile squares as described in 15.1.

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 The last approved version of this historical standard is referenced on www.astm.org.

⁴ Available from Technological Association of the Pulp and Paper Industry (TAPPI), 15 Technology Parkway South, Suite 115, Peachtree Corners, GA 30092, <http://www.tappi.org>.

¹ These test methods are under the jurisdiction of ASTM Committee D14 on Adhesives and are the direct responsibility of Subcommittee D14.30 on Wood Adhesives.

Current edition approved April 1, 2021. Published April 2021. Originally approved in 1983. Last previous edition approved in 2013 as D4300 – 01 (2013). DOI: 10.1520/D4300-01R21E01.

3.2.3 *zone of inhibition, n*—the area on an inoculated agar plate surrounding the adhesive-coated disk or tile, showing a reduced fungal growth or an absence thereof.

3.3 Abbreviations:

3.3.1 *PDA*—potato dextrose agar.

3.3.2 *MSA*—mineral salts agar.

3.3.3 *ZI*—zone of inhibition.

4. Significance and Use

4.1 These test methods are designed to be used to determine the susceptibility of the adhesive film to biodegradation and whether the adhesive will carry into the bond line sufficient anti-fungal properties to prevent growth of fungi frequently present on the gluing equipment, on adherends, or in the adhesive as applied.

4.2 Potato dextrose agar (PDA) provides a complete medium for the growth of fungi, while mineral salts agar (MSA) lacks a carbohydrate source and provides a less favorable medium. Use of PDA tests the adhesive film for its ability to resist the growth of fungi on its surface as well as its ability to repel a copious growth of fungi on the adjacent agar surface. Use of MSA tests the adhesive film primarily for its ability to resist the growth of fungi on its surface. When it is used, there is a reduced possibility that the growth from the agar will be mis-read as coming from the adhesive film, since fungal growth on the adjacent agar will be scant.

NOTE 2—The method given here using the MSA is based on Practice G21, adapted to be used with adhesives. Requirements to meet the approval of government specifications are the use of the MSA described in 10.2, and a mixed species of fungi described in 8.2 for the inoculum.

4.3 The results obtained when using the procedures given in this method apply only to the species used for the testing. The test species listed in Section 8 are frequently used by laboratories to test for antifungal properties, but they are not the only ones which could be used. Selection of the fungal species to test against requires informed judgment by the testing laboratory or by the party requesting the tests. These methods are especially useful when species that have been isolated from contaminated adhesives are used as the test species (see Section 8) to aid in the selection of more effective fungicides.

4.4 The efficacy of some biocides may change in storage due to the chemical and thermal environment to which they are subjected as components of certain adhesives. These test methods are not appropriate for determining the effect of fungal contamination on adhesives under water-soaking conditions, because they are not designed to cover the possibility of water-soluble biocides leaching out of the bond line.

4.5 These test methods are dependent upon the physiological action of living microorganisms under a reported set of conditions. Conclusions about the resistance of the test adhesive to fungal attack can be drawn by comparing the results to simultaneously run controls of known resistance. See X5.2 for statements regarding test repeatability.

5. Apparatus

5.1 In addition to the standard equipment found in any fully equipped microbiological laboratory, items from the following list are needed for various tests. Not all items are needed for each test.

5.1.1 *Chromist Laboratory Spray Unit*.⁵

5.1.2 *Constant Temperature Chamber*, capable of being maintained at $35 \pm 0.5^\circ\text{C}$ ($95 \pm 1^\circ\text{F}$) or $25 \pm 0.5^\circ\text{C}$ ($77 \pm 1^\circ\text{F}$), or two chambers if needed simultaneously.

5.1.3 *Filter Disk, Glass Microfibre, 934-AM*, diameter-21 mm.⁶

5.1.4 *Filter Disk, Sterile Whatman No. 1*.⁶

5.1.5 *Filter Paper Assay Disk*, 1.5 cm diameter, sterile. Schleicher and Schnell, Inc., or the equivalent, has been found satisfactory for this purpose.⁶

5.1.6 *Glass Rods*, 305 mm in length having a diameter of 6.3 mm.

5.1.7 *Glove Bag*, 68 cm in length and width, 38 cm in height.⁷

5.1.8 *Hemocytometer Levy Counting Chamber*, cell depth-0.1 mm, Newbauer rulings.⁶

5.1.9 *Hood, Laminar-Flow Type, Class II Type I*.⁸

5.1.10 *Jar, Screw Cap*, round, approximately 1 L (1 qt, mason type).

5.1.11 *Pipet, Pasteur*.⁶

5.1.12 *Petri Dishes*, sterile, disposable, top-diameter of 150-mm, bottom-height of 15-mm.

5.1.13 *Refrigerator*, capable of maintaining $4 \pm 1^\circ\text{C}$ ($39 \pm 2^\circ\text{F}$).

5.1.14 *Teflon Paper or Grid*, pressure sensitive overlay, coated with TFE-fluorocarbon (PTFE), vinyl sheet backing, to be used at up to 93°C (200°F).⁹

6. Materials

6.1 *Potato Dextrose Agar*, Difco or equivalent.

6.2 *Sterile Deionized or Distilled Water*.

6.3 *Disinfectant Solution*—Amphyll, Alcide, or comparable product.

6.4 *Materials for Mineral Salts Agar*. (See list in 10.2.1.)

6.5 *Sorbitan mono-oleate polyoxyethylene*.¹⁰

7. Precautions

7.1 Assign laboratory personnel trained in correct microbiological techniques to run these tests. These test methods employ live cultures of fungi, some of which are capable of causing disease or allergic reaction in some humans. Use proper microbiological procedures in order to prevent contamination of the cultures or of the work area. Disinfect and sterilize in an approved manner all spills and all equipment

⁵ Available from Gelman Sciences, Ann Arbor, MI.

⁶ Available from laboratory supply houses.

⁷ Available from Instruments for Research and Industry, 108 Franklin Ave., Cheltenham, PA, or most laboratory supply houses.

⁸ The Biogard Hood or similar equipment is available from laboratory supply houses.

⁹ Gelman Sciences, or most laboratory supply houses.

¹⁰ Available commercially as Tween 80.