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Designation: D 4783 – 98a

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## Standard Test Methods for Resistance of Adhesive Preparations in Container to Attack by Bacteria, Yeast, and Fungi<sup>1</sup>

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 ( $\epsilon$ ) 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.

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:

- D 907 Terminology of Adhesives<sup>2</sup>
- D 4299 Test Methods for Effect of Bacterial Contamination on Performance of Adhesive Preparations and Adhesive Films<sup>3</sup>
- D 4300 Test Methods for Ability of Adhesive Films to Support or Resist the Growth of Fungi<sup>2</sup>
- E 640 Test Method for Preservatives in Water-Containing Cosmetics<sup>4</sup>

NOTE 1—Test Method E 640 is under the jurisdiction of ASTM Committee E-35 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<sup>5</sup> 2.3 *CSMA*:

<sup>2</sup> Annual Book of ASTM Standards, Vol 15.06.

Cosmetics Preservation, Method 386

### 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.

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).
- 3.4 For definitions of other terms, see Terminology D 907.

#### 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.

<sup>&</sup>lt;sup>1</sup> 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 D 4783 – 88. Last previous edition D 4783 – 98.

<sup>&</sup>lt;sup>3</sup> Discontinued, see 1989 Annual Book of ASTM Standards, Vol 15.06.

<sup>&</sup>lt;sup>4</sup> Annual Book of ASTM Standards, Vol 11.05.

<sup>&</sup>lt;sup>5</sup> Available from TAPPI, P.O. Box 105113, Atlanta, GA 30348.

<sup>&</sup>lt;sup>6</sup> This method is the same as Test Method E 640.

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.

TABLE 1	Schedule A-Pr	oposed Bacteria ar	nd Yeast Testing	Covering 4-h	. 24-h. 48-h	. 72-h. and 7-I	av Tests
	001100aa1071 111	opoood Buotonia ai	ia roadt rooting		,,	.,	-uy 10010

Day of Week	Day no.	First Challenge	Second Challenge	Third Challenge	Fourth Challenge
Monday	(-1)	inoculate fresh bacterial			
,		or yeast culture			
Tuesday	0	inoculate specimens		<b>.</b>	
Tuesday	0	prepare suspension			
Tuesday	(0 + 4 h)	streak 4-h plate	magrae it	eh ai)	
Wednesday	1	streak 24-h plate	liiuai usili	<u></u>	
Thursday	2	streak 48-h plate			
Friday	3	streak 72-h plate	omt Drovi	CHAN /	
Sat./Sun.	4–5			CVV	
Monday	6		inoculate fresh bacterial		
			or yeast culture		
Tuesday	7		prepare suspension		
Tuesday	7	streak 7-day plate AS1	Vinoculate specimens		
Tuesday	(7 + 4 h)	read 4-h plate	streak 4-h plate	17120277/astm 447	97 08 o
Wednesday	/Catalog8stanuarus	read 24-h plate	streak 24-h plate	1 <u>2,</u> 12a2///aSuiFu4/	0 <u>0</u> -90a
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
- ·	24				culture .
Tuesday	21				prepare suspension
Tuesday	21		read 7-day plate	streak 7-day plate	inoculate specimens
Tuesday	(21 + 4 h)			read 4-n plate	streak 4-n plate
vednesday	22			read 24-n plate	streak 24-n plate
Tridev	23			read 40-n plate	streak 46-n plate
Friday Set /Sun	24			read 72-n plate	streak 72-n plate
Sat./Sun.	25-26				
Ivionday	27				
Tuesday	28 (20 · 4 h)			read 7-day plate	streak 7-day plate
Tuesday	(28 + 4 1)				read 24 h plate
Thursday	29				read 48 h plate
Fridov	30				read 72 h plate
Filuay Set /Sup	<b>।</b> ১০ ১০				reau /2-11 plate
Sal./Sun. Mondov	32-33				
Tuesday	34 25				 road 7 day plata
ruesday	30				reau 7-uay plate