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Standard Guide for Standard Test Methods and Practices Available for Determining Antifungal Activity on Natural or Synthetic Substrates Treated with Antimicrobial Agents¹

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1. Scope

1.1 This guide provides information on various test methods currently available to assess antifungal activity on natural or synthetic substrates.

1.2 Knowledge of microbiological techniques is required for the practice of this guide.

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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.4 *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 Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*²

C1338 Test Method for Determining Fungi Resistance of Insulation Materials and Facings

D2020 Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard (Withdrawn 2009)³

D3273 Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber

D3456 Practice for Determining by Exterior Exposure Tests the Susceptibility of Paint Films to Microbiological Attack

D4300 Test Methods for Ability of Adhesive Films to Support or Resist the Growth of Fungi

D4445 Test Method for Fungicides for Controlling Sapstain and Mold on Unseasoned Lumber (Laboratory Method)

D4576 Test Method for Mold Growth Resistance of Wet Blue and Wet White

D4783 Test Methods for Resistance of Adhesive Preparations in Container to Attack by Bacteria, Yeast, and Fungi

D5259 Test Method for Isolation and Enumeration of Enterococci from Water by the Membrane Filter Procedure

D5590 Test Method for Determining the Resistance of Paint Films and Related Coatings to Fungal Defacement by Accelerated Four-Week Agar Plate Assay

D6329 Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to Support Microbial Growth Using Static Environmental Chambers

D6469 Guide for Microbial Contamination in Fuels and Fuel Systems

D6974 Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels—Filtration and Culture Procedures

D7436 Classification System for Unfilled Polyethylene Plastics Molding and Extrusion Materials with a Fractional Melt Index Using ISO Protocol and Methodology

D7584 Test Method for Evaluating the Resistance of the Surface of Wet Blue and Wet White to the Growth of Fungi in an Environmental Chamber

D7855/D7855M Test Method for Determination of Mold Growth on Coated Building Products Designed for Interior Applications Using an Environmental Chamber and Indirect Inoculation

D7910 Practice for Collection of Fungal Material From Surfaces by Tape Lift

E1326 Guide for Evaluating Non-culture Microbiological Tests

E2111 Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporocidal Potencies of Liquid Chemicals

E2197 Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporocidal Activities of Chemicals

¹ This guide is under the jurisdiction of ASTM Committee E35 on Pesticides, Antimicrobials, and Alternative Control Agents and is the direct responsibility of Subcommittee E35.15 on Antimicrobial Agents.

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

E2471 Test Method for Using Seeded-Agar for the Screening Assessment of Antimicrobial Activity In Carpets

E2756 Terminology Relating to Antimicrobial and Antiviral Agents

E3227 Test Practice for Qualitative Assessment of Antifungal Activity on Textiles

F1094 Test Methods for Microbiological Monitoring of Water Used for Processing Electron and Microelectronic Devices by Direct Pressure Tap Sampling Valve and by the Presterilized Plastic Bag Method

G21 Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi

2.2 *AATCC Standards:*⁴

AATCC 30 Antifungal Activity, Assessment on Textile Materials: Mildew and Rot Resistance of Textile Materials

AATCC TM 90 2016 Antimicrobial Activity Assessment of Textile Materials: Agar Plate Method

AATCC 174 (Part III)-2016 Antimicrobial Activity Assessment of Carpets

2.3 *AWPA Standards:*⁵

AWPA E10-11 Standard Method of Testing Wood Preservatives by Laboratory Soil-Block Cultures

AWPA E24-15 Standard Method of Evaluating the Resistance of Wood Product Surfaces to Mold Growth

2.4 *BSi Standards:*⁶

BS 3900:Part G6:1989 British Standard Methods of test for paints Part G6. Assessment of resistance to fungal growth

BS EN 113:1997 Wood preservatives – Test method for determining the protective effectiveness against wood destroying basidiomycetes – Determination of the toxic values

BS EN 1104:2005 Paper and Board intended to come into contact with foodstuffs – Determination of the transfer of antimicrobial constituents

2.5 *ISO Standards:*⁷

ISO 846 Evaluation of the Action of Microorganisms on Plastics

ISO 16000 Indoor Air Sampling Strategy for Moulds

ISO 16256 Clinical Laboratory Testing and in-vitro diagnostic test systems – Reference method for testing the in vitro activity of antimicrobial agents against yeast fungi involved in infectious disease

2.6 *JIS Standards:*

JIS K 1571:2010 Test methods for determining the effectiveness of wood preservatives and their performance requirements

2.7 *Other Standards:*

Ford Motor Company Specification

MIL-STD-810G Method 508.6 Fungus

TAPPI T-487 Fungus Resistance of Paper and Paperboard

EPA Pesticide Assessment Guidelines – Subdivision G Product Performance, Section 93-30⁸

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this guide, refer to Terminology **E2756**.

4. Significance and Use

4.1 Fungi are known to produce objectionable odors, stains, and premature biodeterioration of various consumer products and construction substrates including textiles, carpet, ceiling tile, gypsum wallboard, lumber, and plasticized vinyl and other polymers.

4.2 Antifungal activity is typically:

4.2.1 Determination of article susceptibility to fungal colonization,

4.2.2 Determination of fungistatic activity (qualitative determination of prevented or delayed fungal colonization), and

4.2.3 Determination of fungicidal/sporicidal activity (quantitative determination of spore kill).

4.3 The degree of required surface examination varies from gross visual examination to detailed microscopic assessment among these methods.

4.4 This guide provides an overview of established methods and suggestions for their applicability, with consideration to the type of substrate treated or the type of antifungal treatment being assessed.

5. Methods Overview

ASTM Standards

5.1 **C1338** Test Method for Determining Fungi Resistance of Insulation Materials and Facings (Qualitative measure of susceptibility and/or fungistatic activity)

5.1.1 *Scope*—This test method covers the determination of the ability of new insulation materials and their facings to support fungal growth.

5.1.2 *Significance and Use:*

5.1.2.1 The type of materials used in the manufacture of insulation products and the type of membrane used to face these products can sometimes affect fungi sustenance in the presence of high humidity.

5.1.2.2 This test method is used to determine the relative ability of an insulation and its facing to support or resist fungal growth under conditions favorable for their development.

5.1.2.3 This test method uses a comparative material to determine the relative ability of a material to support fungal growth. In some specialized product areas, it is required that no growth takes place. In such cases, the use of the comparative material is omitted and the pass/fail criterion is based upon growth.

⁴ Available from American Association of Textile Chemists and Colorists (AATCC), P.O. Box 12215, Research Triangle Park, NC 27709-2215, <http://www.aatcc.org>.

⁵ Available from American Wood Protection Association (AWPA), P.O. Box 361784, Birmingham, AL 35236-1784, <http://www.awpa.com>.

⁶ Available from British Standards Institution (BSI), 389 Chiswick High Rd., London W4 4AL, U.K., <http://www.bsigroup.com>.

⁷ Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, <http://www.ansi.org>.

⁸ Available from United States Environmental Protection Agency (EPA), William Jefferson Clinton Bldg., 1200 Pennsylvania Ave., NW, Washington, DC 20460, <http://www.epa.gov>.

5.2 **D2020** Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard

5.2.1 *Scope*—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.

5.2.2 *Significance and Use*—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 and permanency of protection to attack by paper treatments.

5.2.3 *Summary of the Practice*—This test includes two test methods which can be used singly or in combination. Method A involves direct inoculation of pure test cultures on non-sterile specimens. Method B involves burying test samples in direct contact with soil.

5.2.3.1 Method A is an accelerated screen of both susceptibility and fungistatic activity. Samples are placed on plates of mineral-salt agar (nutrient salts agar) and tested against *Aspergillus niger*, *Aspergillus terreus*, and *Chaetomium globosum*.

5.2.3.2 Assessment of the samples is performed at least once prior to seven days of incubation and again after seven days of incubation. If no growth is observed on specimens after seven days the samples are incubated an additional week.

(1) Samples are rated as fungal resistant, not fungal resistant, or moderately fungal resistant.

5.2.3.3 Method B is an accelerated screen where samples are buried in soil for two weeks. Samples are rated after burial the samples are removed, cleaned, dried, and tensile breaking strength determined.

5.3 **D3273** Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber (Qualitative measure of susceptibility and fungistatic activity)

5.3.1 *Scope*—This test method describes a small environmental chamber and the conditions of operation to evaluate reproducibly in a 4-week period the relative resistance of paint films to surface mold fungi, mildew growth in a severe interior environment. The apparatus is designed so it can be easily built or obtained by any interested party.

5.3.1.1 This test method can be used to evaluate the comparative resistance of interior coating to accelerated mildew growth. Performance at a certain rating does not imply any specific period of time for a fungal free coating. However, a better rated coating nearly always performs better in actual end use.

5.3.1.2 Temperature and humidity must be effectively controlled within the relatively narrow limits specified in order for the chamber to function reproducibly during the short test period. Severity and rate of mold growth on a film is a function of the moisture content of both the film and the substrate.

5.3.2 *Significance and Use*—An accelerated test for determining the resistance of interior coatings to mold growth is useful in estimating the performance of coatings designed for use in interior environments that promote mold growth and in evaluating compounds that may inhibit such growth and the aggregate levels for their use.

5.3.3 *Summary of Method*—This method is favored for creating environmental conditions that are conducive for mold growth. Use of potting soil along with a mixed fungal spore inoculum mimics exposures in soiled and humid environments.

5.3.3.1 Typical industry modifications to this method include evaluation of a variety of substrates beyond interior coatings. These include wood, ceiling tile, gypsum wall board, fabrics and carpet.

5.3.3.2 This method is useful for identification of mold susceptible components of a product.

5.3.3.3 The environmental chamber creates an environment that poses a “worst case scenario” for an incorporated antifungal agent. The method also may be useful in assessing durability of such treatments.

5.4 **D3456** Practice for Determining by Exterior Exposure Tests the Susceptibility of Paint Films to Microbiological Attack (qualitative assessment of microbiological disfigurement of exterior paint films; mold, bacterial or algal)

5.4.1 *Scope*—This practice provides guidelines for determining the susceptibility of paint films to microbiological attack on exterior exposure. While it is recognized that various organisms may occur on an exposed coating, the specific types of organisms are mainly of academic interest. The degree to which microbiological discoloration occurs is the primary concern.

5.4.2 *Summary of the Practice*—Simple observation of a coated object subjected to exterior exposure is considered a practical and reliable method for determining the degree that microorganisms discolor the coating. However, this applies to a specific coated object exposed under a given set of conditions. It should be recognized that there are critical factors that influence the amount of fungal growth that may occur on the same coated object when exposed to other conditions. These factors include the geographic location, local atmospheric conditions such as the dust and pollen content of the air, angle of exposure, degree to which the coating is subjected to weathering, effects of moisture and sunlight, the substrates on which the coating is applied, and the coatings in the paint system under test. The latter factor includes the stability of the coating while packaged in the container, as well as the composition of the coatings included in the total system and the thickness of each coating applied. Thus, while microorganisms occur on the surface of the last film applied, the degree of microbiological growth that will occur is also influenced by the composition of the undercoats. All the above factors should be considered in the selection of a coating resistant to discoloration by microorganisms.

5.5 **D4300** Test Method for Ability of Adhesive Films to Support or Resist the Growth of Fungi

5.5.1 *Scope*—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.

5.5.1.1 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.

5.5.1.2 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.

5.5.2 *Significance and Use*—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 adherents, or in the adhesive as applied.

5.6 **D4445** Test Method for Fungicides for Controlling Sapstain and Mold on Unseasoned Lumber (Laboratory Method)

5.6.1 *Scope*—This (laboratory) method is used for determining the minimum concentration of fungicide, or formulation of fungicides, that is effective in preventing biodeterioration by sapstain fungi and molds in selected species of wood under optimum laboratory conditions.

5.6.2 *Significance and Use*—This method is useful as a screening procedure for selecting fungicides or formulations for more rigorous field evaluation.

5.7 **D4576** Test Method for Mold Growth Resistance of Wet Blue and Wet White

5.7.1 *Scope*—This method covers the determination of mold growth resistance of Wet Blue and Wet White subject to storage and shipping requirements and intended for use in leather manufacturing.

5.7.2 *Significance and Use*—This method provides a technique for evaluating mold growth resistance characteristics of Wet Blue and Wet White, and should assist in the prediction of storage time before molding occurs.

5.7.3 *Summary of Method*—Conclusions about mold growth resistance are drawn from comparisons of the test materials with previously run controls of known resistance.

5.8 **D4783** Test Methods Resistance of Adhesive Preparations in Container to Attack by Bacteria, Yeast, and Fungi

5.8.1 *Scope*—The test methods cover the determination of the resistance of liquid adhesive preparations to microbial attack in the container by challenging adhesive specimens with cultures of bacteria, yeast, or fungi, and checking for their ability to return to sterility. These test methods return qualitative results.

5.8.2 *Significance and Use*—These test methods are used to demonstrate whether an adhesive preparation is sufficiently protected with biocide to resist attack by bacteria, yeast, and fungi during its storage life. They are patterned after methods used by biological laboratories serving the adhesive industry.

5.8.2.1 These test methods may also be used to determine the efficacy of different biocide systems against specific microorganisms.

5.8.2.2 These test methods are especially useful when tested against wild-type microorganisms which have been isolated from contaminated adhesives as an aid in determining the amount and type of biocide necessary to kill or inhibit the growth of the contaminants. If an isolated microorganism not generally used as a challenge organism, is chosen as the inoculum, it is important to identify the organism and deter-

mine on which medium and under what conditions it will grow, in order to demonstrate the efficacy of the biocide.

5.8.2.3 The results obtained when using the procedures given in these methods apply only to the species which are used for the testing. The test species listed in Section 9 (of the method) are frequently used by laboratories to test for antimicrobial properties, but they are not the only ones which could be used. Selection of the species to use for these test methods requires informed judgment by the testing laboratory or by the party requesting the tests. It is also important that species which commonly attack adhesives be used.

5.9 **D5590** Test Method for Determining the Resistance of Paint Films and Related Coatings to Fungal Defacement by Accelerated Four-Week Agar Plate Assay (Qualitative measure of susceptibility and fungistatic activity)

5.9.1 *Scope*—This test method covers an accelerated method for determining the relative resistance of two or more paints or coating films to fungal growth.

5.9.2 *Significance and Use*—Defacement of paint and coating films by fungal growth (mold, mildew) is a common phenomenon, and defacement by algal growth can also occur under certain conditions. It is generally known that differences in the environment, lighting, temperature, humidity, substrate pH, 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.

5.9.3 *Summary of Method*—This test is an accelerated screen of both susceptibility and fungistatic activity. Use of potato dextrose agar provides rapid growth conditions for a mixed spore challenge of *Aspergillus* and *Penicillium* as well as a challenge plate for the slower growing mold *Aureobasidium*.

5.9.3.1 Weekly assessment for four weeks provides data on the susceptibility and or fungistatic activity of a treated paint or coating in laboratory growth conditions.

5.10 **D6329** Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to Support Microbial Growth Using Static Environmental Chamber

5.10.1 *Scope*—Many different types of microorganisms (for example, bacteria, fungi, viruses, algae) can occupy indoor spaces. Materials that support microbial growth are potential indoor sources of biocontaminants (for example, spores and toxins) that can become airborne indoor biopollutants. This guide describes a simple, relatively cost effective approach to evaluating the ability of a variety of materials to support microbial growth using a small chamber method.

5.10.1.1 This guide is intended to assist groups in the development of specific test methods for a definite material or groups of material.

5.10.1.2 Static chambers have certain limitations. Usually, only small samples of indoor materials can be evaluated. Care must be taken that these samples are representative of the materials being tested so that a true evaluation of the material is performed.