



Designation: D 5583 – 00

Standard Test Method for Detection and Estimation of Retention of Wood Preservatives by *Aspergillus* Bioassaying¹

This standard is issued under the fixed designation D 5583; 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 a non-chemical, biological procedure for assaying wood for approximate preservative content (Note 1). It requires minimal equipment and technical proficiency. No costly equipment is needed other than a small autoclave for preparing fungal media. It does not require composite samples, such as called for in Specification D 1760 to ascertain preservation retentions, therefore it is used to assess retention at individual locations.

NOTE 1—With appropriate, simple adaptation, the method can be used with other products besides wood, such as fungicidal paints and glues. The assaying is done wherever it is convenient, since aseptic precautions are not required except for maintaining a pure stock culture of the assay fungus.

1.2 The values stated in SI units are to be regarded as the standard. The inch-pound units 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:²

D 1760 Specification for Pressure Treatment of Timber Products

3. Summary of Test Method

3.1 Small wood specimens of prescribed size are placed on nutrient agar in a Petri dish freshly seeded with spores of *Aspergillus niger*, a black mold, and the culture is allowed to incubate for three to four days. The fungus is unable to produce

its typical black spores around samples containing an inhibitory amount of preservative; this indicates the presence of preservative, and the size of the white area (zone of effect) is the index of preservative retention (see Fig. 1 and Fig. 2). The relation of retention to zone of effect is determined for each preservative, from assays of specimens containing a gradient of retentions of the preservative. (See example for pentachlorophenol in Fig. 3.) Hereafter, this is called the reference relations.

4. Significance and Use

4.1 This test method is intended for use with any preservative that is of uniform composition and not so tightly bound to the wood or so water insoluble that it cannot diffuse in the culture medium on which the assay fungus is grown (1, 2, 3).³ It is especially applicable to monitoring non-pressure treated millwork and other wood items (4), but it can be used to determine approximate preservative retentions at prescribed locations in pressure-treated wood (5). When measuring longitudinal preservative penetration, as is especially useful with millwork, the wood can be sampled on the end grain. The assay also provides a reliable means of observing losses of preservative retention in wood during service (or experimentally subjected to particular environmental conditions). This test method is not intended for comparing preservatives nor for estimating retentions of a preservative of variable composition, such as creosote. It can be used, however, for comparing relative potencies of such preservative.

5. Apparatus

5.1 *Conventional Equipment*, for culturing and aseptic handling of fungi, such as autoclave, refrigerator, transfer needle, and gas or alcohol burner.

5.2 *Glassware*, 100 by 15 mm (approximately) Petri dishes, glass or plastic, 250 mL Erlenmeyer flasks, and a few 150 by 22 mm (approximately) test tubes.

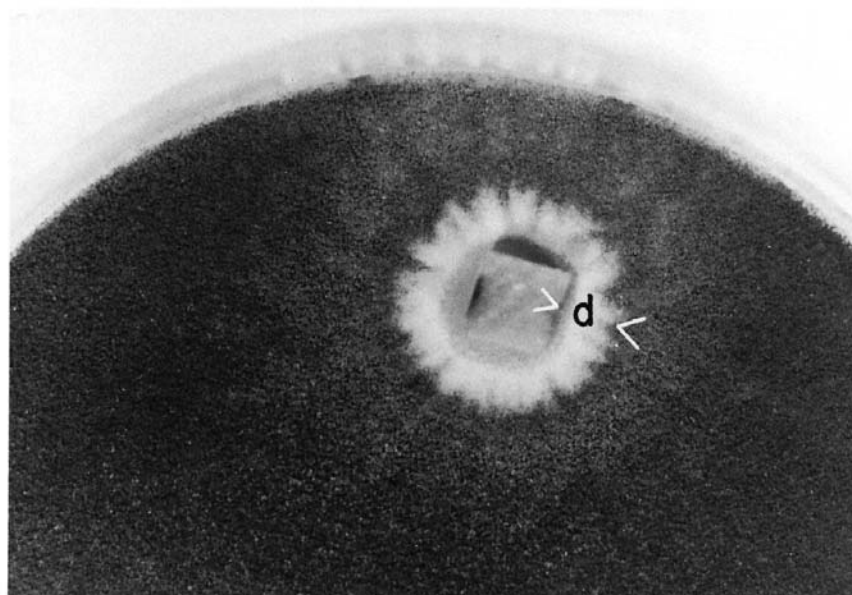
5.3 *Room or Chamber*, in which temperature is controlled at 22 to 27°C (72 to 82°F).

¹ This test method is under the jurisdiction of ASTM Committee D07 on Wood and is the direct responsibility of Subcommittee D07.06 on Treatments for Wood Products.

Current edition approved Oct. 10, 2000. Published December 2000. Originally published as D 5583 – 94. Last previous edition D 5583 – 94.

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

³ The boldface numbers in parentheses refer to the list of references at the end of this test method.



NOTE 1—The black area shows dense coverage by black spores of the fungus *Aspergillus niger*.
 FIG. 1 Zone of Effect (d) in an *Aspergillus* Bioassay of a Pentachlorophenol-Treated Block

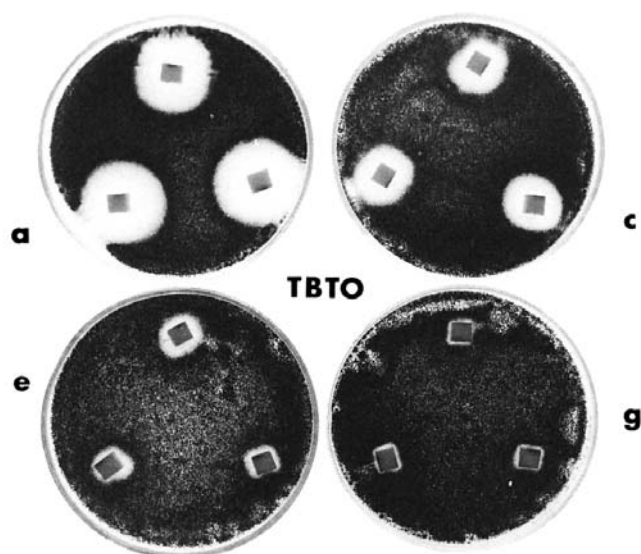


FIG. 2 An *Aspergillus niger* Bioassay of Small-Block Specimens Treated to Selected Retentions With Tributyltin Oxides⁹

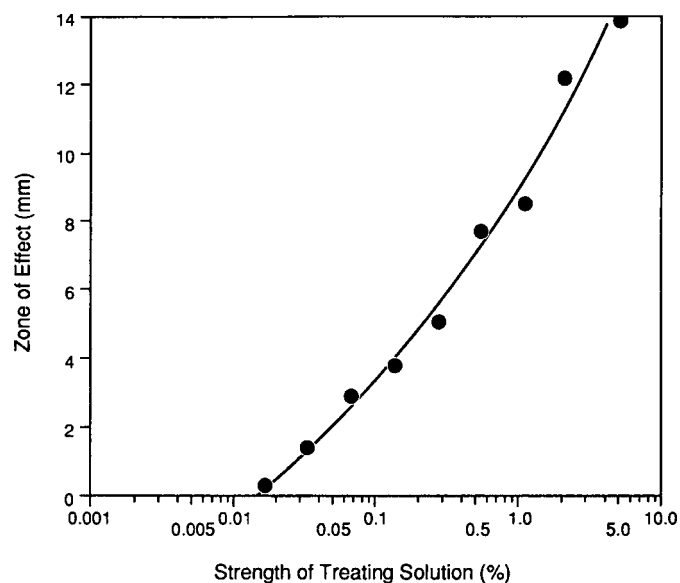
5.4 Atomizer, delivering a fine spray (optional).

5.5 Tools, such as increment borer, plug cutter, saw, and knife for extracting samples and cutting them to desired specimen size.

6. Assay Fungus

6.1 The assay fungus shall be *Aspergillus niger* Van Tiegh⁴ (for example, culture ATCC 64045 from the American Type Culture Collection).

⁴ Cultures are available from ATCC, 12301 Parklawn Drive, Rockville, MD 20852.



NOTE 1—Penta retentions (PCF) here = solution strength $\times$ approximately 0.369.⁹

FIG. 3 Example of Reference Relation Between Strength of Pentachlorophenol Solution and Zone of Effect in an *Aspergillus niger* Bioassay Using Small-Treated Blocks

7. Culture Medium

7.1 The nutrient medium for carrying the stock culture, and for the bioassaying, shall be potato-dextrose agar (PDA) (Note 2). *Aspergillus niger* tends to grow more rapidly and sporulate more profusely on potato-dextrose than on malt-agar. A commercial dry-mix preparation, made up according to directions, is satisfactory.

NOTE 2—The PDA may be prepared in the laboratory by combining the