~~Designation:D4783–98a~~ Designation: D 4783 – 01 (Reapproved 2008)

Standard Test Methods for Resistance of Adhesive Preparations in Container to Attack by Bacteria, Yeast, and Fungi[1]

This standard is issued under the fixed designation D 4783; 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.

This standard has been approved for use by agencies of the Department of Defense.

1. Scope

~~1.1These test methods cover the determination of the resistance of liquid adhesive preparations to microbial attack in the container by challenging adhesive specimens with cultures of bacteria, yeast, or fungi, and checking for their ability to return to sterility. *~~

1.1 These test methods cover the determination of the resistance of liquid adhesive preparations to microbial attack in the container by challenging adhesive specimens with cultures of bacteria, yeast, or fungi, and checking for their ability to return to sterility. These test methods return qualitative results.

1.2 The values stated in SI units are to be regarded as the standard. The values 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.* These test methods are designed to be used by persons trained in correct microbiological technique. Specific precautionary statements are given in Section 8.

2. Referenced Documents

2.1 *ASTM Standards:* [2]

D 907~~Terminology of Adhesives~~ Test Methods for Chemical Analysis of Nickel, Cobalt, and High-Temperature Alloys

D 4299 Test Method~~s~~ for Effect of Bacterial Contamination on Performance of Adhesive Preparations and Adhesive Films[3]

~~D4300Test Methods for Ability of Adhesive Films to Support or Resist the Growth of Fungi[2]~~ 4300 Test Methods for Chemical Analysis of Nickel, Cobalt, and High-Temperature Alloys

E 640~~Test Method for Preservatives in Water-Containing Cosmetics~~ Test Methods for Chemical Analysis of Nickel, Cobalt, and High-Temperature Alloys

NOTE 1—Test Method E 640 is under the jurisdiction of ASTM Committee ~~E-35~~E35 on Pesticides. The procedure in this method outlines a serial dilution method of determining plate count using a pour plate technique.

2.2 *TAPPI Method:*

T 487 Fungus Resistance of Paper and Paperboard[4]

2.3 *CSMA:*

Cosmetics Preservation, Method 38 [5]

3. Terminology

3.1 ~~*Definitions:*~~

~~3.1.1*resistance*, *n—as related to bacteria, yeast, or fungi*, the power or capacity to ward off growth.~~ Definitions—Many terms in these test methods are defined in Terminology D 907.

~~[1] These test methods are under the jurisdiction of ASTM Committee D-14 on Adhesives and are the direct responsibility of Subcommittee D14.30 on Wood Adhesives. Current edition approved Sept. 10, 1998. Published March 1999. Originally published as D4783–88. Last previous edition D4783–98.~~

[1] These test methods are under the jurisdiction of ASTM Committee D14 on Adhesives and are the direct responsibility of Subcommittee D14.30 on Wood Adhesives . Current edition approved April 1, 2008. Published April 2008. Originally approved in 1988. Last previous edition approved in 2001 as D 4783 – 01$^{\varepsilon 1}$.

[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* ~~, Vol 15.06.~~volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Withdrawn.

~~[4] Annual Book of ASTM Standards, Vol 11.05.~~

[4] Available from Technical Association of the Pulp and Paper Industry (TAPPI), 15 Technology Parkway South, Norcross, GA 30092, http://www.tappi.org.

~~[5] Available from TAPPI, P.O. Box 105113, Atlanta, GA30348.~~

[5] This method is the same as Test Method E 640.

***A Summary of Changes section appears at the end of this standard.**

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 ~~*adhesive preparation*~~adhesive preparation, *n*—the adhesive as packaged for distribution, storage, and use.

3.3 ~~*Abbreviations:Abbreviations:*~~ Abbreviations:

3.3.1 *PBS*—phosphate buffered saline.

3.3.2 *PDA*—potato dextrose agar.

3.3.3 *YMPG*—yeast malt peptone glucose (agar).

~~3.4For definitions of other terms, see Terminology D907.~~

4. Summary of Test Methods

4.1 The adhesive specimen is challenged by inoculation with a culture of bacteria, yeast, or fungi, which may be a single species or a mixed culture of several species, following the guidelines given in Note 6. The inoculated adhesive specimen is stored at 21 to 27°C (70 to 80°F) for 7 days, during which time cultures (streak plates) are made at preset intervals. See Note 2. At any point in the series of challenges, if the inoculated specimen shows microbial growth on the streak plates made during the week following the challenge (indicating that it has not returned to sterility), the test is discontinued, and the sample is reported as *not resistant to attack in the container* by the species or combination of species used as the inoculum. If the cultures show no growth, the test is repeated with up to four challenges. If the specimen tests out as sterile following the fourth challenge, it is reported to be *resistant to attack in the container* by the species or combination of species of bacteria, fungi, or yeast used as the inoculum. At the discretion of the biological laboratory, the test may be discontinued after the second or third challenge. See Section 16 for further interpretation.

4.2 The time necessary to kill is determined by noting the earliest streak plate to read sterile. If the 4-h plate is positive and the 24-h plate is negative, the kill time could be narrowed down further by repeating the challenge and making streak plates at intervals of 4, 8, 12, and 24 h following the challenge.

4.3 The testing laboratory has the option of changing the timing of the challenges, the sterility checks, and the incubation period.

NOTE 2—Two proposed schedules for the challenging and sterility checks are shown in Table 1 and Table 2, Schedule A for bacteria and yeast, and Schedule B for fungi. The exact format to be followed will vary, according to the convenience of the schedule to the testing laboratory and special circumstances relating to the problem being addressed.

NOTE 3—A serial-dilution plate-count method of checking for sterility may be used when numerical information is needed on the population of viable organisms or the reduction in population with increasing levels of biocide. Letheen broth is recommended for the diluent and Letheen agar for the pour plate. See Note 1.

5. Significance and Use

5.1 These test methods are used to demonstrate whether an adhesive preparation is sufficiently protected with biocide to resist attack by bacteria, yeast, and fungi during its storage life. They are patterned after methods used by biological laboratories serving the adhesive industry.

5.2 These test methods may also be used to determine the efficacy of different biocide systems against specific microorganisms.

5.3 These test methods are especially useful when tested against wild-type microorganisms which have been isolated from contaminated adhesives as an aid in determining the amount and type of biocide necessary to kill or inhibit the growth of the contaminants. If an isolated microorganism not generally used as a challenge organism, is chosen as the inoculum, it is important to identify the organism and determine on which medium and under what conditions it will grow, in order to demonstrate the efficacy of the biocide.

5.4 The results obtained when using the procedures given in these methods apply only to the species which are used for the testing. The test species listed in Section 9 are frequently used by laboratories to test for antimicrobial properties, but they are not the only ones which could be used. Selection of the species to use for these test methods requires informed judgment by the testing laboratory or by the party requesting the tests. It is also important that species which commonly attack adhesives be used. See 9.4.

5.5 The presence of an active biocide carried over from the adhesive specimen to the agar could have an inhibiting effect on the growth of microorganisms, resulting in no growth during the span of a normal incubation period, when in fact, viable microorganisms are present, but their growth has been slowed down or held in stasis. The use of Letheen agar and broth is recommended to neutralize the effect of this carry-over.

NOTE 4—Letheen agar may be used for the streak plates, or if another agar is chosen for testing, a Letheen agar plate could be streaked as a control to test against the neutralizing effect. Even more effective would be diluting the challenged adhesive specimen with Letheen broth and running Letheen agar pour plates. See Note 1 and Note 3. Extending the incubation period of negative plates would be another safeguard. To neutralize thiazoline-based preservatives, 10 to 50 ppm of sodium thioglycolate can be added to the medium.

5.6 These test methods are dependent upon the physiological action of living microorganisms under a reported set of conditions. Conclusions about the resistance of the test adhesive to microbiological attack can be drawn by comparing the results to simultaneously run controls of known resistance. See X5.2 for statements regarding test repeatability.

6. Apparatus

6.1 In addition to the standard equipment found in any fully equipped microbiological laboratory, the following items are sometimes needed: