Designation: D4196 – 05 (Reapproved 2011)

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

This standard is issued under the fixed designation D4196; 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 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

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.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

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 D1129.

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 D1193.

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.

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

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

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 test may be performed simultaneously with the sterility test of the media. However, the media sterility test will be considered invalid if the growth promotion test shows no growth.

7. Sterility Test Procedure

7.1 **Caution—**Sterility tests should be performed in a laminar flow hood having an air velocity of 30 ± 2 m (90 ± 5 ft)/min. The working surface of the laminar flow bench should be wiped with a suitable disinfectant 30 min prior to performing the test. The exterior surfaces of all containers, equipment, etc., used in conjunction with sterility testing should be disinfected and placed into the laminar flow bench 30 min prior to performing the test. The operator should wear a sterile gown and sterile rubber gloves. The gloves should be disinfected, with a 70 % (V/V) alcohol solution, each time after touching a nonsterile surface.

7.2 Aseptically open the packets of membrane filters.

7.3 Using sterile forceps, aseptically place one membrane filter into each of 20 containers of the fluid thioglycollate medium.

7.4 Using sterile forceps, aseptically place one membrane filter into each of 20 containers of soybean-casein digest medium.

7.5 Incubate the fluid thioglycollate medium at 30 to 35°C for at least 14 days.

7.6 Incubate the soybean-casein digest medium at 20 to 25°C for 14 days.

7.7 Examine all containers for the growth of microorganisms over the 14-day incubation period. Turbidity is indicative of microbial growth.

8. Interpretation of Results

8.1 The membrane filters pass the test for sterility if no growth occurs during the specified incubation period. Results are valid in defined experimental conditions at the time the sample was collected and treated, and for a period that cannot exceed the incubation period used in the method.

9. Retests

9.1 If microbial growth is observed in the sterility tests, the following retests are permitted:

9.2 *First Retest:*

9.2.1 Retest an additional 40 membranes from the lot. If microbial growth does not occur during the specified incubation period, the membrane filters pass the test for sterility.

9.2.2 If microbial growth occurs in the first retest, isolate the microorganism(s) and compare them to the organisms isolated from the sterility test. If they cannot be readily differentiated, a second retest may be performed.

9.3 *Second Retest:*

9.3.1 Test an additional 80 membranes from the lot.

9.3.2 If growth is not apparent, the membrane filters pass the test for sterility. If growth occurs in the second retest, the membrane filters fail to meet the requirements for the test for sterility.

10. Precision and Bias

10.1 Since this is a pass-fail test, a precision statement is not appropriate for this test method.

10.2 Bias of this test method depends upon the strict adherence to good aseptic technique required in performing the tests.

11. Keywords

11.1 filters; membrane; sterility