Designation: C 1338 – 08

Standard Test Method for Determining Fungi Resistance of Insulation Materials and Facings [1]

This standard is issued under the fixed designation C 1338; 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 the determination of the ability of new insulation materials and their facings to support fungal growth.

1.2 *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.*

1.3 The values stated in inch-pound units are to be regarded as standard. The values given in parentheses are mathematical conversions to SI units that are provided for information only and are not considered standard.

2. Significance and Use

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.

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.

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 take place. In such cases, the use of the comparative material is omitted and the pass/fail criterion is based upon growth.

3. Apparatus

3.1 *Glassware*—Sterile disposable petri dishes, 4 or 6 in. (100 or 150 mm) by 0.6 or 0.75 in. (15 or 20 mm) in size are preferred. For larger specimens, trays of borosilicate glass or baking dishes up to 16 by 20 in. (400 by 600 mm) in size may be used.

3.2 *Environmental Chamber or Cabinet*—Equipment for this test method shall maintain a temperature of 82.4 to 86°F (28 to 30°C) and a relative humidity of 95 % (±4 %). Provisions shall be made to prevent condensation from dripping on the test specimen. There shall be free circulation of air around the test chamber.

3.3 *Atomizer*—A chromatography atomizer capable of providing 100 000 ± 20 000 spores/in.^{2} (15 000 ± 3000 spores/cm^2) shall be used for inoculation.

3.4 *Autoclavable Biohazard Bags*, or metal pan able to withstand autoclaving.

4. Reagents and Materials

4.1 *Purity of Water*—Unless otherwise specified, references to water shall be understood to mean sterile distilled water or water of equal purity.

4.2 *Inoculum*:

Fungi	ATCC[2]
Aspergillus niger	9642
Aspergillus versicolor	11 730
Penicillium funiculosum	11 797
Chaetomium globosum	6205
Aspergillus flavus	9643

4.3 *Cultures*—Maintain cultures of the Aspergillus fungi separately on Czapek Dox agar (see Note 1). Culture the Chaetomium globosum on strips of cellulose filter paper on the surface of Czapek Dox agar. Maintain the Penicillium fungi on Sabouraud Dextrose agar. The stock cultures may be kept for not more than 4 months at 43 ± 7°F (6 ± 4°C) at which time subcultures shall be made, and new stocks selected from the subcultures. If genetic or physiological changes occur, obtain new cultures. Incubate subcultures used for preparing new stock cultures or the spore suspension at 86 ± 4°F (30 ± 2°C) for 5 days or longer.

NOTE 1—This media is readily available from any science/microbiological supply house.

5. Specimens

5.1 *Viability Specimens*—Determine the viability of the spore suspension during incubation with these controls: with

[1] This test method is under the jurisdiction of ASTM Committee C16 on Thermal Insulation and is the direct responsibility of Subcommittee C16.31 on Chemical and Physical Properties.

Current edition approved June 1, 2008. Published July 2008. Originally approved in 1996. Last previous edition approved in 2000 as C 1338–00.

[2] The sole source of supply of the cultures known to the committee at this time is American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

each daily group of tests, place one piece of sterilized Whatman 500 filter paper, 1 in.^{2} (6.4 cm^2) on each of two prepared hardened Czapek Dox agar specimens in separate petri dishes. Prepare a third petri dish with Sabouraud Dextrose agar.

5.2 *Comparative Material*—A white birch tongue depressor, 0.75 by 6 in. (20 by 150 mm) or southern yellow pine is the comparative item to determine the relative growth on specimens being tested. The choice of comparative item should be noted. Refer to the appropriate materials standard for relevant comparative materials.

NOTE 2—The comparative item is chosen to reflect the building construction material. In some cases, other material may be more relevant as a comparative item.

5.3 *Test Specimens*:

5.3.1 Prepare triplicate specimens from each test sample.

5.3.1.1 If the test sample is of different construction on each face, prepare triplicate specimens of each face in the up position.

5.3.2 The preferred specimen thickness is 0.75 in. (20 mm). If altering the specimen thickness is required, ensure that the surface to be tested has not been altered and is in the up position. If the normal specimen thickness is less than 0.75 in. (20 mm), then test at its normal thickness. If the specimen container will be covered, the specimen shall not make contact with it.

5.3.2.1 When testing loose materials such as blowing or pouring insulations, refer to the appropriate materials standard for sample preparation recommendations.

5.3.3 It is sometimes desirable to test the adhesive used to bond a facing to the substrate. In such case only, peel back the facing approximately half way across the face of the specimen before testing. Note this change clearly in the report.

6. Procedure

6.1 *Spore Suspension*—Prepare a spore suspension of each of the five fungi by pouring into one subculture of each fungus a 10 mL portion of a sterile solution containing a sufficient quantity, not to exceed 0.10 g/L, of a nontoxic wetting agent such as sorbitan monoleate (Tween-80), sodium dioctyl sulfosuccinate, or sodium lauryl sulfate to prevent clumping of the spores. Gently scrape the surface growth from the culture of the test organism using a sterile platinum or nichrome inoculating wire. Pour the spore charge into a sterile 125-mL glass-stoppered Erlenmeyer flask containing 45 ± 1 mL of sterile water, and 50 to 75 solid glass beads, approximately 0.20 in. (5 mm) in diameter. Vigorously shake the flask to liberate the spores from the fruiting bodies and to break the spore clumps. Filter the dispersed fungal spore suspension through at least a 0.24-in. (6-mm) layer of glass wool contained in a glass funnel, into a sterile flask. This process is intended to remove large mycelial fragments and clumps of agar that could interfere with the spraying process. Centrifuge the filtered spore suspension. Remove all the supernatant down to the surface of the spore pellet, taking care not to remove or disturb the spore pellet. Resuspend the residue in 50 mL of sterile water and centrifuge. (It may be necessary to add a small quantity of nontoxic wetting agent, not to exceed 0.10 g/L, to prevent clumping of the spores.) Wash the spores obtained from each of the fungi in this manner three times. Dilute the final washed residue with distilled water in such a manner that the resultant spore suspension shall contain 1 000 000± 200 000 spores per mL as determined with a counting chamber. Repeat the operation for each organism used in the test and blend equal volumes of the resultant spore suspensions to obtain the final mixed spore suspension. The spore suspension may be prepared fresh each day or may be held at 43 ± 7°F (6 ± 4°C) for not more than 28 days, or until the viability test indicates poor growth, or until growth appears in the sealed storage bottle.

6.2 *Inoculation of Test Specimens, Comparative Material, and Control Specimens*—Precondition the chamber and its contents at 86± 4°F (30 ± 2°C) and 95± 4 % relative humidity for at least 4 h. Place each test, comparative material, and viability control specimen in separate sterile petri dishes. Inoculate each specimen with approximately 0.50 mL of spore suspension by spraying exposed surfaces in the form of a fine mist from a previously sterilized atomizer or nebulizer. If a specimen container is covered, it shall be loose fitting glass to allow air circulation. Place specimens in the chamber and immediately begin incubation.

6.3 *Incubation*—Maintain the test chamber at 86 ± 4°F (30 ± 2°C) and a relative humidity of 95 ± 4 % throughout the test. Keep the test chamber closed during incubation except during inspection. After 3 to 7 days, inspect the control specimens. If control specimens do not show an abundance of growth at this time, repeat the entire test. If growth is present on the control specimens, continue the test for a minimum period of 28 days ± 8 h from the time of incubation. Some materials may require longer periods of incubation, therefore, the test period may be extended. Note all incubation times. Refer to the materials specifications for incubation periods.

7. Interpretation of Results

7.1 *Inspection*—At the end of the incubation period, remove the test specimens and comparative item from the test chamber and examine at 40× magnification.

7.2 *Interpretation of Results*—Test specimens that have growth greater than that on the comparative item shall be considered to have failed. Test specimens on which the growth is not greater than that on the comparative item shall be considered to have passed.

7.2.1 If no growth is the criterion, the observation of any growth on the test specimen shall be considered a failure.

7.3 After completion of inspection, all test specimens and test equipment shall be autoclaved under the manufacturer's autoclave instructions to ensure destruction of vegetative cells and spores to prevent accidental contamination to the laboratory and environment.

8. Report

8.1 Report the following information:

8.1.1 Complete identification of the material tested,

8.1.2 Identification of variable test conditions (comparative item, sample moistening, test duration) criterion used to determine Pass/Fail (comparative item or no growth), and

8.1.3 Results of the test.

9. Precision and Bias

9.1 No statement is made about either the precision or the bias of this fungi resistance test method since the test is a subjective, visual determination of whether the test material differs from a comparative material.

10. Keywords

10.1 batts; blanket; boards; cellulose; facings; foam; fungi resistance; insulation; loose-fill; mineral fiber