Designation: D 4196 – 82 (Reapproved 1998)

An American National Standard

Standard Test Method for Confirming the Sterility of Membrane Filters[1]

This standard is issued under the fixed designation D 4196; 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 describes a test to confirm the sterility of either manufacturer presterilized or user-sterilized analytical membrane filters.

1.2 *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. Referenced Documents

2.1 *ASTM Standards:*

D 1129 Terminology Relating to Water[2]

D 1193 Specification for Reagent Water[2]

2.2 *Other Standard:*

The United States Pharmacopeia, Current Edition[3] (Sections on Sterilization and Sterility Testing)

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminology D 1129.

4. Summary of Test Method

4.1 The membrane filters are immersed in sterile culture media and incubated at temperatures that are suitable for growth of viable bacteria, fungi, and yeasts. Growth of organisms is evidence that the filter has failed the test.

5. Significance and Use

5.1 This test method may be employed to check the sterility of commercially procured sterile membrane filters. The test also confirms that sterilized filters have not been contaminated. Additionally, this test may be used to monitor the efficacy of in-house sterilization procedures. Filter packages that have obvious packaging defects should not be tested because sterility may have been compromised.

6. Reagents and Materials

6.1 *Purity of Water*— Unless otherwise indicated, reference to water shall be understood to mean Type II reagent grade water in accordance with Specification D 1193.

6.2 *Media*—Use commercially available dehydrated media. Dissolve and sterilize by autoclaving, in accordance with the manufacturer's directions.

6.2.1 *Fluid Thioglycollate Medium* (Note)—Dispense 40-mL aliquots into suitable-sized vessels with screw-cap closure, providing a ratio of surface area to depth of medium so that no more than the upper half of the medium has initially undergone a color change indicative of oxygen uptake. When ready for use, not more than the upper one tenth of the medium should be pink. The medium may be restored once by heating in free-flowing steam until the pink color disappears. The pH of the medium, after autoclaving, should be 7.1 ± 0.2.

NOTE 1—If stored at 2 to 5°C in sealed containers, the media may be used for 1 year provided they are tested for the growth-promoting properties every 3 months.

6.2.2 *Soybean-Casein Digest Medium* (Note)—Dispense 40-mL aliquots into suitable vessels with screw-cap closure. The pH after autoclaving should be 7.3 ± 0.2.

6.2.3 Perform a sterility test on each lot of autoclaved medium by incubating ten representative containers of each medium, for not less than 10 days, at the specified test temperature.

6.2.4 Perform a growth-promotion test, as described below, on each lot of autoclaved medium.

6.2.4.1 Inoculate duplicate test containers of each medium separately with less than 100 of each of the below listed microorganisms. Incubate 7 days at the temperatures listed below:

Medium	Test Organisms [A]		Temperature,° C
Fluid thioglycollate	*Bacillus subtilis*	(ATCC 6633) [B]	30 to 35
	Candida Albicans	(ATCC 10231)	30 to 35
Soybean-casein	*Bacillus subtilis*	(ATCC 6633) [B]	20 to 25
	Candida albicans	(ATCC 10231)	20 to 25

[A] Available from the American Type Culture Collection, 12301 Parkview Drive, Rockville, MD 20852.

[B] If a non-spore-forming organism is desired, use Micrococcus Luteus (ATCC 9341).

6.2.4.2 The media are satisfactory if growth of the microorganisms is apparent within 7 days. The growth-promotion

[1] This test method is under the jurisdiction of ASTM Committee D-19 on Water and is the direct responsibility of Subcommittee D19.08 on Membranes and Ion Exchange Materials.

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[2] Annual Book of ASTM Standards, Vol 11.01.

[3] Mack Publishing Co., Easton, PA 18042.