Designation: D 4783 – 01

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*

1.1 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

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

D 4300 Test Methods for Ability of Adhesive Films to Support or Resist the Growth of Fungi

E 640 Test Method for Preservatives in Water-Containing Cosmetics

NOTE 1—Test Method E 640 is under the jurisdiction of ASTM Committee 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*—Many terms in these test methods are defined in Terminology D 907.

3.2 *Definitions of Terms Specific to This Standard:*

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

3.3 *Abbreviations:*

3.3.1 *PBS*—phosphate buffered saline.

3.3.2 *PDA*—potato dextrose agar.

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

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

[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 Oct. 10, 2001. Published December 2001. Originally published as D 4783 – 88. Last previous edition D 4783 – 98a.

[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] Withdrawn.

[4] Available from TAPPI, P.O. Box 105113, Atlanta, GA 30348.

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

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

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

TABLE 1 Schedule A—Proposed Bacteria and Yeast Testing, Covering 4-h, 24-h, 48-h, 72-h, and 7-Day Tests

Day of Week	Day no.	First Challenge	Second Challenge	Third Challenge	Fourth Challenge
Monday	(–1)	inoculate fresh bacterial or yeast culture	...	...	...
Tuesday	0	prepare suspension	...	...	...
Tuesday	0	inoculate specimens	...	...	...
Tuesday	(0 + 4 h)	streak 4-h plate	...	...	...
Wednesday	1	streak 24-h plate	...	...	...
Thursday	2	streak 48-h plate	...	...	...
Friday	3	streak 72-h plate	...	...	...
Sat./Sun.	4–5	...	...	...	...
Monday	6	...	inoculate fresh bacterial or yeast culture	...	...
Tuesday	7	...	prepare suspension	...	...
Tuesday	7	streak 7-day plate	inoculate specimens	...	...
Tuesday	(7 + 4 h)	read 4-h plate	streak 4-h plate	...	...
Wednesday	8	read 24-h plate	streak 24-h plate	...	...
Thursday	9	read 48-h plate	streak 48-h plate	...	...
Friday	10	read 72-h plate	streak 72-h plate	...	...
Sat./Sun.	11–12	...	...	...	...
Monday	13	...	...	inoculate fresh bacterial or yeast culture	...
Tuesday	14	...	...	prepare suspension	...
Tuesday	14	read 7-day plate	streak 7-day plate	inoculate specimens	...
Tuesday	(14 + 4 h)	...	read 4-h plate	streak 4-h plate	...
Wednesday	15	...	read 24-h plate	streak 24-h plate	...
Thursday	16	...	read 48-h plate	streak 48-h plate	...
Friday	17	...	read 72-h plate	streak 72-h plate	...
Sat./Sun.	18–19	...	...	...	...
Monday	20	...	...	...	inoculate fresh bacterial or yeast culture
Tuesday	21	...	...	...	prepare suspension
Tuesday	21	...	read 7-day plate	streak 7-day plate	inoculate specimens
Tuesday	(21 + 4 h)	...	...	read 4-h plate	streak 4-h plate
Wednesday	22	...	...	read 24-h plate	streak 24-h plate
Thursday	23	...	...	read 48-h plate	streak 48-h plate
Friday	24	...	...	read 72-h plate	streak 72-h plate
Sat./Sun.	25–26	...	...	...	...
Monday	27	...	...	...	...
Tuesday	28	...	...	read 7-day plate	streak 7-day plate
Tuesday	(28 + 4 h)	...	...	...	read 4-h plate
Wednesday	29	...	...	...	read 24-h plate
Thursday	30	...	...	...	read 48-h plate
Friday	31	...	...	...	read 72-h plate
Sat./Sun.	32–33	...	...	...	...
Monday	34	...	...	...	...
Tuesday	35	...	...	...	read 7-day plate