Designation: D 5583 – 94

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An American National Standard

Standard Test Method for Detection and Estimation of Retention of Wood Preservatives by Aspergillus Bioassaying[1]

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] This test method is under the jurisdiction of ASTM Committee ~~D-7~~ D7 on Wood and is the direct responsibility of Subcommittee D07.06.03 on Evaluation of Preservatives.

Current edition approved ~~July 15, 1994.~~ Oct. 10, 2000. Published ~~September 1994.~~ December 2000. Originally published as D 5583 – 94. Last previous edition D 5583 – 94.

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:*

D 1760 Specification for Pressure Treatment of Timber Products[2]

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

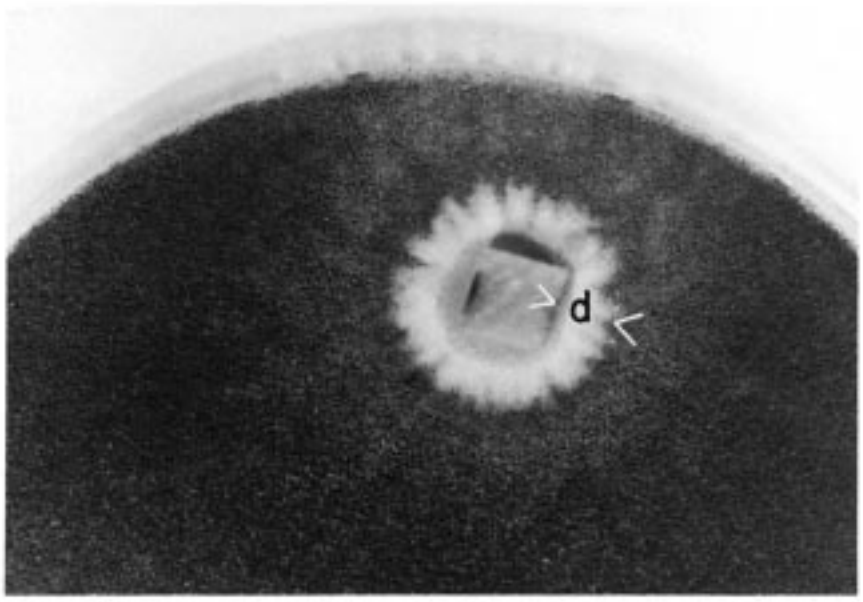
4.1 This test method is intended for use with any preservative that is of uniform composition and not so tightly bound to the wood or so water insoluble that it cannot diffuse in the culture medium on which the assay fungus is grown **(1, 2, 3)**.[3] It is especially applicable to monitoring non-pressure 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.

[2] *Annual Book of ASTM Standards*, Vol 04.10.

[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 j 6.
FIG. 1 Zone of Effect (d) in an Aspergillus Bioassay of a Pentachlorophenol-Treated Block

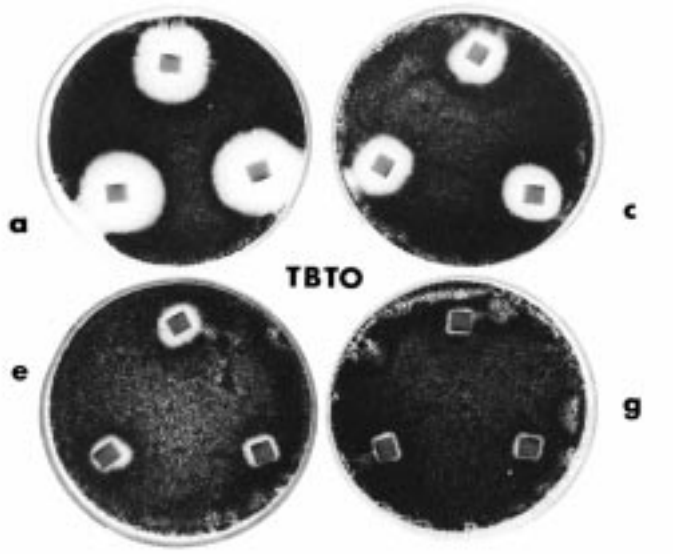


FIG. 2 An Aspergillus niger Bioassay of Small-Block Specimens Treated to Selected Retentions With Tributyltin Oxides[9]

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).

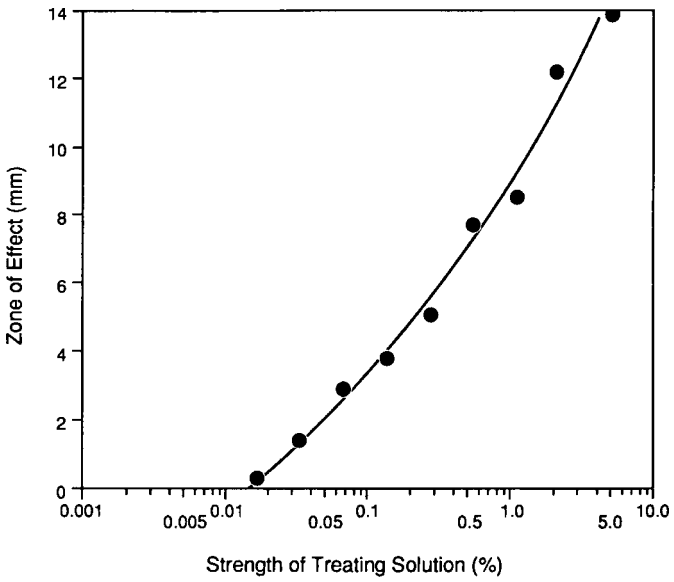
5.4 *Atomizer*, delivering a fine spray (optional).

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 Tiegh[4] (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 × 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 hot water extract of 200 g of potatoes, 20 g of dextrose (glucose), and 15 g of agar per litre.

8. Assay Samples

8.1 For standard use, acquire samples of the wood being assayed with a conventional increment borer or plug cutter.

8.2 When monitoring treated products for quality control of the treating, always take samples at the same location on the product.

8.3 The number of samples required may vary with product and precision needed (see 11.1).

9. Assay Specimens

9.1 Reduce the samples to small specimens for the assaying. For standard use, the specimens shall be increment core or circular wafers cut from plugs. The core segments shall be at least 6 mm (¼ in.) long. Wafers shall be complete or cut in half, and 3 cm (⅛ in.) thick. If preservative retentions are to be estimated, the specimens should be of the form size and kind of wood as those used to derive the reference relation.

9.2 To assay the preservatives at various depths in the sampled wood, take the increment core segments and identify serially.

10. Procedure

10.1 Maintain a stock culture.

10.1.1 Maintain the assay fungus by transferring a small plug of an actively sporulating culture to an 18.20 × 150 mm test tube containing a fresh slant of potato-dextrose agar. The slant should be approximately 10 cm long. If more rapid coverage of the new slant is desired, inoculate the surface by streaking it with the spore-bearing transfer needle.

10.2 Use sterilized culture dishes.

10.2.1 Sterilize glass petri dishes for 1 ¼ h at 160°C, or use pre-sterilized plastic dishes as obtained from the supplier. Provide dishes in sufficient numbers to carry out the desired bioassay when using three or four specimens per dish. More specimens per inch may be permissible (see 10.6.1).

10.3 Add culture medium to the dishes.

10.3.1 Heat the potato-dextrose medium at about 100°C until the ingredients are dissolved and uniformly dispersed.

10.3.2 Autoclave the medium for 15 min at approximately 121°C (15 lb (6.8 g) steam pressure).

10.3.3 Fill the sterilized dishes to a depth of about 7 mm with the hot medium, and allow the dishes to cool with covers on. Do this procedure in a comparatively dust free space with minimal air disturbance.

10.4 *Prepare Spore Suspension*:

10.4.1 Mix three or four drops of kitchen detergent (pretested for nontoxicity to *A. niger*) in a flask containing 250 mL of sterile water.

10.4.2 Using the detergent solution that will keep spores from clumping, wash the loose spores from a test-tube culture into the flask. Repeat, using the spore suspension, until the 250 mL suspension becomes slightly cloudy.

10.5 *"Seed" the Petri Dishes*—Seed the culture medium using one of the methods given in 10.5.1 and 10.5.2.

10.5.1 *Seeding by Spraying*—Seed the culture medium by spraying it with the spore suspension, using an atomizer that delivers fine droplets. This operation is facilitated by holding the dishes slightly off the vertical. One or two bursts from the atomizer is sufficient to provide suitably dense and uniform spore coverage. To prevent spores escaping into the laboratory, this operation should be done under a fume hood (Note 3).

NOTE 3—Remaining spore suspension can be stored up to six to eight weeks for further use if kept near 5°C.

10.5.2 *Seeding by Flooding*—Keep the spores uniformly suspended by shaking the flask and pour about 4 mL of the spore suspension onto the hardened culture medium in each dish. Gently rock the dish a few seconds to distribute the spores uniformly over the medium and pour off the excess suspension.

10.6 *Insert Assay Specimens*:

10.6.1 Immediately after seeding, place three or four assay specimens on the agar medium (Note 4). Space the specimens uniformly around the dish, approximately 1 cm from the edge. Replace the dish covers and mark them to identify the individual specimens.

NOTE 4—More specimens can be used per plate if the preservative diffusion is moderate enough to preclude interference between zones of effect.

10.6.2 Once the dishes have been seeded, no precautions against contamination are needed other than to keep the lids on. The specimens need not be sterilized prior to testing since the heavy spore suspension usually prevents obstructing contamination. If contamination is experienced, minimize it by very lightly flaming the specimens.

10.7 *Final Steps*:

10.7.1 Incubate the dishes at 22 to 27°C (72 to 82°F) until the surface of the nutrient medium is densely covered with black spores (commonly after three to four days) except for the white zones of effect around the specimens where darkening of the spores was prevented by toxic diffusible preservative.

10.7.2 Record the zones of effect by measuring the distance between the edge of each specimen and the margin of the "white" zone (see Fig. 1). Make measurements from any side of a specimen except the one nearest the dish edge. Measure the zones by light transmitted through the dish from below or by reflected window light.

11. Calculations

11.1 To estimate preservative retention from the zone of effect, first determine the relation of zone of effect to retention (reference relation). It is convenient to first determine the relation of zone of effect to the strength of solution used to impregnate specimens, as illustrated in Fig. 3. Knowing the specimen volume and the amount of solution absorbed (both nominally uniform throughout the treating range) convert solution strengths to retentions by these formulas:

$$\textit{retention } lb/ft^3 = G \times \textit{solution strength } (\%) \times (62.4) / 100V$$

$$\textit{retention } kg/m^3 = (G \times \textit{solution strength } (\%)/V) \times 10$$

where:

G = grams of solution absorbed by the specimens,
V = specimen volume (cm3), and
62.4 = factor for converting grams per cubic centimetre to pounds per cubic foot.

11.2 For the closest estimate, ascertain the relation on specimens of the same dimension and kind of wood as are to be assayed. Treat specimens to nominal refusal, using vacuum and atmospheric pressure. Include untreated control specimens in order to discern a zone of effect that is not caused by the treatment chemical but, instead, by a natural fungus inhibitor in the wood. Before bioassaying, air-dry the treated specimens overnight, and oven-dry at 50°C for two days to eliminate any significant amount of the preservative carrier. Base the number of specimens needed to define a zone of effect-retention relation on the range and spacing of retentions used and the required precision of estimating retention (Note 5).

NOTE 5—A number of specimens closely distributed over the retention range is better than the same numbers serving as replications at fewer retentions.

11.3 There shall be at least three specimens for each concentration of treating chemical. The primary concern shall be to see that the number of specimens and spread of treatment concentrations is sufficient to clearly define the relation between retention and zone of effect.

12. Use of Zone of Effect

12.1 For routine monitoring of a product for adequacy of treatment, estimation of retention is not needed. Determination that a preservative is present and in the required amount can be based simply on the zone of effect (Note 6).

NOTE 6—A minimum acceptable zone of effect for the sampling location (see 7.1) is an individual matter and can be established on material that has been accurately treated in accordance with specifications.

13. Precision and Bias

13.1 *Sensitivity*—The assay has exhibited sensitivity for detecting pentachlorophenol at spectroscopically determined levels in wood as low as 0.02 % (w/w). It has indicated similar sensitivity to tributyltin oxide (TBTO) and 3-iodo-2-propynyl butylcarbamate.

13.2 *Consistency*—In a series of trials at different times and places, the zone of effect for a given preservative and concentration proved to be consistent. Standard deviations were prevalently 1.6 to 1.8 mm with pentachlorophenol, 1.1 to 1.7 mm with TBTO, and 0.6 to 1.0 mm with a mixture of the two chemicals.

TABLE 1 Zones of Effect in an *Aspergillus* Bioassay of Douglas-fir Sapwood Blocks Treated to Varying Retentions of Chromated Copper Arsenate (CCA) then Dipped in Water or 85 % Lactic Acid Prior to Testing

Treatment Solution Concentration (%)[A]	Average CCA Retention (Kg/m^3)	Zone of Effect (mm)[B]	
		Water Dipped	Lactic Acid Dipped
0	0	2–3	0
0.062	0.5	2–3	11
0.125	1.0	2–3	14
0.250	1.9	2–3	17
0.500	3.9	2–3	18
1.000	7.4	2–3	20
2.000	15.6	2–3	22
4.000	33.0	2–3	21

[A] Oxide basis.
[B] Values represent averages of two replicates.

14. Keywords

14.1 Aspergillus bioassaying; wood preservatives

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