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METHOD FOR MEASURING MICROBIAL RESISTANCE FROM VARIOUS SOURCES USING STATIC ENVIRONMENTAL CHAMBERS

Prepared for use by
The GREENGUARD Certification Program[SM]

By
Air Quality Sciences, Inc.

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Foreword

The GREENGUARD Environmental Institute began certifying products for low chemical emissions in 2001. A pilot program was initiated in 2004 to evaluate the potential of measuring mold resistant properties of materials used in building construction and manufactured for use in the built environment. Starting with the guidance of ASTM Standard 6329, the pilot study further developed a test method suitable for assessing the resistance of materials to fungal colonization (mold growth) with a quantitative measurement scale. The test method was validated for a number of representative product types and found to be reliable and reproducible. Quantitative measurement results have been defined in a product rating scale of 1-4, based on the amount of mold growth resulting on a product under controlled and defined laboratory conditions.

The following Method incorporates the best-learned practices for using a known mold inoculum for challenging and evaluating building products for the GREENGUARD Microbial Resistance Listing Program, including paints and coatings, adhesives and sealants, wallboard, insulation, flooring, ceilings, window components, and many other dry and wet materials. Elements of the Method include sample handling and shipment, sample preparation, product inoculation, environmental chamber exposure, analytical measurement techniques, and mold amplification levels.

The assessment relies upon a challenge using a widely occurring biodeteriogenic mold and conditions favorable for the growth of mold. This combination of challenge conditions permits an accelerated test of the ability of a material to resist mold growth. The assessment of mold growth relies upon established mold quantification procedures and yields an objective value of the degree of mold amplification that developed during the challenge. The configuration of the challenge was developed according to ASTM Standard D 6329, “Standard Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to support Microbial Growth Using Static Environmental Chambers.”

What is presented applies specifically to the assessment requirements for the GREENGUARD Microbial Resistance Listing Program. However, this method can be expanded for various specific product applications, to include environmental insults such as soiling, or additional mold types, etc.

This practice ensures that product users are able to compare general product categories and particular products within categories by comparing the rating assigned by the GREENGUARD Microbial Resistance Listing Program evaluation scale.

This document will be updated as new test protocols and standards become available.

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SECTION 1
BACKGROUND INFORMATION

1.0 Scope

Building materials exposed to environmental conditions and/or with poor maintenance can lead to mold growth. Some materials, especially those that are porous, are more likely than others to support microbial growth. As a result, these materials can become potential indoor sources of bio-contaminants including mold. Common materials susceptible to mold growth include porous materials and those with cellulose substrates. These may include: gypsum wallboard, ceiling tile, insulation, textiles, wallcoverings, floor coverings, upholstered furniture, and office panels. In some cases, materials may be treated with anti-microbial agents as a preventive step. The ability of these materials to support or to resist mold growth is often not well documented.

A testing protocol has been established to determine how susceptible or resistant a product may be to mold growth. Based on ASTM Standard D 6329, this test is designed specifically to address indoor air quality (IAQ) issues. It involves the study of molds most likely to contaminate products and, consequently, contribute to poor indoor air quality. Materials are inoculated with mold and placed in static environmental chambers with elevated humidity conditions. Mold growth is measured over time, and the results will indicate if a product is likely to support mold growth under these pre-defined environmental conditions. This test is being conducted by a number of manufacturers to further document their products' IAQ performance and to rank products as to their ability to resist mold growth.

1.0.1 This method establishes the procedures for product sample collection, microbial resistance testing and analysis, and reporting.

1.0.2 This method is an application of ASTM Standard D 6329, "Standard Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to support Microbial Growth Using Static Environmental Chambers."

1.0.3 The methodology provides a standard means of reproducibly and accurately testing building material under realistic, yet highly controlled, environment conditions.

1.0.4 The methodology with standardized measurement and analysis provides consistent testing of materials within a product group and across product groups.

1.0.5 This methodology is applicable for newly manufactured products before they are used in construction. Products taken from inventory or from within buildings can also be studied but these may be soiled or precontaminated. Resultant data may not be considered representative of newly manufactured products.

1.0.6 This method is applicable to newly manufactured products and may not serve as a predictor of mold resistance based on long term usage of product, with exposure to soil and varying environmental conditions.

1.0.7 While this method categorizes a new building material's resistance to mold growth, it does not assess the human risk involved with use of the materials as an end user.

1.0.8 This practice does not purport to address safety concerns, if any, associated with the use of this practice. It is the responsibility of the user of this protocol to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1.0.9 This method can be expanded to evaluate environmental insults such as soiling, or additional mold types

1.1 Objectives and Use

1.1.1 Objectives:

1.1.1.1 Measure mold resistance of building materials, furnishings and finishes.

1.1.1.2 Application of ASTM Standard D 6329 for quantitatively measuring the mold resistance of building materials, furnishings, and finishes.

1.1.1.3 Measurements of mold resistance that can be used to rank products.

1.1.1.4 Provide material mold resistance data to manufacturers for assistance in product development.

1.1.1.5 Provide mold resistance material rating to assist specifiers and users in selecting proper materials for building use.

1.1.1.6 Provide mold resistance material rating for building moisture management programs and certifications.

1.1.2 Use:

1.1.2.1 Provide measurements of mold growth with spiked (inoculated) mold on defined materials.

1.1.2.2 Use of multiple chambers with different environmental parameters, such as a range of relative humidities, permits the evaluation of multiple microenvironments and allows investigation of material under differing environmental conditions.

1.2 References and Documents

ASTM Standard D 1193-06 Standard Specification for Reagent Water." American Society for Testing and Materials, West Conshohocken, PA, 2006.

ASTM Standard D 1356-05 Standard Terminology Relating to Sampling and Analysis of Atmospheres." American Society for Testing and Materials, West Conshohocken, PA, 2005.

ASTM Standard D 6329-98 (Reapproved 2003). "Standard Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to support Microbial Growth Using Static Environmental Chambers." American Society for Testing and Materials, West Conshohocken, PA, 2003.

ASTM Standard E 104-85 (Reapproved 1991). "Standard Practice for Maintaining Constant Relative Humidity by Means of Aqueous Solutions." American Society for Testing and Materials, West Conshohocken, PA, 1991.

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1.3 Terminology

Acronyms and Abbreviations

ASTM – American Society for Testing and Materials
AQS – Air Quality Sciences, Inc.
BQL – Below quantifiable limit
CFU – Colony forming unit
COC – Chain of Custody
DL – Detection limit
EPA – U.S. Environmental Protection Agency
GEI – GREENGUARD Environmental Institute
IAQ – Indoor air quality
ISO – International Organization for Standardization
LOQ – Limit of quantitation, lower
MDF – Medium density fiberboard
MRT – Microbial resistance testing
MSDS – Material safety data sheet
OSB – Oriented strand board
QL – Quantifiable limit
RH – Relative humidity in percent
SEC – Static Environmental Chamber
VCT – Vinyl composition tile

1.4 Definitions

Amplification – The act or result of increasing the quantity of microorganisms.

Absolute Humidity (H) –A measure of the amount of water vapor in the air; expressed as grams of water per grams of air.

Accuracy - The degree of conformity of a value generated by a specific procedure to the assumed or accepted true value; includes both precision and bias.

Chain-of-Custody (COC) – A document providing written evidence of the transfer of a product sample, air sample, or another document from one organization to another organization or from one individual to another individual within the same organization. The document is signed and dated by each party involved in the transfer.

Concentration – The number of colony forming units or spores per unit of air or water volume expressed at standardized conditions for temperature and humidity (i.e., 298K, 101.3 kPa or as cfu/ml)

Colony - Macroscopically visible growth.

Colony Forming Unit – The measure of viable fungal units, such as spores.

Coupon – A representative sample of material used for testing, cut to the required size for MRT analysis.

Inoculation – The act of introducing a microorganism (inoculum) onto the test material.

Inoculum – Viable test microorganism introduced onto a material by implanting a small amount on the surface or substrate.

Humidity (H) –A measure of the amount of water vapor in the air.

Mold colonization – Growth of mold on or in the materials; typically materials colonized by mold degrade and/or disfigure.

Manufacturer's Identification Number - Unique identifier given to a product by the manufacturer.

Penicillium brevicompactum - One of the molds used for MRT analysis.

Plate - Petri dish containing microbiological agar media on which microorganisms are grown.

Positive Control Material - Testing material not resistant to mold growth under provided test conditions.

Precision - The degree of agreement of repeated measurements of the same property. The precision of a method is expressed quantitatively as the standard deviation computed from the results of a series of controlled determinations.

Product Category – A general group of similar products intended for a particular application and performance, such as adhesives, flooring, furniture, or paints and coatings.

Product Subcategory – A group of products within a product category having similar chemistry, construction, weight, formulation and manufacturing process and which may have a similar microbial resistant rating.

Relative Humidity (RH) - The ratio of the amount of water vapor actually present in the air to the greatest amount possible at the same temperature; expressed as percent saturation.

Representative Product Sample – A product sample, which is representative of the product manufactured and produced under typical operating conditions.

Sampling Period – The established time for the determination of mold concentration on the test specimen.

Static Environmental Chamber - A test apparatus with controlled environmental parameters used for the purpose of providing accurate and reproducible environmental conditions for microbial resistance testing.

Surface Area – The result of multiplying the width times the length of a single side of a product.

Susceptibility – The vulnerability of a material or surface to colonization by microorganisms.

Temperature (T) – A measure of the heat content of a material expressed in degrees Celsius.

Test Specimen – A portion of representative sample prepared for microbial resistance.

Time Zero – The initial time at which a test specimen is sealed in a chamber for microbial resistance testing.

1.5 Symbols

Symbol	Description	Units
A	Projected surface area	cm^2
CFU/cfu	Colony forming unit	cfu/ml
RH	Relative humidity	%
T	Temperature	°C
Ts	Time after start of test	hr or day or week
t	Time	hr
V	Volume	ml or m^3
W	Weight	g

SECTION 2
COLLECTION, PACKAGING, SHIPMENT, & DOCUMENTATION OF PRODUCT SAMPLES

2.0 Collection, Packaging, Shipment & Documentation Of Product Samples

2.0.1 Purpose

Guidelines are established for the collection, handling and documentation of material samples to ensure the samples tested are reliable, representative, uncontaminated, and well preserved.

2.1 Personnel

2.1.1 Personnel responsible for sample collection must perform the task carefully and conscientiously and according to specific instructions, if supplied. Improper sample collection may impact the integrity of the sample and invalidate analysis, data and use of data.

2.1.2 Individuals engaged in sample collection and handling must be qualified by training and experience and possess an understanding of the relevant practices and techniques, or at a minimum, be under the direct supervision of such an individual.

2.2 Representative Sample

2.2.1 Materials selected or requested for testing are to be representative of similar materials produced by the manufacturer. These materials shall be treated no differently than similar materials or components produced in the normal course of business and available in the marketplace.

2.3 Sample Preservation

2.3.1 Special care shall be taken to prevent contamination of the product sample from any external source including moisture, prior to, during and subsequent to the sample collection procedure.

Powder free latex gloves are recommended during collection and packaging of the sample. Latex gloves minimize the risk of sample contamination by bio-contaminants on the hands of sample collection personnel.

2.3.2 Product samples may be packaged in two ways: 1) using the manufacturers standard product packaging materials, including sealed containers (as provided to distributors and/or customers); or 2) using contaminant-free, airtight, specialized Mylar or polyethylene lined foil barrier bags provided by the laboratory (specialized sample bags). In each case, care shall be taken to ensure that the sample package is tightly sealed to minimize contamination from external sources during shipment and storage. If the manufacturer's standard product packaging does not meet sealing requirements, then other specialized packaging must be used.

2.3.3 The product will remain in its packaging as received, or transferred to a specialized bag (see Section 2.3.3), foil bag or otherwise sealed to preserve the integrity of the sample, until immediately prior to test specimen preparation and loading into the static environmental chamber. Until it is unpacked, the product will be stored in an environmentally controlled indoor environment free of contamination with temperature control of 20º – 25ºC and relative humidity no greater than 60% RH.

2.4 Location of Sampling

2.4.1 Generally, samples are to be collected directly from the manufacturing or packaging line. The most appropriate location is dependent on the product and packaging process employed by the manufacturer. When collecting samples directly off the manufacturing line, the collection location shall be chosen to ensure easy access so that a representative selection of the material is obtained. Sample collection personnel shall document the sample collection location and any relevant observations. This information shall be included on the chain of custody (COC) form.

2.5 Sample Age

2.5.1 Samples shall be packaged no more than 1 hour following collection off the manufacturing line or immediately following completion of the manufacturer's product packaging process. However, the sample shall not be packaged until it has reached room temperature. If additional time is required for the product to reach room temperature beyond the one hour, note this on the chain of custody.

2.5.2 Samples shall be shipped from the manufacturing facility within 24 hours of collection and packaging.

2.5.3 Samples shall arrive at the testing laboratory within 7 days of shipment, although overnight shipment is recommended.

2.5.4 Timing of sample collection shall be coordinated between the manufacturing facility and the testing laboratory to ensure that preparation and loading of samples can occur within 21 days of receipt at the laboratory.

2.5.5 The schedule for sample collection, shipping, specimen preparation, and testing is summarized below.

Table 1 - Building Material Products

Manufacturing Date	Date product comes off of final manufacture line
Sample Collection	Same as Manufacturing Date
Shipment to Laboratory	Within 24 hours of sample collection
Arrival at Laboratory	Not to exceed 7 days of shipment date
Testing Date	Not to exceed 21 days after arrival and product acceptance at laboratory

2.6 Sample Collection Procedures

2.6.1 Tile and Plank Products - Tile and plank products are collected directly from the manufacturing or packaging line. If standard manufacturer packaging materials are not used, a minimum of four representative tiles, strips or planks, each with a minimum surface area of at least 64 square inches, shall be collected. The tiles, strips or planks shall be stacked tightly together face to back and immediately placed in specialized packaging as described in Sections 2.3 and 2.5. Following packaging, the GREENGUARD COC must be fully completed. The white and yellow copies of the completed three-part form (Section 2.8) shall be attached to the outside of the sample package. The pink copy of the COC is a record retained by the manufacturer. No more than 1 hour shall elapse between the time of sample collection and packaging.

2.6.2 Sheet and Roll Goods - Sheet and roll goods are collected directly from the manufacturing or packaging line. Sheet and roll goods shall be cut at a minimum width of 12" across the entire width of the roll. Following cutting, the product shall be tightly rolled and immediately placed in specialized packaging as described in Sections 2.3 and 2.5 (unless such size material can be collected from the packaging line using the manufacturer's standard packaging materials). Wallcovering and other fabric may be collected as a full or partial production roll. In these cases, the roll shall have at least 10 layers of material and may be provided as collected from the packaging line or placed in specialized packaging as described in Sections 2.3 and 2.5. Following packaging, the GREENGUARD COC must be fully completed. The white and yellow copies of the completed three-part form shall be attached to the outside of the sample package. The

pink copy of the COC is retained as a record for the manufacturer. No more than 1 hour shall elapse between the time of collection and packaging.

2.6.3 Rigid Panel Products - Rigid Panel Products are collected directly from the manufacturing line. For large panel products, the entire panel may be shipped to the testing laboratory as long as the sample is appropriately sealed in the manufacturer's normal packaging. As an alternative, smaller samples may be collected from the larger panel. These samples shall be collected at least 6 inches away from all edges of the larger panel. Within this boundary, the smaller panel samples shall be cut into approximate 12" x 12" squares. A minimum of four squares is required. The squares shall be stacked tightly together face to back and immediately placed in specialized packaging as described in Sections 2.3 and 2.5. Following packaging, the GREENGUARD COC must be fully completed. The white and yellow copies of the completed three-part form shall be attached to the outside of the sample package. The pink copy of the COC is retained as a record for the manufacturer. No more than 1 hour shall elapse between the time of collection and packaging.

2.6.4 Insulation - In order to best represent the finished product, air that may be exchanged in, and evacuated from, the packaging process is simulated as much as possible. The cooling of the product is also a factor when the collection point is established. Creating realistic timeframes and conditions for sample collection ensures that the product is representative of insulation materials standard production procedures.

2.6.4.1 Batts and Rolls - Most, if not all of these materials are packaged in bags as an integral point of manufacturing. Further, air is generally evacuated from the package to reduce the physical size of the packaged product for shipping purposes. Samples shall be collected after 15 minutes (±2 minutes) following packaging. The product should be removed from its original packaging and placed into the specialized packaging. Manufacturers who wish to continue to collect samples directly from the production line may continue to forego packaging and may establish an earlier point of sample collection as long as the sample is cool. Samples shall be submitted per the following size requirements: 18" x 30", samples of the same material may be cut into smaller sizes (no smaller than 12" x 12") and placed in the same packaging.

2.6.4.2 Boards and Foam Products - As board products are generally boxed, opening the final package would be both difficult and cause excessive waste. In this case, the sample should be removed immediately from the production line and allowed to condition for 15 minutes (±2 minutes) under standard laboratory conditions in the plant prior to packaging for microbial resistance testing. Manufacturers who provide GREENGUARD Certified products for furniture panel applications will be expected to deliver product for the purposes of Microbial resistance testing in the same fashion as standard board products. Manufacturers who wish to continue to collect samples directly from the production line may continue to forego packaging and may establish an earlier point of sample collection. Samples shall be submitted per the following size requirements: 18" x 30", samples of the same material may be cut into smaller sizes (no smaller than 12" x 12") and placed in the same Mylar packaging bag.

2.6.4.3 Blowing Wools and Loose Fill Products - It has been determined that the collection of loose fills or blowing wools is not easily achieved directly from the production line, dependent on the manufacturer. The sample collection procedure shall be consistent with Batts and Rolls. Sample collection, in this case, requires collection of product from the compressed or evacuated product package, 15 minutes (±2 minutes) following packaging. Manufacturers who wish to continue to collect samples directly from the production line may continue to forego packaging and may establish an earlier point of sample collection. Samples shall be submitted per the following size

requirements: Loose fill insulation shall be placed in a specialized packaging bag, filling roughly 65% to 75% of the bag.

2.6.5 Containerized and Wet Products - Containerized and wet products can be supplied in original, standard 1-quart or 1-gallon consumer containers. Adhesives can be supplied in their consumer packaging such as an applicator tube or can (if less than 1 gallon). Alternatively, adhesive and paint samples can be collected in clean, unused paint cans (1-pint or 1-quart size). Special care is required to assure that these samples are representative of the larger batches from which they are collected. Containers shall be filled so there is minimal unfilled headspace. The collection procedure shall be documented. Following packaging, the GREENGUARD COC must be fully completed. The white and yellow copies of the completed three-part form (Section 2.8) shall be attached to the outside of the sample package. The pink copy of the COC is retained as a record for the manufacturer. Samples of containerized products sent to a laboratory shall also be accompanied by a Material Safety Data Sheet (MSDS) and a specification sheet that describe the products, list the major chemical ingredients, identify the intended uses and describe the application methods. Disposal recommendations should also be provided.

2.6.6 Furniture and Other Large Products - Furniture and other large products shall be collected immediately following manufacturing and packaging due to product size, the manufacturer's standard packaging materials and processes are often use for large products such as chairs, tables, and desks. This is typically shrink wrapped followed by placement in a cardboard box. For multi-component systems, a consolidation area must be established that is clean with controlled environmental conditions. As additional components are packaged, they shall be stored in the consolidation area until all components have been manufactured and packaged. Following packaging of individual samples or completion of consolidation of multi-component systems, the GREENGUARD COC must be fully completed. The white and yellow copies of the completed three-part form shall be attached to the outside of the sample package. The pink copy of the COC is retained as a record for the manufacturer.

2.7 Packaging and Shipment of Samples

2.7.1 Samples are shipped to the testing laboratory in sealed Mylar or polyethylene lined foil barrier bags (specialized sample bags) provided by the testing laboratory, in the manufacturer's standard packaging, or in otherwise sealed containers. The type of packaging used must ensure that the sample is tightly sealed to minimize contamination from external sources during shipment and storage (also see Section 2.3)

2.7.2 Samples must be packaged to avoid cross contamination. Different types of products should be packaged individually for shipping.

2.7.3 Samples are to be shipped to the testing laboratory within 24 hours of collection and packaging. Products shall arrive at the testing laboratory within 7 days of shipment, although overnight shipment is recommended.

2.8 Chain of Custody Documentation

2.8.1 The manufacturer is responsible for the completing GREENGUARD Product Documentation/Chain of Custody form. This form must be completed by the responsible manufacturer's employee/representative.

2.8.2 The chain of custody form includes the following information:

2.8.2.1 Manufacturer/Company Details – Name, Street Address, City, State/Province, Country, Zip/Postal Code.

2.8.2.2 Contact Details – Contact Name, Title, Phone Number, Fax Number, Email Address

2.8.2.3 Sample Details – Sample ID, Product Category, Product Subcategory (if applicable), Product Name, Manufacturers Identification Number, Date Manufactured, Sample Collection Location, Sample Collection Date and Time, Sample Collected By.
2.8.2.4 Shipping Details – Packed By, Shipping Date, Carrier, Airbill Number (Carrier and Airbill Number may be filled in by Laboratory upon receipt).
2.8.2.5 Ship to Laboratory – Name, Street Address, City, State/Province, Country, Zip/Postal Code, Phone Number, Fax Number.
2.8.2.6 Laboratory Receiving Details – Received By, Received Date, Condition of Shipping Package, Condition of Sample, Assigned Laboratory Material Tracking Number.
2.8.2.7 Signature Tracking Details – Relinquished By, Received By, Signature, Company, Date and Time.

2.9 Receipt of Samples by Laboratory

2.9.1 Once the laboratory receives the product sample, the packages will be checked against the shipping invoice to ensure all packages have been received.
2.9.2 The laboratory will visually inspect the shipping containers upon arrival to ensure the containers are intact and to verify sample integrity.
2.9.3 The sample custodian shall note the condition of the package or container on the chain-of-custody form and sign and date the form.
2.9.4 If packages or containers are damaged or missing, the laboratory will notify the manufacturer as soon as feasible.
2.9.5 If a package or container is significantly damaged or other criteria are not met, the laboratory shall reject the sample as described in Section 2.10.
2.9.6 Valid samples are assigned a unique identifier and entered into an electronic data management system for sample and data tracking purposes.

The product is to remain in its original packaging (as received) until immediately prior to preparation for loading into the static environmental chamber. The product is to be stored in a normal indoor environment not expected to introduce contamination.

2.10 Rejection of Samples by Laboratory

2.10.1 The testing laboratory has the right to reject a product sample for testing due to, but not limited to, any of the following reasons:
2.10.1.1 Shipping package is severely damaged upon arrival.
2.10.1.2 Product container (i.e., external bag, foil package, can, tube, etc) is damaged upon arrival so that integrity of the sample is compromised.
2.10.1.3 Chain of Custody form is missing or incomplete.
2.10.1.4 Product is wet, contaminated with debris or initial mold growth.
2.10.1.5 Product sample arrives with insufficient time to initiate testing within the required time frame.
2.10.2 When a product sample is rejected, the testing laboratory shall inform the manufacturer immediately and provide the reason for rejection.
2.10.3 The manufacturer has the right to collect a new sample and resubmit it for testing, subject to the conditions described within this method. All costs for recollection and shipment shall be the responsibility of the manufacturer.

2.11 Storage of Samples by Laboratory Prior to and Following Testing

2.11.1 Before Testing: Samples are stored in original, sealed packaging in a controlled environment not expected to introduce contamination. This environment must be free of contamination and environmentally controlled at 20º - 25ºC and not greater than 60% RH.

2.11.2 After Testing: Following testing and report issuance, unused product is stored for 30 days. After this time, the product is either returned to the manufacturer or disposed of depending on the request of the client. The yellow copy of the chain of custody form is returned or destroyed with the product.

SECTION 3
LABORATORY SAMPLE PREPARATION AND ANALYSES

3.0 Test Specimen Preparation and Apparatus

3.0.1 Test Specimen Preparation

3.0.1.1 Specific details on the preparation of the sample depend upon the characteristics of the material to be tested.

3.0.1.2 Replicate test specimens are used for testing and analysis.

3.0.1.3 The size of the test specimen may also vary and can be as small as 2 x 4 cm.

3.0.1.4 Test specimens are sterilized (or sanitized) before inoculation to ensure that the test organism is the only viable fungal source being evaluated. Applicable methods of sterilization used are; 1) autoclaving, 2) dry heat and 3) wiping with 70% iso-propanol. For test specimens not amenable to these methods, the test specimen keep as clean and dry as possible.

3.0.1.5 Test specimens are equilibrated in the static environmental chamber prior to inoculation with the test organism for a minimum time of 24 hours or until constant weight is attained.

3.0.1.6 Inoculated test specimens are placed in Petri dishes or other appropriate holders on the shelves of the static environmental chamber.

3.0.1.7 Control samples are included for QA/QC evaluations and comparison with the test sample.

3.0.1.8 Placement of inoculated test specimens in the static environmental chamber is considered the zero time for MRT.

3.0.1.9 Temperature and humidity control: The temperature of the static environmental chamber shall be maintained at 25 ±2°C throughout the test period. The humidity of the chamber air shall be maintained at 95% RH.

3.0.2 Apparatus

Static environmental chamber or other suitable container (See Section 3.9)
Autoclave
Compound Microscope and stereoscope
Glass Petri plates 100mm x 15mm or other suitable containers
Hemacytometer
Incubator capable of 25°C setting (or other temperature as appropriate)
Salts appropriate to the desired RH (ASTM Standard E 104-85) – review MSDS and select least harmful salt appropriate to desired RH range
Positive control material (filter paper – Whatman #4)
Organism appropriate to test material
Peptone Tween
Pipette and pipette tips
RH measurement device
Sterile spreaders
Sterilization pouch (Crosstex®) or other appropriate wrapping material
Thermometer (NIST traceable)
Calibrated analytical balance

3.1 Static Environmental Chamber Preparation, Equilibration and Monitoring

3.1.1 Rinse the environmental chambers with tap water thoroughly.

3.1.2 Clean and disinfect with BACDOWN© detergent. Do not use alcohol.

3.1.3 Prepare the environmental chamber to maintain a relative humidity of 95% RH by adding a salt solution in accordance with ASTM Standard E 104-85.

3.2 Preparation of Salt Solutions and Temperature & Relative Humidity Monitoring

3.2.1 Sodium phosphate heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) or any other appropriate salt is used to achieve 95% RH.

3.2.2 A salt bed is poured into a tray or flat dish until the tray is about halfway full of the salt.

3.2.3 Water is slowly added in 10 to 20 ml increments and stir each aliquot into the salt until liquid covers the surface of the salt bed. This is evident when the salt bed will no longer absorb the liquid.

3.2.4 100-200 ml of salt solution is generally adequate to stabilize the relative humidity in the static environmental chamber.

3.2.5 The solution should cover the entire bottom of the tray.

3.2.6 A data logger and Humidity Card® are placed in each environmental chamber to monitor temperature and relative humidity for the duration of the MRT. Temperature is maintained at 25°C.

3.2.7 To avoid contamination, test samples for different projects shall not be placed in the same static environmental chamber at any given time.

3.2.8 Temperature and relative humidity data are logged throughout for the duration of the MRT.

3.3 Test Specimen and Control Preparation

3.3.1 Test specimens, as well as control material (filter paper) are normally cut in 2 to 4 cm x 4 to 4 cm coupons. Other sizes ma be cut to maintain sample representativeness. Depending on the type of material being tested, wood may be more appropriate as the control material.

3.3.2 A set of three (3) test coupons and three (3) control coupons are prepared for each time point to be tested. In a standard test with 2 time points, 6 coupons are prepared. This requires 6 coupons for the test material and 9 control coupons for a standard test of a material at 95% RH and evaluated at time-zero, 1 week (control only) and 3 week time points.

3.3.3 Additional coupons are cut as needed for additional time points or additional organisms.

3.3.4 Test and control coupons are placed into a sterilization pouch and autoclaved for 15 minutes, or otherwise sanitized.

3.3.5 Alternate sterilization (or sanitizing) processes are allowed as long the material integrity is unaltered and the product is deemed sufficiently clean for testing. Records shall be maintained

3.3.6 Containers that will hold the coupons in the humidity chamber are selected to accommodate the size of the coupons.

3.3.7 All containers must be sterile or sterilized prior to use.

3.3.8 Glass Petri dishes are the primary container used.

3.3.9 Appropriate methods are using deeper dishes, cutting the material in a manner that does not affect the testing surface, or altering the material by another means approved by the laboratory manager or supervisor that will not affect the integrity of the sample. Contact of the test coupons with the container lid shall be kept to minimum. Deeper containers may be used. Alternatively, the test specimens may be cut in a manner that will minimize coupon to lid contact.

3.4 Inoculum Preparation and Adjustment of the Concentration

3.4.1 Flood a seed plate of a freshly grown (7 day old) primary test organism (*P. brevicompactum*) subculture with 10 ml sterile 0.9% NaCl solution. The seed plate must be pure. Other organisms may be used as necessary.

3.4.2 Gently loosen spores with a sterile spreader.

3.4.3 Collect spores with a pipette, dispense into a 50 ml sterile test tube and vortex for 30 seconds to wash spores.

3.4.4 Centrifuge the mixture at 2641 x g for 5 minutes. Discard the supernatant and re-suspend the spore residue in 10 ml sterile 0.9% NaCl solution. Vortex for 30 seconds and repeat centrifugation. Discard supernatant and re-suspend the pore residue in 10 ml sterile 0.9% NaCl solution.

3.4.5 Pipette enough of the inoculum from the test tube onto the grid area of a hemacytometer and count the spores in the grid at 400 X with a compound microscope.

3.4.6 Calculate the number of spores for each organism in 1 ml. Adjust the dilution as needed with additional Peptone Tween solution. The concentration should not exceed 2 x 10^5 spores/cc. Inoculum can be stored and used for up to 1 week with a sealed cap, maintained at 4°C.

3.5 Inoculation of Test and Control Specimen, Harvesting and Dilution Plating

3.5.1 Wear examination gloves and use aseptic techniques throughout process.

3.5.2 In a bio-hood, place the prepared coupons in the sterilized containers. There will only be 1 coupon per container.

3.5.3 Pipette 0.2 ml of the inoculum drop by drop onto east test and control coupon in order to cover as much surface as possible.

3.5.4 Allow as much inoculum to absorb into the test coupon as possible. Cover the inoculated coupon in the container.

3.5.5 Place the containers with the inoculated coupons into the appropriate static environmental chamber and incubate at 25°C.

3.5.6 Harvesting and plating is done aseptically, gloved, and under a bio-hood.

3.5.7 Time Zero (1 hour of incubation after inoculation):

3.5.7.1 Remove 1 set of each type of coupon (3 test coupons and 3 control coupons) from each static environmental chamber.

3.5.7.2 Visually assess coupons. Record observations.

3.5.7.3 With sterile forceps remove a coupon from its container.

3.5.7.4 Place the coupon in a sterile 50 ml centrifuge tube. An autoclaved flask or beaker with foil cover may be used depending upon the coupon. Do not cut or alter the coupon.

3.5.7.5 Wash spores from coupon in 10 ml of Peptone Tween.

3.5.7.6 Add more Peptone Tween to the material as needed in 5 ml increments until the material is submerged and record the final volume used.

3.5.7.7 For certain materials the recommended elution volume has been established. See Table 2.

Table 2: Recommended Elution Volumes for Common Materials

Material	Elution volume (ml)
Filter Paper (Control)	10
Ceiling tile	40
Drywall/ gypsum board	40
Textile (Upholstery)	10
Carpet	25

3.5.7.8 Place the centrifuge tube on a rotary shaker for 2 minutes or vortex for 10 seconds at high speed.
3.5.7.9 Plate a dilution series (10^{-1} to 10^{-4} suggested) for each coupon. The dilutions are plated in duplicate.
3.5.7.10 Repeat the harvesting and dilution plating for the other two coupons in each set.
3.5.7.11 Incubate the plates at 25°C.
3.5.7.12 Inspect the colonies in 4-7 days for counts and identifications.
3.5.7.13 Select the plates of countable dilution and count the number of viable colonies.
3.5.7.14 Calculate the CFU/coupon, and record the results.
3.5.7.15 Follow 3.5.7.1 through 3.5.7.14 for all time points.

3.6 Calculations

3.6.1 Using a hemacytometer count the spores determine the spore concentration of the collected spore suspension
3.6.1.1 The hemacytometer is used to prepare the required dilution of the spore suspension (e.g. 2×10^4 spores/ml).
3.6.2 Adjust the concentration by dilution ($C_1V_1=C_2V_2$) of the suspension to be used as inoculum.
3.6.3 Measure the CFU/coupon by dilution plating

$$\text{CFU/coupon} = \frac{(\text{average \# colonies/plates})(\text{elution volume})}{(\text{decimal plating factor})(\text{decimal dilution factor})}$$

3.7 Quality Assurance / Quality Control

3.7.1 RH is monitored by means of a hygrometer and/or RH data logger.
3.7.2 Saturation of the salt solution is determined visually by the presence of excess salt.
3.7.3 Temperature of the incubator is monitored with a NIST traceable thermometer, or thermometer verified against NIST traceable thermometers.
3.7.4 Verification of the inoculum viability is confirmed with positive control coupons, and plates inoculated with inoculum suspension.
3.7.5 If the Time Zero results vary by more than 25% among triplicate coupons the test set-up will be repeated.
3.7.6 Hemacytometer counts (top vs. bottom) are to be within 20% of each other. If the initial count is unacceptable, the counts are repeated after vortexing the suspension.
3.7.7 All records (including temperature and relative humidity) stall be maintained and available for review.

3.8 Preconditioning of Products Prior to Testing

3.8.1 Test specimens are equilibrated in the static environmental chamber before inoculation with the test organism for a minimum time of 24 hours or until constant weight attained.

3.9 Microbial Resistance Testing Equipment and Facilities

3.9.1 Facilities

3.9.1.1 Microbial Challenge - A facility designed and operated to assess microbial growth on building material and indoors finishes should contain humidity challenge chambers, sample storage areas, microbial incubation and handling equipment, monitoring and control systems, sample processing and analysis equipment, data acquisition systems, and data reporting systems.

3.9.2 Equipment

3.9.2.1 Environmental Humidity Challenge Requirements – the chamber requirements are defined in the ASTM Standards D 6329 and E 104.

SECTION 4
MOLD GROWTH RATES
AND PRODUCT RATINGS

4.0 Mold Resistance Rating System

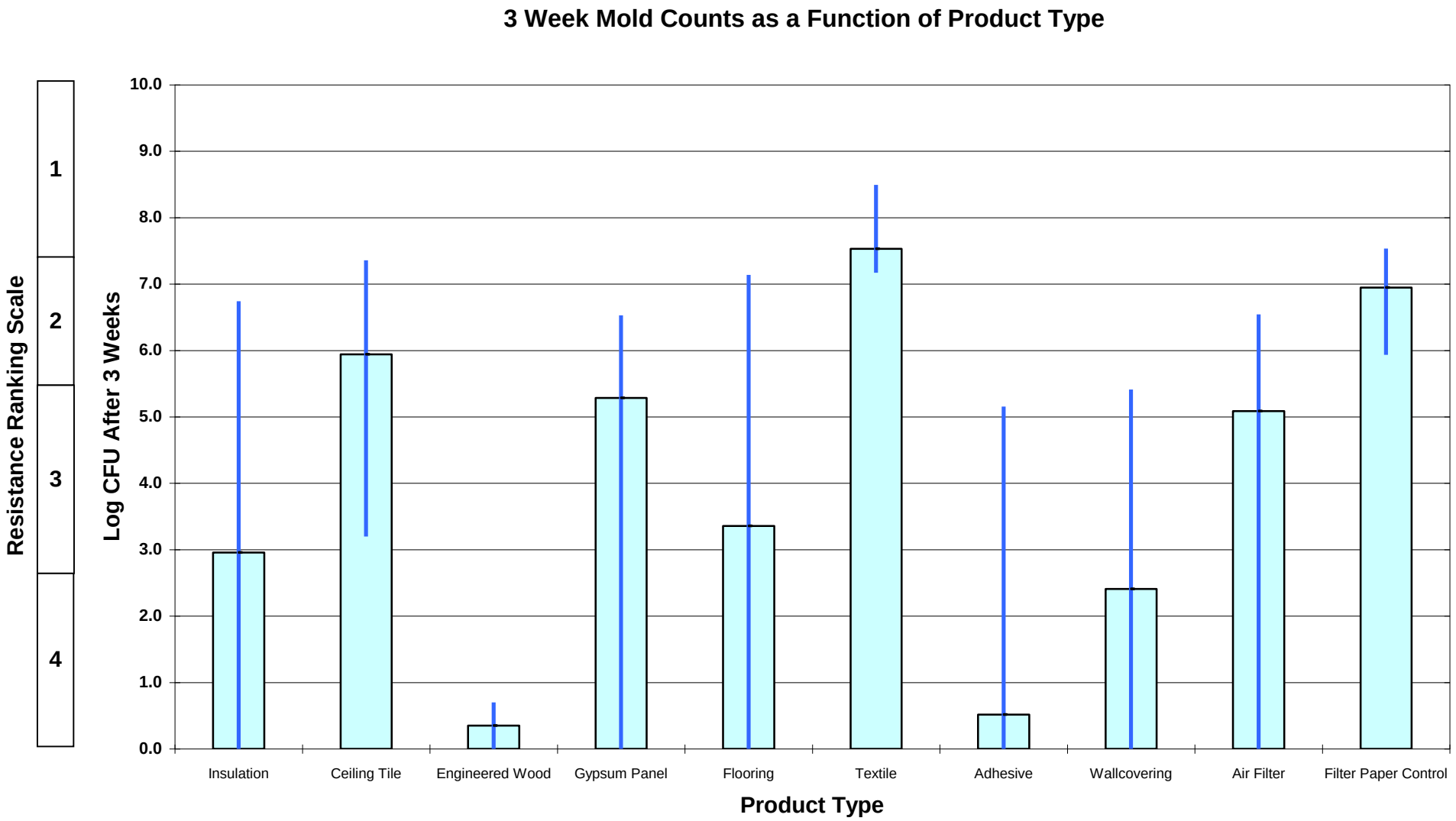
A rating system for mold resistant properties of materials has been developed. Verification studies indicated that the number of microbial colony forming units present after three weeks of incubation provides a reliable measure.

4.1 The GREENGUARD Microbial Resistant Rating following a three week incubation period at 95% RH and 25°C.

Rating	Name	Definition
1	Highly Susceptible to Mold Growth	Growth comparable to highly susceptible materials. Log(CFU) > 7.5 at 3 weeks.
2	Susceptible to Mold Growth	Growth comparable to susceptible materials. Log(CFU) ≤ 7.5 and > 5.5 at 3 weeks.
3	Resistant to Mold Growth	Growth comparable to resistant materials. Log(CFU) ≤ 5.5 and > 2.5 at 3 weeks.
4	Highly Resistant to Mold Growth	Growth comparable with highly resistant materials. Log(CFU) ≤ 2.5 at 3 weeks.

4.1.1 Figure 1 shows the 3-week average CFU measurements as a function of product of type, along with the GREENGUARD Resistant Microbial Rating Scale. Also included on the graph are bars indicating the range of values typically measured for product types.

FIGURE 1



SECTION 5
REQUIRED ELEMENTS OF THE LABORATORY TEST REPORT

5.0 The Report of the Test Results Should Contain the Following Sections:

5.0.1 Laboratory identification: Name, address, phone number and other contact information for the laboratory.

5.0.2 Manufacturer, product and sample identification:

5.0.2.1 Product name, product number, product category and subcategory (if applicable)

5.0.2.2 Manufacturer's ID number and other identification numbers (if applicable)

5.0.2.3 Manufacturing date, collection date, shipment date and date of arrival at laboratory (on chain of custody)

5.0.2.4 Laboratory sample ID or tracking number.

5.0.3 Testing conditions: Temperature, relative humidity, exposed area of test specimen (or other relevant test specimen measurement parameter), test specimen preparation details, conditioning period start date and duration (if applicable), and test period start date and end date.

5.0.4 Chamber methodology: Referenced methods/practices followed to operate chambers; description of the chamber used, how relative humidity and temperature maintained and monitored.

5.0.5 Data analysis procedures: Analytical methods used to calculate the colony forming unit at time zero and time three weeks intervals.

5.0.6 Test results: For GREENGUARD Certification tests, for all time points list temperature, relative humidity, test organism control material used and test specimen identification.

5.0.7 Provide the following information:

5.0.7.1 Provide GREENGUARD rating system scale for three week time interval MRT.

5.0.7.2 Certification of the Report with date including authorized laboratory.

5.0.7.3 Report any additional facts, which may have influenced the test results. These may include, but are not limited to, the following:

5.0.7.4 Dates of most recent internal and external calibrations, methods.

5.0.7.5 Dates of most recent proficiency evaluation(s) and corrective actions taken, if any.

5.0.7.6 Any deviations of laboratory parameters from specified values.

5.0.7.7 Any other relevant observations.

5.0.8 Attach a copy of the completed and signed chain-of-custody (COC) form with the laboratory report.