Designation: D7855/D7855M – 13

Standard Test Method for Determination of Mold Growth on Coated Building Products Designed for Interior Applications Using an Environmental Chamber and Indirect Inoculation[1]

This standard is issued under the fixed designation D7855/D7855M; 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 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.

1.2 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.

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

1.4 The 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 for 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.

1.5 The values stated in either SI units or inch-pound units are to be regarded separately as standard. The values stated in each system may not be exact equivalents; therefore, each system shall be used independently of the other. Combining values from the two systems may result in non-conformance with the standard.

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

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D16 Terminology for Paint, Related Coatings, Materials, and Applications

D1193 Specification for Reagent Water

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

E177 Practice for Use of the Terms Precision and Bias in ASTM Test Methods

E691 Practice for Conducting an Interlaboratory Study to Determine the Precision of a Test Method

3. Terminology

3.1 *Definitions*—For definitions of terms refer to Terminology D16.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *chamber control, n*—open Petri dish containing appropriate agar to demonstrate viability of fungal organisms within the environmental chamber.

3.2.2 *coated building product, n*—a building material having a liquid, liquefiable or mastic composition that is converted to a solid protective, decorative, or functional adherent film after application as a thin layer onto a building fabric.

3.2.3 *interior, n*—any surface not exposed to exterior environments in end use.

3.2.4 *interior finish, n*—interior wall and ceiling finish and interior floor finish.

3.2.5 *material control, n*—untreated representative substrate.

3.2.6 *sample, n*—a portion of material taken from a larger quantity for the purpose of estimating properties or composition of the larger quantity.

3.2.7 *sample tests, n*—a group of samples (one or more).

[1] This test method is under the jurisdiction of ASTM Committee D01 on Paint and Related Coatings, Materials, and Applications and is the direct responsibility of Subcommittee D01.28 on Biodeterioration.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3.2.8 *test run, n*—the evaluation of coated building products in accordance with the procedure outlined in this test method.

3.2.9 *test specimen, n*—a portion of a test unit needed to obtain a single test determination.

4. Summary of Test Method

4.1 This test method is an indirect inoculation to a coated interior building product of two fungal organisms, *Aspergillus niger* and *Penicillium citrinum*. Test specimens are placed in an environmental chamber maintained at 30 ± 2°C [86 ± 3.6°F] and at greater than 90 % relative humidity for four weeks. Humidity is maintained by adding sufficient sterile DI water to the bottom of the covered test chamber. A continuous fungal inoculation is provided by open Petri dishes supporting seven day cultures of the two test organisms placed on a rack below the test pieces. Test specimens are removed from the chamber after four weeks exposure and examined for fungal growth. The evaluation is a macroscopic inspection of the test pieces with indirect lighting.

5. Significance and Use

5.1 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.

5.2 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.

6. Interferences

6.1 Proper lab ventilation, hygiene, and aseptic technique must be followed to ensure fungal cultures are pure and no cross contamination of fungal strains or growth media occurs.

6.2 The exposure of test specimens to environmental conditions including temperature, humidity, and light can impact test results. To minimize variability of test results consistent handling and storage of test specimens is important.

7. Apparatus

7.1 *Environmental Chamber*—A non-corrosive covered box containing standing water placed in an incubator at 30 ± 2°C [86 ± 3.6°F] will expose the test specimens to a controlled environment of temperature and humidity. Containers found suitable include glass, polycarbonate[3] or other plastic storage containers which are generally available. For example a nineteen liter container measuring approximately 460 mm long by 300 mm wide by 230 mm high [18 in. long by 12 in. wide by 9 in. high] can accommodate fifteen 75 by 100 mm [3 by 4 in.] test specimens suspended from rods using cable ties. Opaque chambers shall have a viewing port that permits observation of chamber controls. Chamber shall permit temperature and humidity monitoring without opening the chamber lid. Examples would include wireless or wired probes.

7.1.1 The test chamber shall be designed so that no condensate forming on the top interior surface will drip onto the test specimens. For stand-alone chambers, this can be accomplished by designing the top so that the interior surface is at an angle of at least 30 degrees, relative to the plane of the bottom of the chamber. A sheet of polycarbonate secured at an angle, or hinged or joined sheets of polycarbonate attached to the underside of the lid will direct the condensation away from the test samples. Condensation inside the test chamber is not a concern as it indicates that humidity is being maintained.

7.1.1.1 A non-corrosive open grid is placed at the bottom of the test chamber above the water level supporting 100 by 15 mm [4 by ⅝ in.] sterile Petri dishes alternating the two fungal organisms. See Fig. 1 for non-corroding open grid example. Place sufficient Petri dishes on the rack to fill the grid. The plastic grid designed to cover recessed ceiling fixtures or similar works well.

7.1.1.2 Position test specimens by suspending them from rods using plastic cable ties. Samples must be 50 to 100 mm [2 to 4 in.] above the inoculated Petri dishes. The minimum distance between adjacent specimens and between test specimens and chamber walls shall be at least 25 mm [1 in.]. Materials used as the rods or mounting racks shall be non-corroding and of sufficient strength to support specimens throughout the duration of the test. Use of engineered plastics such as polycarbonate has been found suitable. Fig. 2 shows a photo of typical chamber construction. Fig. 3 illustrates use of cable ties to hang test specimens from rods.

7.2 *Measurement Instruments,* capable of accurate and precise measures of temperature and humidity. See Section 12.

7.3 *Incubator or Controlled Temperature Room* maintained at 30 ± 2°C [86 ± 3.6°F].

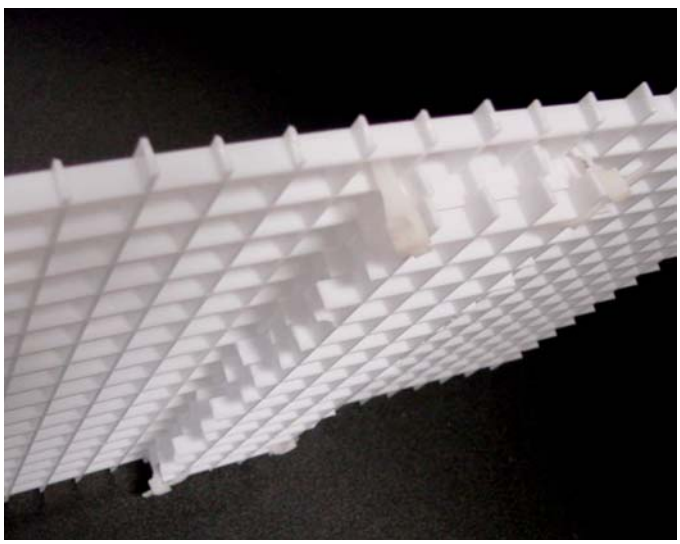


FIG. 1 Non-corroding Open Grid for Placement in Bottom of the Environmental Chamber

[3] The 5.0 Gallon Rectangular Food Storage Containers from various suppliers have been found to work well.

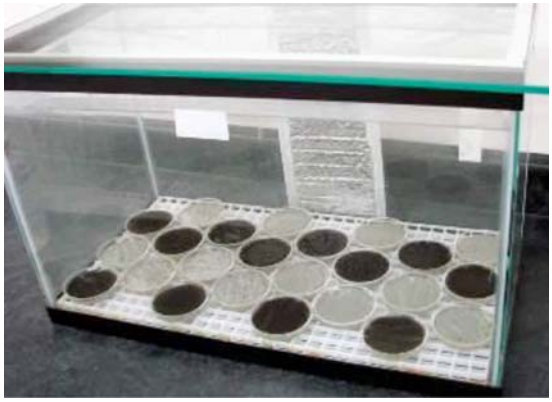


FIG. 2 Typical Environment Chamber Set Up

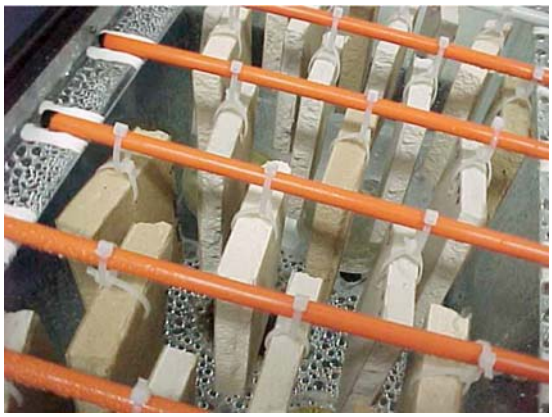


FIG. 3 Cable Ties used to Hang Test Specimens from Rods Inside of the Environmental Chamber

8. Reagents and Materials

8.1 *Cultures—Aspergillus niger*, ATCC[4] 6275 or IMI/CABI Bioscience[5] 45551, *Penicillium citrinum* ATCC 9849 or IMI/CABI Bioscience 321326.

8.1.1 Selection of the appropriate test organisms is extremely important and must be representative of the types of organisms found or likely to be found on the interior coated building products being tested. The organisms named in 8.1 are not representative of all potential fungal organisms that may be found growing on interior coated building products. Other fungal organisms may also be used in separate evaluations, but the specified organisms in 8.1 shall be used and reported. The potential for interferences between non-specified fungal test organisms shall be considered when using organisms other than those named in 8.1.

NOTE 1—Subcommittee D01.28 reviewed the published study listed in the Reference section of this document and determined the organisms in 8.1 as appropriate.

8.2 *Chamber Controls*—Open PDA plates placed on the bottom of the chamber at opposite corners and near the center.

8.3 *Material Controls,* if available, see 3.2.

8.4 *Purity of Reagents*—Water shall be distilled water or higher purity. See Specification D1193.

8.5 *Sabouraud Dextrose Agar* or media appropriate for fungi selected.

8.6 *Sterile Disposable Cotton-tipped Swabs.*

8.7 *Sterile 100 by 15 mm (4 by ⅝ in.) Petri Dishes.*

9. Hazards

9.1 This test must be performed by trained individuals in laboratories specially equipped for conducting microbiological tests.

10. Sampling and Test Specimens

10.1 Sampling shall be representative of the product being evaluated.

10.2 *Test Specimens:*

10.2.1 A minimum of three test specimens shall be cut from each sampled coated interior building product to be evaluated. The number of test specimens will be reported in the results.

10.2.2 Additional replicates should be available to rerun the test if necessary.

11. Preparation of Apparatus and Inoculum

11.1 Clean and sanitize the environmental chambers prior to use. Add approximately 25 mm [1 in.] of water to the bottom of the container. Ensure the water level is sufficient to provide humidity through the duration of the test. If the test specimens absorb the water or water is lost through the seal of the lid, additional water must be added to ensure the relative humidity remains at 90 % or greater for the duration of the test. The water level should be at least 25 mm [1 in.] below the rack supporting the Petri dishes of the fungal test organisms.

NOTE 2—Petroleum jelly or similar product may be used between the lid and the container to improve the seal.

11.2 Insert the temperature/humidity sensor or data logger into the environmental chamber and set in an incubator or other temperature controlled chamber set at 30 ± 2°C [86 ± 3.6°F] to equilibrate for 24 h before starting the test. Record the temperature and humidity not less than every 7 days. If temperature and humidity readings are outside the parameters set in 4.1, results shall be discarded and testing restarted with new test pieces. Temperature and humidity measurements shall be included in the final report.

11.3 Prepare spore suspensions of each test fungi from 7 to 14 day old well sporulating cultures. Maturation of the fungal organisms designated in 8.1 may not occur at the same rate. *Penicillium citrinum* typically takes longer to sporulate than *Aspergillus niger* so cultivation should begin earlier assuring both organisms are sporulating when placed in the environmental chamber. Stock cultures may be kept for no more than four months at 3 to 10°C [37 to 50°F].

11.3.1 To prepare the inoculum, dislodge fungal spores from agar by rolling a sterile cotton-tipped swab moistened with sterile distilled water across the sporulating fungi. Transfer the spores from the cotton tipped swab to a test tube containing 5

[4] Cultures can be obtained from American Type Culture Collection, P.O. Box 1549, Manassass, VA 20108 or Mycological Services, P.O. Box 1056, Crawfordsville, IN 47933.

[5] Cultures can be obtained from IMI/CABI Bioscience, Nosworthy Way, Wallingford, Oxfordshire, OX108DE UK.

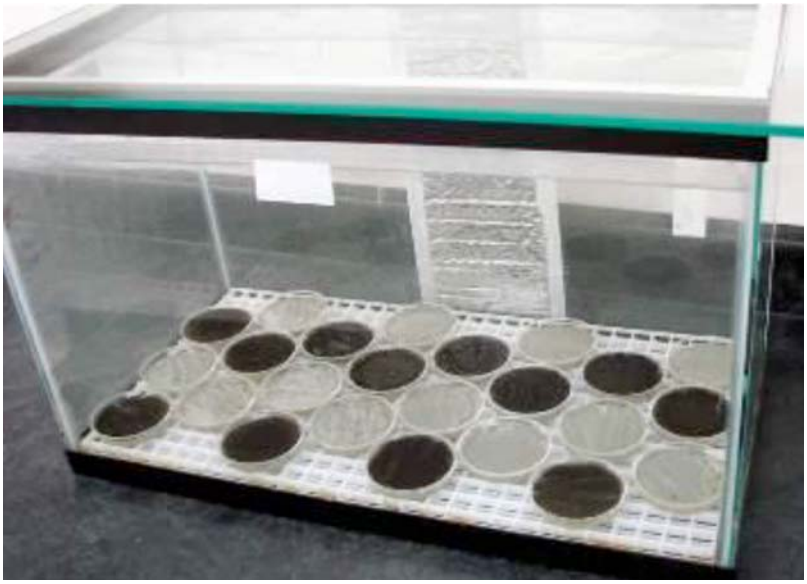


FIG. 4 Example of Sporulating Fungal Plates Arranged in an Alternating Pattern in the Environmental Chamber

ml of sterile distilled water and a nontoxic wetting agent for each test organism. Blend the fungal spore suspension on the vortex mixer for 10 s to liberate spores from hyphae and to break up spore clumps. Repeat this procedure for each fungal organism used.

11.4 Pour 25 mL [1.0 oz.] of Sabouraud Dextrose or appropriate agar for test organisms named in 8.1 into 100 by 15 mm [4 by ⅝ in.] sterile Petri dishes and allow the agar to solidify. Prepare sufficient Petri dishes to fill the rack placed at the bottom of each environmental chamber. For the chamber size referenced in 7.1, twelve 100 by 15 mm [4 by ⅝ in.] Petri dishes are appropriate.

11.5 Transfer 200 to 300 µL (microlitres) of a single spore suspension to each Petri dish. Spread the inoculum across the surface of the agar with a cell spreader or equivalent, cover and incubate at 28 to 30°C [82 to 86°F] for 7 to 14 days or until dense sporulating fungal growth is present. Prepare an equal number of Petri dishes for both test organisms in sufficient quantity to cover the rack placed at the bottom of the environmental chamber.

11.6 If growth is sparse or absent after 14 days, prepare fresh inoculums as discussed in 11.3 and repeat inoculation of new agar plates.

11.7 Place the sporulating agar plates on the rack of the equilibrated environmental chamber referenced in 11.2 and remove covers. Place Petri dishes alternating between the two test organisms to ensure equal exposure of the test specimens to both fungal strains. Fig. 4 shows the alternating pattern of agar plates on the rack at the bottom of the environmental chamber.

12. Calibration and Standardization

12.1 Instruments for temperature measurement shall measure with a precision of ±2°C [±3.6°F] over the full range of instrument capability.

12.2 Instruments for humidity measurement shall measure with a precision of ±5 % RH at 95 % RH.

12.3 Both types of measurement instruments shall be calibrated no less than annually by a laboratory using standards that are documented traceable to NIST standards.

13. Conditioning

13.1 Equilibrate triplicate test specimens of each sample and condition to room temperature before starting test.

14. Procedure

14.1 *Sample Preparation*—Wear disposable powder-free plastic gloves or equivalent when handling test pieces. Approximate test specimen size shall be approximately 75 by 100 mm [3 by 4 in.] or approximately 75 cm^2 [12 $in.^2$].

NOTE 3—A 25 mm [1 in.] grid may be drawn with a pencil on the surface of the test specimen to help standardize the evaluation of the sample for fungal growth.

14.2 Add approximately 25 mm [1 in.] of distilled water to the bottom of the test chamber. Remove the lids of the 7 to 14 day old sporulating culture plates and place them on the tray in the bottom of the chamber above the water level.

14.3 *Exposure*—Place test specimens and chamber controls randomly in the environmental chamber allowing free air circulation. Test specimens shall be placed so there is no contact between them, the inside walls or the lid of the chamber. Required minimum spacing between test specimens is 25 mm [1 in.]. Place environmental chamber in the incubator or temperature room maintained at 30 ± 2°C [86 ± 3.6°F]. Record twelve regularly spaced temperature and humidity observations over the 4 week incubation period.

14.3.1 After four weeks exposure remove the test specimens from the environmental chambers and determine the percent fungal growth across the surface. Rate the test specimen in accordance with the scale in 15.3 and record the temperature and humidity at the time of test completion.

14.4 If fungal growth is not seen on the chamber controls (8.2) at one week, or if readings for temperature and humidity are outside the parameters set in 4.1, data shall be discarded and testing restarted with new test pieces

14.5 A separate test shall be run if additional test organisms besides those named in 8.1.1 are desired.

15. Calculation or Interpretation of Results

15.1 Macroscopically evaluate test specimens after four weeks. Look carefully for evidence of fungal growth across the entire surface to determine the percent coverage as characterized by fungal spores and mycelia. All fungal growth shall be considered when rating the test specimen including organisms which are not part of the inoculum. Care shall be taken to report fungal growth only initiating on the surface of the test specimen.

15.2 Illuminate the surface of the test specimen with light pipes or other low angle light sources and visually consolidate any discontinuous growth to estimate the percent growth on the test piece where 100 % growth would completely obscure the surface. Standard 150 watt illuminators with self supporting light pipes and adjustable light intensity work well. See Fig. 5. Test specimens shall be evaluated from different directions to identify inconsistencies in the inoculated surface. Look for inconsistencies of color or texture, or both, of the inoculated surface with the naked eye only. Use a stereoscope at 20 to 40X magnification or other low level magnification, that is, task light, as pictured in Fig. 6 to confirm that any inconsistencies of texture or color identified during macroscopic evaluation as fungal growth are in fact microbial in nature. Do not use magnification to search for fungal growth on the sample surface, only to confirm that any questionable defacement is fungal growth. A note indicating the number of different types of organisms present based on general appearance may be made in the report.

15.2.1 Where a grid is used to aid in the evaluation of the test specimen, consider each square in the grid for evidence of fungal growth as characterized by mycelium or fruiting bodies, or both. Fungal organisms including but not limited to the inoculated organisms shall be included in the rating. Tally the percent of fungal growth (maximum 100 %) in each square of the grid and divide by the total squares in the grid to determine percent total growth. Rate the test specimen in accordance with the scale in 15.3.

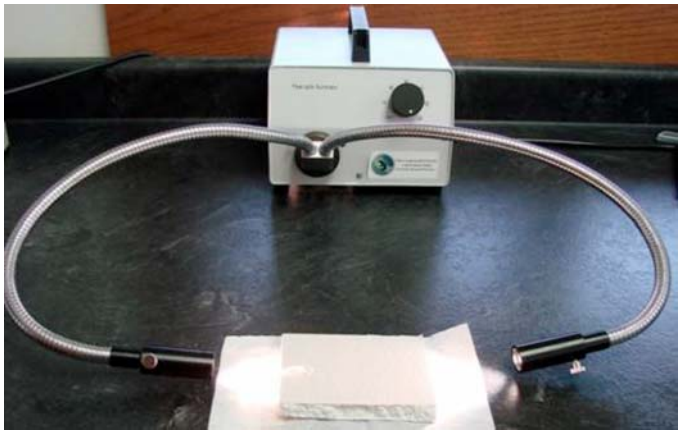


FIG. 5 Light Pipes Illuminating Surface of Test Specimen

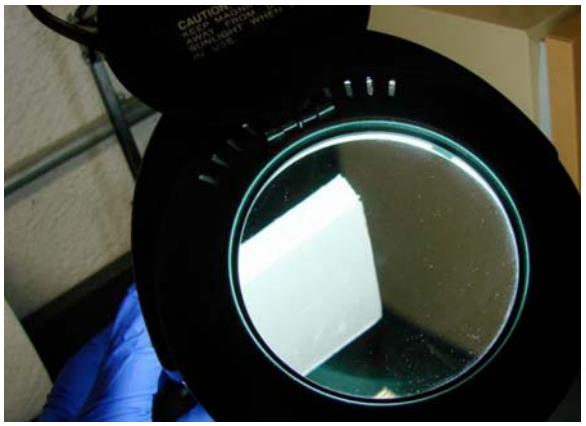


FIG. 6 Task Light Providing Low Level Magnification of Test Specimen

15.3 *Rating:*

Rating	Percent Growth
1	no visible growth
2	less than 5 % growth
3	6 to 20 % growth
4	21 to 80 % growth
5	>80 % growth

15.3.1 The images in Fig. 7 are examples of test pieces with 5 %, 20 % and 80 % mold growth over the surface representing the maximum fungal growth permitted to achieve ratings of 2, 3, and 4 respectively.

16. Report

16.1 Report the results at the end of the 4-week exposure including the % growth and numerical rating of the three test specimens and the names of the test organisms used in the inoculum. Include a record of the chamber humidity and temperature as recorded by the data loggers or temperature and humidity probes, or combination thereof, taken weekly. Include the instrument's accuracy in the record.

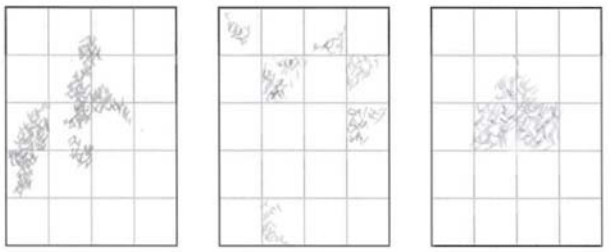
17. Precision and Bias[6]

17.1 The precision of this test method is based on an inter-laboratory study of Test Method for Determination of Mold Growth on Coated Building Products Designed for Interior Applications Using an Environmental Chamber and Indirect Inoculation conducted in 2011. Eight laboratories tested a total of four different materials. Every "test result" represents an individual determination. Practice E691 was followed for the design and analysis of the data; the details are given in ASTM Research Report No. RR:D01-1172.

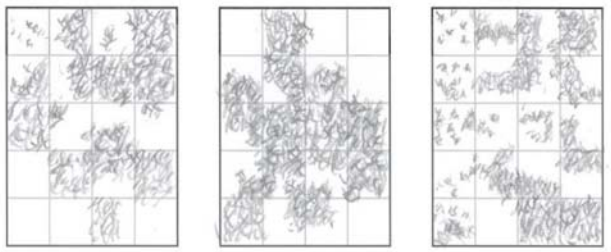
17.1.1 *Repeatability Limit (r)*—Two test results obtained within one laboratory shall be judged not equivalent if they differ by more than the "*r*" value for that material; "*r*" is the interval representing the critical difference between two test

[6] Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR:D01-1172. Contact ASTM Customer Service at service@astm.org.

Rating 2 - Less than 5% growth over surface (0.6 of one square)



Rating 3 - 6% to 20% growth over surface (> .6 up to 2.4 squares)



Rating 4 - 21% to 80% growth over surface (> 2.4 squares up to 9.6 squares)

FIG. 7 Examples of Test Pieces with 5 %, 20 %, and 80 % Mold Growth over the Surface and the Appropriate Ratings

results for the same material, obtained by the same operator using the same equipment on the same day in the same laboratory.

17.1.1.1 Repeatability limits are listed in Table 1 and Table 2.

17.1.2 *Reproducibility Limit (R)*—Two test results shall be judged not equivalent if they differ by more than the "*R*" value for that material; "*R*" is the interval representing the critical difference between two test results for the same material, obtained by different operators using different equipment in different laboratories

17.1.2.1 Reproducibility limits are listed in Table 1 and Table 2.

17.1.3 The above terms (repeatability limit and reproducibility limit) are used as specified in Practice E177.

TABLE 1 % Growth

Material	Average $\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation S_R	Repeatability Limit r	Reproducibility Limit R
A	85.08 %	8.16	18.97	22.86	53.12
B	96.92 %	5.72	5.72	16.02	16.02
C	0.38 %	0.71	0.94	1.98	2.63
D	4.17 %	3.73	6.86	10.45	19.21

TABLE 2 Scale Rating

Material	Average $\bar{X}$	Repeatability Standard Deviation s_r	Reproducibility Standard Deviation S_R	Repeatability Limit r	Reproducibility Limit R
A	4.625	0.29	0.60	0.81	1.68
B	4.958	0.20	0.20	0.57	0.56
C	1.167	0.29	0.39	0.81	1.09
D	1.750	0.29	0.88	0.81	2.46

17.1.4 Any judgment in accordance with statements 17.1.1 and 17.1.2 would have an approximate 95 % probability of being correct.

17.2 *Bias*—At the time of the study, there was no accepted reference material suitable for determining the bias for this test method, therefore no statement on bias is being made.

17.3 The precision statement was determined through statistical examination of 380 results, from eight laboratories, on four materials. These four materials were the following:

Material A: ½ in. gypsum board

Material B: Same coated with commercial multi-purpose primer

Material C: ½ in. gypsum board coated with mold-resistant paint

Material D: Fiberglass mat gypsum panel

18. Keywords

18.1 coated building product; environmental chamber; fungal growth; fungal resistance

ANNEX

(Mandatory Information)

A1. SAFE HANDLING OF MICROORGANISMS

A1.1 Refer to 4th Edition "Biosafety in Microbiological and Biomedical Laboratories (BMBL)," published by U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health, Fourth Edition, May 1999.

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