



Designation: D 4576 – 01 (Reapproved 2006)^{ε1}

Standard Test Method for Mold Growth Resistance of Wet Blue¹

This standard is issued under the fixed designation D 4576; 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.

^{ε1} NOTE—Footnote 3 was revised in July 2008.

1. Scope

1.1 This test method covers the determination of mold growth resistance of wet blue 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 *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. Terminology

2.1 *Definition of Term 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.

3. Summary of Test Method

3.1 Wet blue 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 surface covered by mold.

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

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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4. Significance and Use

4.1 This test method provides a technique for evaluating mold growth resistance characteristics of wet blue, 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 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 ($\pm 2^\circ\text{C}$) temperature within the 26 to 30°C range.

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

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.

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.

² A product that meets the requirements of this method is Potato Dextrose Agar stock no. 0013-01-4, available from Difco Labs, P.O. Box 1058A, Detroit, MI 28232.

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.