Designation: E3152 – 23

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D2020 Test Methods for Mildew (Fungus) Resistance of Paper and Paperboard (Withdrawn 2009)[3]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
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 Sporicidal Potencies of Liquid Chemicals
E2197 Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal 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.

Current edition approved April 1, 2023. Published April 2023. Originally approved in 2018. Last previous edition approved in 2018 as E3152 – 18. DOI: 10.1520/E3152-23.

[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 93-30[8]

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.

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[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 of 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 know 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 determine 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.

5.10.1.3 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.

5.10.1.4 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.

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

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

5.10.2.2 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.

5.10.2.3 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.

5.10.2.4 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.

5.10.2.5 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.

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

5.11.1 *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 ≤ 24 mm^2s^{-1} at ambient temperature.

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

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

5.11.1.3 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.

5.11.1.4 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.

5.11.2 *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.

5.11.2.1 *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.

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

5.11.2.3 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.

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

5.12.1 *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.

5.12.1.1 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.

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

5.12.1.3 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

[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

performance has not been validated, nor have the limitations for such use been determined.

5.12.2 *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.

5.12.3 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.

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

5.13.1 *Scope*—This environmental chamber method measures the resistance of the treated Wet Blue and Wet White to the germination of spores and subsequent vegetative growth over a period of four weeks. The test method is useful in estimating the performance of fungicides and should assist in the prediction of storage time of Wet Blue and Wet White before fungal growth begins. The apparatus is designed so it can be easily built or obtained by any interested party and duplicate the natural environment in which Wet Blue and Wet White is inoculated with fungal spores. Spores that germinate on untreated or treated Wet Blue and Wet White can produce fungal growth, resulting in disfigurement or discoloration, or both, of the Wet Blue and Wet White.

5.14 D7910 Practice for Collection of Fungal Material from Surfaces by Tape Lift

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

5.14.1.1 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.

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

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

5.14.2 *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).

5.14.2.1 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.

5.14.2.2 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.

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

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

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

5.15.1 *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.

5.15.1.1 This test method is designed to have survivors and also to be used with a performance standard. The surviving microorganisms on each test carrier are compared to the mean of no less than three control carriers to determine if the performance standard has been met. To allow proper statistical evaluation of results, the size of the test inoculum should be sufficiently large to take into account both the performance standard of 6-$\log_{10}$ reduction in the viability titer of the test organism used, and an inoculum size of 10^7 CFU, then theoretically a maximum of ten survivors per carrier is permitted; however, because of experimental variability, the exact target may need to be higher than 10^6 CFU/carrier, thus fewer survivors would be permitted.

5.15.2 *Significance and Use*—This test method is fully quantitative and it also avoids any loss of viable organisms through wash off. This makes it possible to produce statistically valid data using many fewer test and control carriers than other quantitative methods based on most probable number (MPN).

5.15.2.1 The design of the carriers makes it possible to place into each a precisely measured volume of the test suspension. The use of the threaded stir bars allows for efficient recovery of the inoculum even after is exposure for several hours to strong fixatives such as glutaraldehyde.

5.15.2.2 The membrane filtration step allows processing of the entire eluate from the test carriers and therefore the capture and subsequent detection of even low numbers of viable organisms that may be present.

5.15.2.3 This test can be performed with or without a soil load to determine the effect of such loading on microbicide performance. The soil load developed for this test is a mixture of three types of proteins (high molecular weight proteins, low molecular weight proteins, and mucous material) to represent the body secretions, excretions, or other extraneous substances that chemical microbicides may encounter under field conditions. It is suitable for working with the various test organisms included here. The components of the soil load are readily available and subject to much less variability than animal sera.

5.15.2.4 Since the quality of tap water varies considerably both geographically and temporally, this test method incorporates the use of water with a specified and documented level of hardness to prepare use-dilutions of test products. The U.S. Environmental Protection Agency's Scientific Advisory Panel (SAP) on Germicide Test Methodology has recommended the use of water with a standard hardness of 400 ppm of $CaCO_3$.

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

5.16.1 *Scope*—This test method is designed to evaluate the ability of test substances to inactivate vegetative bacteria, viruses, fungi, mycobacteria, and bacterial spores on disk carriers of brushed stainless steel that represent hard, nonporous environmental surfaces and medical devices. It is also designed to have survivors that can be compared to the mean of no less than three control carriers to determine if the performance standard has been met. For proper statistical evaluation of the results, the number of viable organisms in the test inoculum should be sufficiently high to take into account both the performance standard and the experimental variations in the results.

5.16.1.1 The test protocol does not include and wiping or rubbing action. It is, therefore, not designed for testing any wipes.

5.16.2 *Significance and Use*—The design of this test eliminates any loss of viable organisms through wash off, thus making it possible to produce statistically valid data using many fewer test carriers than needed for methods based on simple MPN estimates.

5.16.2.1 The stringency in the test is provided by the use of soil load, the microtopography of the brushed stainless steel carrier surface, and the smaller ratio of test substance to surface area typical for many disinfectant applications. Thus, the test substance being assessed is presented with a reasonable challenge while allowing for efficient recovery of the test organisms from the inoculated carriers. The metal disks in the basic test are also compatible with a wide variety of actives.

5.16.2.2 The design of the carriers makes it possible to place onto each a precisely measured volume of the test organism (10 µL) as well as the control fluid or test substance (50 µL).

5.16.2.3 The inoculum is placed at the center of each disk whereas the volume of the test substance covers nearly the entire disk surface, thus virtually eliminating the risk of any organisms remaining unexposed.

5.16.2.4 In all tests, other than those against viruses, the addition of 10 mL of an eluent/diluent gives a 1:200 dilution of the test substance immediately at the end of the contact time. While this step in itself may be sufficient to arrest the microbicidal activity of most actives, the test protocol permits the addition of a specific neutralizer to the eluent/diluent, if required. Except for viruses, the membrane filtration step allows processing of the entire eluate from the test carriers and, therefore, the capture and subsequent detection of even low numbers of viable organisms that may be present. Subsequent rinsing of the membrane filters with saline also reduces the risk of carrying on inhibitory residues over to the recovery medium. Validation of the process of neutralization of the test substance is required by challenge with low numbers of the test organism.

5.16.2.5 The soil load in this test is a mixture of three types of proteins (high molecular weight proteins, low molecular weight proteins, and mucous material) designed to represent the body secretions, excretions, or other extraneous substances that microbicidal chemicals may encounter under field conditions. It is suitable for working with all types of test organisms included here. The components of the soil load are readily available and subject to much less variability than animal sera.

5.16.2.6 If distilled water or other diluent is not to be specified on the product label, the diluent for the test substance is assumed to be tap water. Since the quality of tap water varies considerably both geographically and temporally, this test method incorporates the use of water with a specified and documented level of hardness to prepare use-dilutions of test substance that require dilution in water before use. While water with a hardness of at least 300 ppm as $CaCO_3$ is recommended consult local regulations regarding use of hard water prior to testing.

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

5.17.1 *Scope*—This test method is designed to evaluate (qualitatively) the presence of antimicrobial activity in or on carpets. Use this method to qualitatively evaluate both antibacterial and antifungal activity.

5.17.1.1 Use half strength (nutrient and agar) tryptic soy agar as the inoculum vehicle for bacteria and half strength potato dextrose agar as the inoculum vehicle for mold conidia. Use of half strength agars may reduce undue neutralization of an antimicrobial due to excessive organic load.

5.17.1.2 This method simultaneously evaluates (both visual and stereo-microscopic) antimicrobial activity both at the fiber layer and at the primary backing layer of carpet.

5.17.1.3 Use this method to assess the durability of the antimicrobial treatments on new carpets, and on those repeatedly shampooed or exposed to in-use conditions.

5.17.2 *Significance and Use*—This method provides for rapid screening of antimicrobial treatments located in or on the carpet face fiber or incorporated into the backing structure of the carpet (or both).

5.17.2.1 This method simulates actual use conditions that may occur on carpets (for example, food and beverage spills, soiling from foot traffic, prolonged moisture exposure).

5.17.2.2 This method provides a means to screen for activity and durability of an antimicrobial treatment under conditions of organic loading.

5.17.2.3 This method provides for the simultaneous assessment of multiple carpet components for antimicrobial activity.

5.18 E3227 Standard Practice for Qualitative Assessment of Antifungal Activity on Textiles

5.18.1 *Scope*—This test practice determines the relative fungal growth inhibition properties of materials treated with an active biocidal agent. Samples of porous materials, such as textiles, are inoculated with a defined suspension of fungal conidia or spores and then incubated. The inhibition of growth or visible growth present on treated compared to identical untreated materials is used to measure relative antifungal properties of the treated identical materials.

5.19 G21 Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi (Qualitative measure of susceptibility and/or fungistatic activity)

5.19.1 *Scope*—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,