Designation: E3152 – 18

Standard Guide for Standard Test Methods and Practices Available for Determining Antifungal Activity on Natural or Synthetic Substrates Treated with Antimicrobial Agents[1]

This standard is issued under the fixed designation E3152; 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 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D2020 Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard (Withdrawn 2009)[3]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

D4141/D4141M Practice for Conducting Black Box and Solar Concentrating Exposures of Coatings

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

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 Sporicidal Potencies of Liquid Chemicals

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

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

[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.

Current edition approved Feb. 1, 2018. Published March 2018. DOI: 10.1520/E3152–18

[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.

E2722 Test Method for Using Seeded-Agar for the Screening Assessment of Antimicrobial Activity in Fabric and Air Filter Media
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 30-2015 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

[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.

3. Significance and Use

3.1 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.

3.2 Antifungal activity is typically:
3.2.1 Determination of article susceptibility to fungal colonization,
3.2.2 Determination of fungistatic activity (qualitative determination of prevented or delayed fungal colonization), and
3.2.3 Determination of fungicidal/sporicidal activity (quantitative determination of spore kill).

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

3.4 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.

4. Methods Overview

ASTM Standards

4.1 C1338-14 Test Method for Determining Fungi Resistance of Insulation Materials and Facings (Qualitative measure of susceptibility and/or fungistatic activity)
4.1.1 *Scope*—This test method covers the determination of the ability of new insulation materials and their facings to support fungal growth.
4.1.2 *Significance and Use:*
4.1.2.1 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.
4.1.2.2 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.
4.1.2.3 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.

4.2 D2020-92(2003) Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard
4.2.1 *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.
4.2.2 *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.
4.2.3 *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.

4.2.3.1 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*.

4.2.3.2 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.

4.2.3.3 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.

4.3 D3273-16 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)

4.3.1 *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 of obtained by any interested party.

4.3.1.1 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.

4.3.1.2 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.

4.3.2 *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.

4.3.3 *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.

4.3.3.1 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.

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

4.3.3.3 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.

4.4 D3456-12 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)

4.4.1 *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.

4.4.2 *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.

4.5 D4141/D4141M-14 Practice for Conducting Accelerated Outdoor Exposure Tests of Coatings

4.5.1 *Scope*—This practice covers two accelerated outdoor exposure procedures for evaluating the exterior weather resistance of coatings applied to substrates.

4.5.2 *Significance and Use*—As with any accelerated test, the increase in rate of weathering compared to in service exposure is material dependent. Therefore, no single acceleration factor can be used to relate two different types of outdoor weathering exposures. The weather resistance rankings of coatings provided by these two procedures may not agree when coatings differing in composition are compared. These two procedures should not be used interchangeably.

4.5.2.1 The procedures described in this practice are designed to provide greater degradation rates of coatings than those provided by fixed angle open-rack outdoor exposure racks. For many products, fixed angle exposures will produce higher degradation rates than the normal end use of the material.

4.5.2.2 The use of Procedure A (Black Box) instead of an open-rack direct exposure is a more realistic test for materials with higher temperature end use service conditions. For many coatings, this procedure provides greater rates of degradation than those provided by 5°, equator-facing, open-rack exposures because the black box produces higher specimen temperatures

during irradiation by daylight and longer time of wetness. The black box specimen temperatures are comparable to those encountered on the hoods, roofs, and deck lids of automobiles parked in sunlight. The relative rates of gloss loss and color change produced in some automotive coatings by exposures in accordance with Procedure A are given in ASTM STP 781.[8]

4.5.2.3 The acceleration of Procedure C is produced by reflecting sunlight from ten mirrors onto an air-cooled specimen area. In the ultraviolet portion of the solar spectrum, approximately 1400 MJ/m² of ultraviolet radiant exposure (295 to 385 nm) is received over a typical one-year period when these devices are operated in a central Arizona climate. This compares with approximately 333 MJ/m² of ultraviolet radiant exposure from a central Arizona at-latitude exposure and 280 MJ/m² of ultraviolet radiant exposure from a southern Florida at-latitude exposure over the same time period. However, the test described by Procedure C reflects only direct beam radiation onto test specimens. The reflected direct beam of sunlight contains a lower percentage of short wavelength ultraviolet radiation than global daylight because short wavelength ultraviolet is more easily scattered by the atmosphere, and because mirrors are typically less efficient at shorter ultraviolet wavelengths. Ultraviolet radiant exposure levels should not be used to compute acceleration factors since acceleration is material dependent.

4.5.2.4 The weather resistance of coatings in outdoor use can be very different depending on the geographic location of the exposure because of differences in ultraviolet (UV) radiation, time of wetness, temperature, pollutants, and other factors. Therefore, it cannot be assumed that results from one exposure in a single location will be useful for determining relative weather resistance in a different location. Exposures in several locations with different climates that represent a broad range of anticipated service conditions are recommended.

4.5.2.5 Because of year-to-year climatological variations, results from a single exposure test cannot be used to predict the absolute rate at which a material degrades.

NOTE 1—Several years of repeat exposures are typically needed to get an "average" test result for a given location.

4.5.2.6 The degradation profile for many polymers is not a linear function of exposure time or radiant exposure. When short exposures are used as indications of weather resistance, the results obtained may not be representative of those from longer exposures.

4.6 D4300-01 (2013) Test Method for Ability of Adhesive Films to Support or Resist the Growth of Fungi

4.6.1 *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.

4.6.1.1 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.

4.6.1.2 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.

4.6.2 *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.

4.7 D4445-10 Test Method for Fungicides for Controlling Sapstain and Mold on Unseasoned Lumber (Laboratory Method)

4.7.1 *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.

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

4.8 D4576-16 Test Method for Mold Growth Resistance of Wet Blue and Wet White

4.8.1 *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.

4.8.2 *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.

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

4.9 D4783-01 (2013) Test Methods Resistance of Adhesive Preparations in Container to Attack by Bacteria, Yeast, and Fungi

4.9.1 *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.

4.9.2 *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.

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

4.9.2.2 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

[8] Symposium on Permanence of Organic Coatings, *ASTM STP 781*, ASTM, 1982.

inoculum, it is important to identify the organism and determine on which medium and under what conditions it will grow, in order to demonstrate the efficacy of the biocide.

4.9.2.3 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.

4.10 D5590-10 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)

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

4.10.2 *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.

4.10.3 *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*.

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

4.11 D6329-98 (2015)) Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to Support Microbial Growth Using Static Environmental Chamber

4.11.1 *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.

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

4.11.1.2 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.

4.11.1.3 Static chambers provide controlled laboratory microenvironment conditions. These chambers are not intended to duplicate room conditions, and care must be taken when interpreting the results. Static chambers are not a substitute for dynamic chambers or field studies.

4.11.1.4 A variety of microorganisms, specifically bacteria and fungi, can be evaluated using these chambers. This guide is not intended to provide human health effect data. However, organisms of clinical interest, such as those described as potentially allergenic, may be studied this approach.

4.11.2 *Significance and Use*—The static chambers have several different applications.

4.11.2.1 The static chambers can be used to compare the susceptibility of different materials to the colonization and amplification of various microorganisms under defined conditions.

4.11.2.2 Chambers operated at high relative humidity's may be used to perform worst case scenario screening tests on materials by providing an atmosphere where environmental conditions may be favorable for microbial growth.

4.11.2.3 Use of multiple chambers with different environmental parameters, such as a range of relative humidity's, permits the evaluation of multiple microenvironments and allows investigation of materials under differing environmental conditions.

4.11.2.4 Drying requirements for wetted materials may also be investigated. This information may be relevant for determining material resistance to microbial growth after becoming wet. These conditions may simulate those where materials are subjected to water incursion through leaks as well as during remediation of a building after a fire.

4.11.2.5 Growth rates of microorganisms on the material may also be investigated. Once it has been established that organisms are able to grow on a particular material under defined conditions, investigations into the rate of organism growth may be performed. These evaluations provide base line information and can be used to evaluate methods to limit or contain amplification of microorganisms.

4.12 D6974-16 Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels-Filtration and Culture Procedures

4.12.1 *Scope*—This practice covers a membrane filter (MF) procedure for the detection and enumeration of Heterotrophic bacteria (HPC) and fungi in liquid fuels with kinematic viscosities $\leq 24 mm^2 s^{-1}$ at ambient temperature.

4.12.1.1 This quantitative practice is drawn largely from IP Method 385[9] and Test Method D5259-14.

4.12.1.2 This test may be performed either in the field or in the laboratory.

[9] F. Passman, Ed., *Fuel and Fuel System Microbiology: Fundamentals, Diagnosis, and Contamination Control*, MNL47-EB, ASTM International, West Conshohocken, PA, 2003, https://doi.org/10.1520/MNL47-EB

4.12.1.3 The ability of individual microbes to form colonies on specific growth media depends on the taxonomy and physiological state of the microbes to be enumerated, the chemistry of the growth medium, and incubation conditions. Consequently, test results should not be interpreted as absolute values. Rather they should be used as part of a diagnostic or condition monitoring effort that includes other test parameters, in accordance with Guide D6469.

4.12.1.4 This practice offers alternative options for delivering fuel sample microbes to the filter membrane, volumes or dilutions filtered, growth media used to cultivate fuel-borne microbes, and incubation temperatures. This flexibility is offered to facilitate diagnostic efforts. When this practice is used as part of a monitoring program, a single procedure should be used consistently.

4.12.2 *Significance and Use*—Biodeteriogenic microbes infecting fuel systems typically are most abundant within slime accumulations on system surfaces or at the fuel-water interface (Guide D6469). However, it is often impractical to obtain samples from these locations within fuel systems. Although the numbers of viable bacteria and fungi recovered from fuel-phase samples are likely to be several orders of magnitude smaller than those found in water-phase samples, fuel-phase organisms are often the most readily available indicators of fuel and fuel system microbial contamination.

4.12.2.1 *Growth Medium Selectivity*—Guide E1326 discusses the limitations of growth medium selection. Any medium selected will favor colony formation by some species and suppress colony formation by others. As noted, physical, chemical and physiological variables can affect viable cell enumeration test results. Test Method D7436-16 provides a non-culture means of quantifying microbial biomass in fuels and fuel associated water.

4.12.2.2 Since a wide range of sample sizes, or dilutions thereof, can be analyzed by the membrane filter technique (Test Methods D5259-14 and F1094-12), the test sensitivity can be adjusted for the population density expected on the sample.

4.12.2.3 Enumeration data should be used as part of diagnostic efforts or routine condition monitoring programs. Enumeration data should not be used as fuel quality criteria.

4.13 D7855/D7855M-13 Test Method for Determination of Mold Growth on Coated Building Products Designed for Interior Applications Using an Environmental Chamber and Indirect Inoculation

4.13.1 *Scope*—This test method covers an environmental chamber and the conditions of operation to evaluate in a 4-week period the relative resistance to mold growth and microbial surface defacement on coated building products designed for interior application using an indirect inoculation method. The apparatus is designed so it can be easily built or obtained by any interested party.

4.13.1.1 This test method can be used to evaluate the comparative resistance of coated building products to accelerated mold growth. Ratings do not imply a specific time period that the coated building product will be free of fungal growth during installation in an interior environment.

4.13.1.2 This test method is not intended for use in the evaluation of public health claims.

4.13.1.3 This test method is intended for the accelerated evaluation of mold growth on a coated building product designed for interior use. This method is not intended for evaluation of surfaces designed exterior applications or uncoated surfaces. Use of this test method for evaluating exterior performance has not been validated, nor have the limitations for such use been determined.

4.13.2 *Significance and Use*—An accelerated test for determining the resistance of interior coated building products to mold growth is useful in estimating the relative performance for use in interior environments under conditions favorable to fungal growth.

4.13.3 Static or environmental chambers provide controlled laboratory micro-environment conditions. These chambers are not intended to duplicate room conditions, and care must be taken when interpreting the results. Static chambers are not a substitute for dynamic chambers or field studies.

4.14 D7910-14 Practice for Collection of Fungal Material from Surfaces by Tape Lift

4.14.1 *Scope*—This practice describes the protocols for collection of surface samples using tape lifts and their delivery to the laboratory.

4.14.1.1 The purpose of this practice is to support the field investigator in differentiating fungal materials from non-fungal material such as scuffs, soot deposits, stains, pigments, dust, efflorescence, adhesives, and water stains.

4.14.1.2 The samples collected by this practice are appropriate for either qualitative or quantitative analysis by direct microscopy.

4.14.1.3 This practice does not address the development of a formal hypothesis or the establishment of sampling objectives.

4.14.2 *Significance and Use*—This practice defines a consistent procedure for collecting surface material using clear, transparent, single sided adhesive collection medium, typically tape (also known as tape lift).

4.14.2.1 A tape lift sample collected according to this practice is intended to be used to assess the material present at one specific location on a surface for fungal content.

4.14.2.2 A tape lift sample collected from a point of interest can be used for qualitative analysis or to quantify fungal material per sample or per unit area. Note that the recovery efficiency of material from the surface sampled is unknown and a likely source of uncertainty for quantitative analyses.

4.14.2.3 A tape lift sample collected according to this practice can be analyzed by direct microscopy.

4.14.2.4 This practice may help supplement consistency in mold sample during an indoor air quality investigation.

4.15 ASTM E2111-12 Quantitative Carrier Test Method to Evaluate the Bactericidal Fungicidal or Mycobactericidal, and Sporicidal Potencies of Liquid Chemicals

4.15.1 *Scope*—This test method is designed for use in product development and for the generation of product potency data. This test method permits the loading of each carrier with a known volume of the test organism. The incorporation of controls can also determine the initial load of colony forming units (CFU) of organisms on the test carriers and any loss in CFU after the mandatory drying of the inoculum.