Standard Test Method for
Determining the Resistance of Paint Films and Related Coatings to Fungal Defacement by Accelerated Four-Week Agar Plate Assay

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

1.2 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

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

2. Referenced Documents
2.1 ASTM Standards:
D 3273 Test Method for Resistance to Growth of Mold on the Surface of Interior Coatings in an Environmental Chamber
D 3456 Practice for Determining by Exterior Exposure Tests the Susceptibility of Paint Films to Microbiological Attack
D 4141 Practice for Conducting Accelerated Outdoor Exposure Tests of Coatings
D 4587 Practice for Conducting Tests on Paint and Related Coatings and Materials Using a Fluorescent UV-Condensation Light- and Water-Exposure Apparatus
D 5031 Practice for Conducting Tests on Paints and Related Coatings and Materials Using Enclosed Carbon-Arc Light and Water Exposure Apparatus
G 21 Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi

3. Summary of Test Method
3.1 This test method outlines a procedure to (1) prepare a suitable specimen for testing, (2) inoculate the specimen with the proper fungal species, (3) expose the inoculated samples under the appropriate conditions for growth, and (4) provide a schedule and guidelines for visual growth ratings. This test method is not designed to include all the necessary procedures to maintain the proper microbiological techniques required to provide the most accurate results.

4. Significance and Use
4.1 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.

NOTE 1—It is hoped that a ranking of relative performance would be similar to that ranked from outdoor exposures. However, this test method should not be used as a replacement for exterior exposure (that is, Practice D 3456) since many other factors, only a few of which are listed will affect those results.

NOTE 2—Several companies have reported reasonable correlation of results from this test with actual use when testing film-forming, pigmented coatings. Round-robin testing of this test method versus exterior exposure is planned.

4.2 Familiarity with microbiological techniques is required. This test method should not be used by persons without at least basic microbiological training.

5. Apparatus and Materials
5.1 Balance, capable of weighing to 0.10 g.
5.2 Incubator, or other device capable of maintaining a constant temperature between 25 and 30°C, relative humidity of ±85%.
5.3 Refrigerator, or other device capable of maintaining a
temperature of 4 ± 2°C.

5.4 Petri Dishes, 100 by 15 mm (3.9 by 0.6 in.).

5.5 Autoclave, capable of producing 103 kPa (15 psi) of steam pressure at 121°C and maintaining it for a minimum of 15 min. An autoclave is not necessary if pre-prepared media plates are used.

5.6 Paint Brush, coarse bristle, 12 to 19 mm (½ to ¾ in.).

5.7 Substrate, Filter Paper (Glass fiber, Grade 391, 4.2 cm (1.65 in.)) or draw-down paper (unlaquered chart paper 216 by 280 mm (8.5 by 11 in.), cut into 10 216 by 28-mm (8.5 by 1.1-in. strips).

5.8 Atomizer or Chromatography Sprayer.

5.9 Sterile Glass Rods, Forceps, 250-mL Glass Erlenmeyer Flasks, Test Tubes, and other routine microbiological equipment.

5.10 Potato Dextrose Agar (PDA) or Malt Agar.

5.11 Nutrient-Salts Agar. (see Practice G 21, 6.3.)

5.12 Nutrient-Salts Solution, (see 5.11 without agar).

5.13 Counting Chamber (Hemocytometer).

6. Reagents and Materials

6.1 Purity of Reagents—Reagent grade chemicals should be used in all tests. Unless otherwise indicated, it is intended that all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided they are first ascertained to be of sufficiently high purity to permit use without decreasing the accuracy of the determination.

6.2 Purity of Water—Unless otherwise indicated, references to water are understood to mean distilled water or water of equal or higher purity.

6.3 PDA or Malt Agar plates can be purchased prepared, or the PDA and Malt Agar powder can be purchased and prepared according to the instructions using standard microbiological techniques and equipment.

7. Preparation of the Fungal Spore Inocula

7.1 Fungal Cultures—Use the following test fungi in preparing the inocula:

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5 Pre-prepared plates are available from microbiological supply companies, or they may be prepared using standard microbiological equipment and techniques.

6 Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see Analar Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, U.K., and the United States Pharmacopoeia and National Formulary, U.S. Pharmacopoeial Convention, Inc. (USPC), Rockville, MD.

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

8 The supply of penicillium funiculosum, known to the committee at this time is the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD, 20852 or the Mycological Services (MYCO), Box 1056, Crawfordsville, IN, 47933. Either can be used. If you are aware of alternative suppliers, please provide this information to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend.

9 Historically known as Pulsatilla pallidula.
9. Procedure

9.1 Inoculation of the Test Specimens:

9.1.1 The *A. niger* and *P. funiculosum* may be tested together on the same plates. The *A. pullulans* must be tested separately to ensure its survival.

9.1.2 Combine an equal portion of the *A. niger* and *P. funiculosum* spore suspensions.

9.1.3 Run a count of the spores using a counting chamber to confirm the inoculum count for each test (see 7.5).

9.1.4 Apply a thin coat of fungal suspension to each specimen using a sterile atomizer or pipet, making sure the surface is covered, but not to oversaturate the samples. Alternately, a separate sterile cotton swab may be used to apply and evenly spread the inoculum over the surface of each test sample. Be certain that the amounts of inoculum used are the same between each of the various samples under test. This should be done using the same method by the same applicator at the same time for all samples.

9.1.5 Incubate all plates at 28°C under 85 to 90 % relative humidity for 4 weeks.

10. Evaluation of Results

10.1 Rate the growth weekly for four weeks according to the following:

<table>
<thead>
<tr>
<th>Observed Growth on Specimens</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>Traces of growth (&lt;10 %)</td>
<td>1</td>
</tr>
<tr>
<td>Light growth (10–30 %)</td>
<td>2</td>
</tr>
<tr>
<td>Moderate growth (30–60 %)</td>
<td>3</td>
</tr>
<tr>
<td>Heavy growth (60 % to complete coverage)</td>
<td>4</td>
</tr>
</tbody>
</table>

Note 9—These ratings are for microbial growth, not coating performance, so as not to be confused with exterior evaluations that run from 10 to 0. The lower growth ratings should correspond to longer time periods of fungus-free surface under actual use conditions between the samples compared in a given test (if the samples are leached/weathered). Comparisons of actual ratings between samples tested at different times should be avoided since changes in inocula, substrate content, or other conditions can affect the growth rating. Comparisons of relative rankings of performance between samples tested at different times should be valid.

10.2 Notations should be made for “zones of inhibition” of growth on the surrounding agar if present in addition to a “0” growth rating on the sample. Such zones can be designated by a Z prefix with a number following it. The number would correspond to the average width in millimetres of the zone around the sample. A large zone of inhibition indicates good biocidal effectiveness against the test organism(s), but it also suggests that the biocide is rapidly migrating out of the coating (high potential for leaching). Leached samples showing a significant decrease in efficacy (increase in growth rating or decrease in zone of inhibition) versus the corresponding unleached sample indicate that the biocide is leaching from the coating to some extent. This may indicate the potential for diminished exterior performance.

11. Report

11.1 Report the following information or as otherwise agreed upon between the parties involved in the testing:

11.1.1 The date, fungal species used, incubation conditions, and some means of sample identification.

11.1.2 The corresponding results of weekly observations, including: dates; notation of any unusual occurrences; and the rating of degree of defacement.

11.1.3 Complete description of exposure cycle, time of exposure, and device(s) utilized for any preconditioning of specimens. If an ASTM method is used for preconditioning, all appropriate information as required by that method must be reported.

12. Precision and Bias

12.1 Precision—It is not practical to specify the precision of the procedure in this test method for measuring fungal resistance of a coating because the actual rating numbers for samples tested at different times or in different laboratories will be affected by changes in inoculum strength, substrate, or other
conditions that affect the fungal growth. In addition, differ-
ences in the perception and experience of the individual
determining the growth ratings may effect the actual rating
numbers assigned. Comparisons may be made between
samples tested at the same time using the same inoculum with
a given laboratory. A relative ranking in order of the perfor-
mance ratings (that is, good, better, best) should remain the
same between samples tested at different times or in different
laboratories. Comparisons of the actual rating numbers be-
tween samples tested at different times or in different labora-
tories should be avoided.

12.2 Bias—No information can be presented on the bias of
the procedure in this test method for measuring fungal resis-
tance of a coating because materials having acceptable refer-
ence values are not available.

13. Keywords

13.1 agar plate assay; fungi; fungal resistance; mildew; mold