Designation: D 1413 – 05b

Standard Test Method for Wood Preservatives by Laboratory Soil-Block Cultures[1]

This standard is issued under the fixed designation D 1413; 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 determination of the minimum amount of preservative that prevents decay of selected species of wood by selected fungi under optimum laboratory conditions.

1.2 The requirements for preparation of the material for testing and the test procedure appear in the following order:

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 841 Specification for Nitration Grade Toluene

D 1193 Specification for Reagent Water

D 1758 Test Method for Evaluating Wood Preservatives with Stakes

D 3345 Test Method for Laboratory Evaluation of Wood and other Cellulostic Materials for Resistance to Termites

D 3507 Test Method for Penetration of Preservatives in Wood and Differentiating Between Heartwood and Sapwood.

E 11 Specification for Wire Cloth and Sieves for Testing Purposes

2.2 *Other Standards:*[3]

AWPA E-1-01 Testing Wood Preservatives by Soil Block Cultures

3. Summary of Test Method

3.1 Conditioned blocks of wood are impregnated with different concentration solutions of a preservative in water or suitable organic solvent to produce a series of retentions of the preservatives in the blocks. After periods of conditioning or weathering, the impregnated blocks are exposed to one or more strains of wood-destroying fungi, one fungus for each test series. The minimum amount of preservative that in the prescribed testing protects the impregnated blocks against decay by a given test fungus is defined as the threshold retention for that organism. Failure to protect is evidenced by loss of wood from the treated wood blocks, as indicated by a loss in weight.

3.2 Provision must be made for coordinated preparation of the test cultures and for impregnation, conditioning, or weathering and conditioning, of the test blocks.

4. Significance and Use

4.1 This test method is useful in the development of new wood preservatives and preservative systems by evaluating the minimum preservative retention to prevent decay under laboratory conditions. The results are used to facilitate target retentions in subsequent tests for effectiveness against termites (see Test Method D 3345) and in field stakes (see Test Method D 1758). The sections on Treatment and Preservative Permanence are referenced by other ASTM standards. The test

[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.

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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] American Wood Preservers' Association (AWPA), P.O. Box 388, Selma, Alabama 36702 (www.awpa.com).

method assumes that the test blocks exposed to certain conditions after treatment will achieve equilibrium, and will return to the same equilibrium after exposure to fungi. This assumption may lead to weight loss that is not due to decay. The test uses live cultures of fungal organisms that require careful colonization, storage, and feeding to remain viable strains.

5. Apparatus

5.1 *Conditioning Chamber or Room*, maintained at a selected temperature between 20 and 30°C (68 and 86°F) and a selected relative humidity between 25 and 75 %. The selected temperature shall not vary more than ±1°C (±2°F) and the selected humidity not more than ±2 %.[4]

5.2 *Incubation Room or Incubation Cabinet*, maintained at a selected temperature between 25 and 27°C (77 and 81°F) and a relative humidity between 65 and 75 %. The selected temperature shall not vary more than ±1°C (±2°F) and the selected humidity percentage not more than 2.

5.3 *Drying Oven*—A suitable, vented oven, maintained at a temperature of 105 ± 2°C (220 ± 4°F).

5.4 *Steam Sterilizer*.

5.5 *Balances*, fast-acting types preferred, sensitive and accurate to 0.01 g.

5.6 *Vacuum Pump or Water Suction Pump*, capable of reducing pressure to 100 mm (3.94 in.) Hg, or less.

5.7 *Impregnation Apparatus*—A suitable desiccator or bell jar shielded to protect personnel in event of breakage, provided with suitable separatory funnel or auxiliary flask for holding the treating solution and vacuum gage or manometer (Fig. 1).

5.8 *Trays or Racks, or Pin Bars*—Trays or racks made from suitable screening to permit free air movement around each block during initial drying and for convenient handling of the test blocks. Pin bars facilitate handling (see 7.2).

5.9 *Weathering Apparatus*:

5.9.1 *Forced Draft Oven.* [5]

5.9.2 600 cm^3 breakers for weathering of oil-type preservatives.

5.9.3 225 cm^3 wide-mouth screw cap bottles for weathering water-borne preservatives.

5.10 *Culture Bottles*, cylindrical or square (Note 1), capacity nominal 225 or 450 cm^3 (8 or 16 oz), fitted with screw caps without liners (Fig. 2). An alternate lid fitted (Note 2) with a 25-mm autoclavable filter with a pore size of 0.2 microns is permitted to reduce or prevent mite infestation during the test.

NOTE 1—*Culture Bottles:*

(1) 225-cm^3 (8-oz) French square, for use with one block only.

(2) 225-cm^3 (8-oz) cylindrical, for use with one or two blocks.

(3) 450-cm^3 (16-oz) cylindrical, for use with two blocks only.

NOTE 2—Prepare the lids by drilling a 0.64-mm hole in the center and lightly sanding the underside with medium grit paper. Adhere the filter with a small amount of high temperature silicon or slow-curing epoxy and cure overnight. Ensure that the adhesive does not covered the drilled hole.

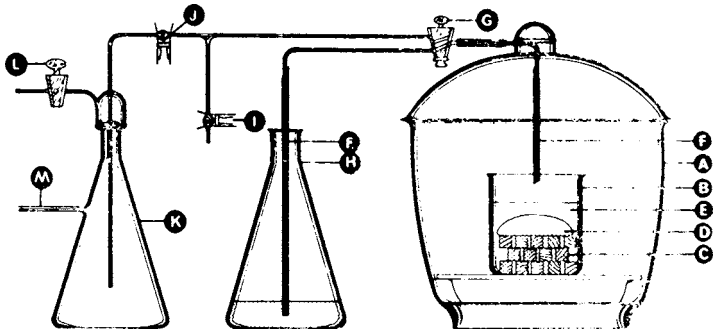
5.11 *Soil Sieves*—U.S. No. 6 sieve in accordance with Specification E 11.

6. Reagents

6.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the Specifications of the Committee on Analytical Reagents of the American Chemical

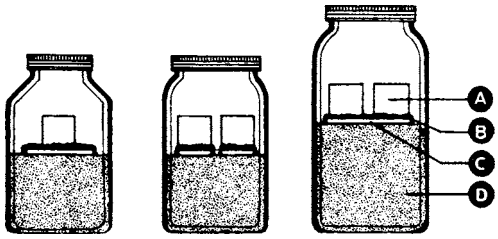
[4] Scheffer, T. C., "Humidity Controls for Conditioning Rooms," Forest Products Laboratory Report No. 2048, U.S. Forest Service, 4 pp., 5 Figs., January 1956.

[5] Blue M Model OV 490 A, or equivalent, has been found satisfactory.



A—Vacuum desiccator, internal diameter 250 mm.
B—Plastic or glass treatment beaker.
C—Test wood blocks.
D—Glass or other suitable weight.
E—Treating solution.
F—Polyethylene tubing.
G—Three-way stopcock with TFE-fluorocarbon plug.
H—Flask containing treating solution.
I—Glass joint with O-ring leading to either vacuum gage or mercury manometer.
J—Glass joint with O-ring.
K—Flask for vacuum trap.
L—Stopcock to atmosphere.
M—Line to source of vacuum.

FIG. 1 Apparatus for Vacuum Impregnation



A—Wood cubes, 19-mm or 0.75-in.
B—Test fungus growing over feeder block.
C—Wood feeder strip, one feeder strip for each culture bottle..
D—Soil.

FIG. 2 French Square and Cylindrical 225 cm³(8 oz) and cylindrical 450-mm (16-oz) Culture Bottles with Metal Screw Lids

Society, where such specifications are available.[6] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

6.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type IV of Specification D 1193.

6.3 *Toluene*, conforming to Specification D 841.

7. Wood

7.1 *General Properties*—The selection of either a hardwood or softwood is dependant on the products to be treated for the selected preservatives. Selected wood shall be free of knots and visible concentration of resins, and showing no visible evidence of colonization by mold, stain, or wood-destroying fungi, with 2½ to 4 rings/cm (6 to 10 rings/in.). Whenever practicable, begin selection of the wood for the test blocks at the sawmill. Quarter-sawed boards are preferable. Newly cut boards, nominally 25 mm (1 in.) thick, that are immediately kiln dried without antistain treatment provide chemical-free wood that has had minimum opportunity for fungus infection prior to use in the soil-block culture test.

7.1.1 For softwoods, Pine sapwood shall be used for tests intended to show comparative wood preserving values under the test. If southern pine is used, it should be 40 to 50 % summerwood, Report the species of wood and growth characteristics of selected specimens.

7.1.2 For hardwoods, use sapwood from sweet-gum (*Liquidambar styraciflua* L.) or yellow poplar (*Liriodendron tulipeifera* L.).

7.1.3 *Sapwood Identification*—When the boundary between heartwood and sapwood is difficult to recognize, use a color test (see Test Method D 3507 to distinguish between the two. Sample blocks shall be all sapwood.

7.2 *Test Blocks* (Note 3) Mill-test blocks as accurately as possible to 19 mm on each face. The volume of the blocks (without holes) shall be 6.9 ± 0.2 cm³ as determined by calipers.

[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 Pharmacopeia and National Formulary,* U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

NOTE 3—For convenience in handling, blocks may be drilled through the center of a tangential face with a 3-mm drill (approximately 0.125 in. or a No. 30 drill). Pin bars may then be used for handling. Store working stocks of test blocks and feeder strips in the conditioning room. It is desirable to weigh the blocks after they come to approximate equilibrium moisture content in storage or in the conditioning room, and to sort them into fairly narrow-range weight groups. Since the blocks are cut accurately to size this division into weight groups is, in effect, a segregation into density groups (see 11.4).

7.3 *Feeder Strips*:

7.3.1 *General Considerations*—One feeder strip is required for each culture bottle (Fig. 2). If test blocks other than pine are used for special investigations, the sapwood selected for feeder strips shall be capable of furnishing a satisfactory growth of the test fungus; for example, sweetgum sapwood often is used with hardwood test blocks.

7.3.2 *Size*—The feeder strips are cut 3 by 28 by 35 mm (⅛ by 1⅛ by 1⅜ in.) with the grain of the wood parallel to either of the longer dimensions. The exact dimensions are not critical, but in bottles with two test blocks, the blocks shall not contact each other.

8. Test Fungi

8.1 *General Considerations*—Always include a comparatively tolerant fungus (see 8.2 and 8.3) in testing a preservative.

NOTE 4—Other economically important fungi may be use din addition to the tolerant fungus in special investigations, or in some cases, substituted for it. (see AWPA E-10). The following numbers refer to standard strains of test fungi maintained in the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852.

8.2 *Fungus Species for Softwood Sapwoods*:

8.2.1 *Neolentinus lepideus (Fr:Fr.) Redhead and Ginns. (Madison 534, ATCC No. 12653)*—A fungus particularly tolerant to creosote or to mixtures containing creosotes.

8.2.2 *Gloeophyllum trabeum* (Pers. ex. Fr.) Murr. = [*Lenzites trabea* Pers. ex. Fr.] (Madison 617, ATCC No. 11539)—A fungus particularly tolerant to phenolic and arsenic compounds.

8.2.3 *Postia placenta* (Fr.) M. Larsen et Lombard = [*Poria monticolor* Murr.] (Madison 698, ATCC No. 11538)—A fungus particularly tolerant to copper and zinc compounds. Suggested for testing mercury compounds.

8.3 *Fungus Species for Hardwood Sapwoods*:

8.3.1 The three fungi listed in 8.2.

8.3.2 *Trametes versicolor* (L. ex Fr.) Pilát = [*Polyporus versicolor* L. ex. Fr.] (ATCC No. 42462), a white-rot fungus prevalent on hardwood products.

9. Culture Media

9.1 *Malt Agar Substrate*—For both stock test-tube and petri dish cultures of the test fungi, use a nutrient medium consisting of about 2 weight % malt extract and 1.5 weight % agar. Sterilize the medium at 103 kPa (15 psi) for 20 min and allow to cool before inoculations.

9.2 *Soil Substrate*—Use a soil substrate with a water-holding capacity between 20 and 40 % (Note 5) and pH between 5.0 and 8.0 and weighing not less than 90 g/120 cm³.

9.2.1 *Determination of Water-Holding Capacity of Soil*—After breaking up all clumps, mix and screen the soil through

the U.S. No. 6 sieve and store in large covered containers. The soil should not be so wet when it is sifted that the particles again stick together. Pass a sample of air-dry soil through a U.S. No. 6 sieve. Determine the water-holding capacity as follows. Use the sieved soil to fill a small Buchner funnel approximately 50 mm in diameter and 25 mm in depth, and fitted with rapid-filtering paper, to somewhat more than capacity. Compact the soil by dropping the funnel three times through a height of 10 mm (0.4 in.) on a wooden tabletop. Level the soil surface by cutting off excess soil with a spatula at the top of the funnel without further compaction. Then place the filled funnel in a 400-cm^3 beaker and retain in an upright position by wedges at the sides of the funnel. Add water to the beaker to a depth slightly beyond the level of the filter paper. Allow the soil to wet by capillarity so as to reduce the danger of entrapping air within the column. When the upper soil surface shows signs of wetting, add more water until the water level approximates the upper surface of the funnel. Place a cover over the beaker, and allow the soil to soak for 12 h or overnight. Then place the funnel in a suction flask which is connected to a water aspirator or vacuum pump, and apply full suction for 15 min. During suctioning, cover the funnel with a moist cloth on which an inverted cup is placed to prevent evaporation of water from the exposed soil surface. After 15 min remove the funnel from the suction flask, scrape the soil into a weighed receptacle, and weigh to obtain the wet weight, W_1. Ovendry for 24 h at 105 ± 2°C (220 ± 4°F) and reweigh soil (W_2). Determine soil moisture content (water-holding capacity) based on the ovendry weight of soil.

$$\text{Water-holding capacity } (WHC), \% = [(W_1 - W_2)/W_2] \times 100 \quad (1)$$

NOTE 5—The water-holding capacity of a soil is that percentage of water, based on the ovendry weight of the soil, that is retained after subjecting the soil to the following procedure based on a method of Bouyoucos, G. J. A., "A Comparison Between the Suction Method and the Centrifuge Method of Determining the Moisture Equivalent of Soils." *Soils Science,* Vol 40, 1935, pp. 165–170.

9.2.2 *Preparation of Soil Culture Bottles*—Half fill a culture bottle with sifted soil substrate and lightly compact by tapping. This amount of soil, about 120 cm^3 for an 8-oz culture bottle, should weigh not less than 90 g when ovendried. Use a proportionate volume when the larger 450 cm^3 bottles are used. The water in the completed soil culture bottle should be 130 % of the water-holding capacity of the soil. To determine the amount of additional water needed, weigh the volume of soil that will be used to half-fill a culture bottle, W_3. Dry this soil at 105 ± 2°C (220 ± 4°F) for 12 h and reweigh, W_4. Calculate the amount of water to be added to each culture bottle with that particular soil as follows:

$$\text{Water required, g} = (WHC \times 0.013 \times W_4) + W_3 - W_4 \quad (2)$$

where WHC is entered as a number, not a decimal.

9.2.3 Add the required amount of water to each culture bottle. Use a funnel bottle with a stem of large diameter that reaches nearly to the bottom of the culture bottles to add the corresponding volume of soil to minimize dust settlement on the glass. Level the soil surface and place directly on the soil one sapwood feeder strip for each test block to be used. Steam sterilize the prepared bottles, with caps loosened, at 103 kPa (15 psi) for 30 min. This sequence of steps generally leaves the inside surfaces of the culture bottles clean above the soil level and the water diffuses through the soil during sterilization without puddling.

10. Preparation of Test Cultures

10.1 After the sterilized soil culture bottles are thoroughly cooled, cut approximately 10-mm square fungus inoculum sections from a petri dish culture that is not more than 3-weeks-old (Note 6). Immediately place the square of inoculum in contact with an edge of the feeder strip on the soil. Close the culture bottles with lids released one-fourth turn from a tightened position (see 5.10, Note 2), and incubate at the desired temperature for approximately 3 weeks or until the feeder strips are covered by mycelium. The culture bottles are now ready to receive the test blocks.

NOTE 6—When not in active use, store the test cultures in test tube agar slants in a refrigerator maintained between 2 and 5°C (35 and 40°F). When the slants are used to inoculate petri dishes, inoculate and incubate replacement slants until the surface of the slant is covered by mycelium prior to refrigeration. Test tubes that are 150 by 16 mm in diameter, equipped with a plastic screw cap work well. It is recommended that the liner in the cap be removed before using. Depending on the type of refrigerator used, check the agar slants every 1 to 2 months for loss of moisture. When the culture appears excessively dry, prepare new slants and inoculate (see 9.1). It is suggested that three test tube slants of each test fungus be maintained as outlined above. It is sometimes helpful to place small sapwood sticks into the plants to maintain fungal viability.

11. Preparation and Impregnation of Test Blocks

11.1 *Initial Conditioning and Initial Weights*—Before impregnation, condition the test blocks by either of the following methods:

11.1.1 *Conditioning at Specified Temperature and Relative Humidity*—Mark each block (for example, with waterproof ink) and bring the test blocks to a constant moisture equilibrium in the conditioning room. Weigh the blocks to the nearest 0.01 g just before treatment. This weight (T_1) is referred to as the initial or untreated weight of the test block (Note 7). After weighing keep the test blocks in the conditioning room until they are to be impregnated with the preservative.

NOTE 7—Coding the different weights as T_1, T_2, and so forth, avoids confusion and simplifies recording data. The suggested system of T designations is as follows, record all weights in grams:

T_1 = initial weight of the conditioned or oven-dried test block before impregnation,
T_2 = weight of the test block immediately after impregnation and wiping (equals T_1 plus grams of treating solution absorbed),
T_3 = weight of test block plus remaining preservative after conditioning and before exposure to the test fungus,
T_{3w} = weight of the test block plus remaining preservative after weathering or leaching and conditioning and before exposure to the test fungus,
T_m = weight of the test blocks immediately after removal from the test bottle and after adherent mycelium has been brushed off, and
T_4 = weight of the test block after test and after final conditioning.

11.1.2 *Ovendrying*—Dry the marked blocks in the drying oven (see 5.3) for 24 h. Remove the blocks to a desiccator and