Designation: D4576 – 16 (Reapproved 2021)

Standard Test Method for Mold Growth Resistance of Wet Blue and Wet White[1]

This standard is issued under the fixed designation D4576; 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 mold growth resistance of Wet Blue and Wet White subject to storage and shipping requirements and intended for use in leather manufacturing. This test method may not be suitable to evaluate fungicides that are inactivated by proteins. This includes alkyldimethylbenzyl ammonium chlorides.

1.2 Conclusions about mold growth resistance are drawn from the results by comparing the test with a simultaneously run control of known resistance. Success or failure is determined by the amount of mold growth relative to the control.

1.3 To allow use of this test method by any laboratory, flexibility has been permitted in times, temperature, and humidity of incubation, inoculum, hide sampling area, and choice of control. These may be adjusted to fit local conditions but must be standardized.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Terminology

2.1 *Definitions of Terms Specific to This Standard:*

2.1.1 *Wet Blue*—hide or skin, or split of a hide or skin, tanned with basic chromium sulfate, containing approximately 50 % moisture and having an acidic pH.

2.1.2 *Wet White*—a hide or skin, or split of a hide or skin tanned with organic or non-organic tanning agents (excluding chromium or iron containing agents and vegetable extracts), containing approximately 50 % moisture.

3. Summary of Test Method

3.1 Wet Blue and Wet White test specimens are surrounded by but not covered with agar, inoculated, and incubated.

3.2 After various incubation periods, mold growth is rated as a percentage of the Wet Blue and Wet White surface covered by mold.

3.3 Resistance to mold growth of the Wet Blue or Wet White test specimen is determined by comparison with Wet Blue or Wet White of known resistance characteristics (the control), that is tested simultaneously.

4. Significance and Use

4.1 This test method provides a technique for evaluating mold growth resistance characteristics of Wet Blue and Wet White, and should assist in the prediction of storage time before molding occurs.

4.2 The degree of correlation between this test and commercial quantities of Wet Blue and Wet White in storage or shipment situations, or both, has not been fully determined.

5. Interferences

5.1 A common interference is contamination of plates, agar, or samples by unwanted organisms that settle in from the environment.

5.2 *Volatility and Leachability of Biocides*—A "zone of inhibition" where no mold grows on the agar adjacent to the specimen indicates that the fungicide may leach.

6. Apparatus

6.1 *Petri Dishes,* 120 mm diameter. Sterile plastic disposable dishes are preferred.

6.2 *Incubator,* or location providing similar conditions being free of drafts, and capable of a constant (±2 °C) temperature within the 26 to 30 °C range.

6.3 *Medicine droppers,* disposable plastic type delivering 30 to 35 drops per mL.

[1] This test method is under the jurisdiction of ASTM Committee D31 on Leather and is the direct responsibility of Subcommittee D31.02 on Wet Blue.

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7. Reagents and Materials

7.1 *Potato Dextrose Agar,* [2] a dehydrated plating medium used in culturing yeasts and molds from dairy products.

7.2 *Inoculum,* [3] Aspergillus niger 1×10^6 spores per mL, or other organism or a combination of organisms known to be indigenous to the storage area of the Wet Blue and Wet White.

8. Sampling, Test Specimen, and Test Units

8.1 Take test specimens from equivalent hide locations (for example, butt area) for both test and control.

8.2 If unable to test immediately, hold test specimens in separate plastic bags and keep cool.

8.3 Test specimens should be a square, with a side of 25.4 mm (1 in.).

8.4 Use three test specimens for each test unit of Wet Blue or Wet White surface to be evaluated.

9. Procedure

9.1 *Agar Preparation:*

9.1.1 *Agar Requirements*—A split Wet Blue or Wet White test specimen requires about 25 mL solution and an unsplit Wet Blue or Wet White test specimen requires about 40 mL. Calculate number of millilitres of agar required for tests to be performed, allowing 50 mL for vitality check.

9.1.2 Weigh out 3.9 g potato dextrose agar for every 100 mL of agar required.

9.1.3 Pour a volume of water equivalent to total millilitres of agar solution to beaker. Bring water to boiling on hot plate equipped with magnetic stirrer mechanism. While stirring, slowly add dry agar.

9.1.4 Boil agar for 20 min.

NOTE 1—Pressure cooking for 20 min. is preferable to open boiling.

9.1.5 Cover with aluminum foil to prevent contamination and cool to 50 °C before pouring.

NOTE 2—This temperature is critical, as 50 °C allows some water of condensation to develop on petri dish cover providing humidity for growth of fungus.

9.2 *Agar Plate Preparation:*

9.2.1 Place one Wet Blue and Wet White test specimen in center of each petri dish with the surface to be tested facing up.

9.2.2 Carefully lift cover from each dish and pour agar just up to, but not over, the top surface of the test specimen.

9.2.3 Prepare one dish with agar only (without Wet Blue) for evaluation of the vitality of the inoculum.

9.2.4 Let agar solidify for about 20 min.

9.3 *Inoculation:*

9.3.1 Reduce working stock of 1×10^6 spores per mL to 1×10^5 by diluting 1 volume to 10 volumes with water.

9.3.1.1 Use tap water, that has been freshly boiled for 20 min. and cooled to room temperature, for making dilutions.

9.3.1.2 Prepare only enough diluted suspension for use in a 48-hour period.

9.3.1.3 Keep organism stock suspensions refrigerated at about 4 °C. Do not freeze.

9.3.2 Use three drops of 1×10^5 spores per milliliter per plate using a plastic disposable medicine dropper. Deposit one drop directly on the sample and one drop to either side as shown in Fig. 1.

9.3.3 Let dishes set about 1 h.

NOTE 3—If moved too quickly the inoculum runs over the specimen surface.

NOTE 4—Keep work area as clean and aseptic as possible. Work in an area of minimal air circulation while handling Wet Blue or Wet White, pouring agar, and inoculating plates. Keep covers on petri dishes at all times except when pouring and inoculating.

9.4 Incubate up to three weeks at constant temperature in a clean location where they will not be disturbed.

9.4.1 Constant temperature is more important than the precise temperature. A temperature of 26 to 30 °C is acceptable and should not vary more than ±2 °C.

NOTE 5—Storage in a clear plastic box in a boiler room may be sufficient. Use separation to prevent cross contamination.

NOTE 6—More rapid growth occurs at higher temperature.

9.5 Control Wet Blue and Wet White specimens of known mold resistance must be done simultaneously with test specimens. A successful test will have less mold growth than the control.

NOTE 7—After completion of work, test specimens should be sterilized by autoclaving. If that is not practical, cook for 30 min. in a pressure cooker and discard in a trash container.

10. Interpretation of Results

10.1 The following rating scale of 0 to 4 can be used where each number represents the degree of growth observed on the specimen (not on the agar) at any selected period.

0–No growth on specimen,

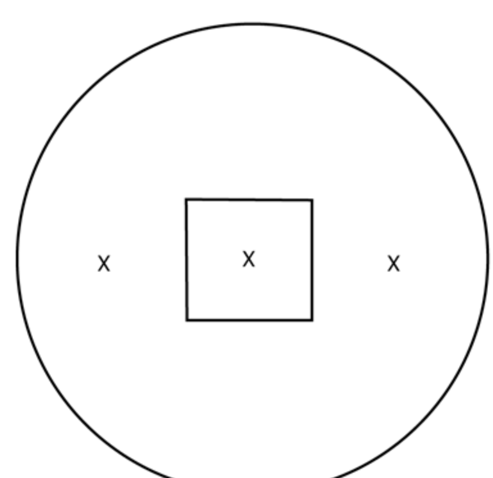

FIG. 1 Specimen with Inoculum Locations Shown (X)

[2] The sole source of supply of a product that meets the requirements of this method known to the committee at this time is Potato Dextrose Agar stock no. 0013-01-4, available from Difco Labs, P.O. Box 1058A, Detroit, MI 28232. 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.

[3] An inoculum that meets the requirements of this method is available as ATCC (American Type Culture Collection) 16404, and is available from several sources for laboratory supplies.