



Designation: F838 – 05

Standard Test Method for Determining Bacterial Retention of Membrane Filters Utilized for Liquid Filtration¹

This standard is issued under the fixed designation F838; 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 determines the bacterial retention characteristics of membrane filters for liquid filtration using *Pseudomonas diminuta* as the challenge organism. This test may be employed to evaluate any membrane filter system used for liquid sterilization.

1.2 *This standard may involve hazardous materials, operations, and equipment. 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

D1193 Specification for Reagent Water

3. Terminology

3.1 *Definitions:*

3.1.1 *log reduction value*—the logarithm to the base 10 of the ratio of the number of microorganisms in the challenge to the number of organisms in the filtrate.

4. Summary of Test Method

4.1 After sterilization, the test filter is challenged with a suspension of *Pseudomonas diminuta* (ATCC 19146)³ at a concentration of 10^7 organisms per cm^2 of effective filtration area (EFA) at a maximum differential pressure across the test filter of 30 psig (206 kPa) and a flow rate of 0.5 to 1.0 GPM per ft^2 of effective filtration area (2 to 4×10^{-3} LPM per cm^2). The entire filtrate is then filtered through an analytical membrane filter disc which is subsequently incubated on a solidified

growth medium. Organisms that are not retained by the filter being tested will develop into visible colonies on the analysis membrane and can then be enumerated.

5. Significance and Use

5.1 Since all sterilizing filtration processes are performed under positive pressure, this test method is designed to assess the retentivity of a sterilizing filter under process conditions.

5.1.1 A challenge of 10^7 bacteria per cm^2 of effective filtration area is orders of magnitude higher than one would expect to encounter in a sterilizing filtration process. This level was selected in order to provide a high degree of assurance that the filter would quantitatively retain large numbers of organisms. This concept is important, in view of the requirement to provide a quantitative assessment in validating a sterilization process.

5.1.2 The analytical procedure utilized in this test method provides a method to assign a numerical value to the filtration efficiency of the filter being evaluated. This value, coupled with a knowledge of the number and types of organisms (bioburden) indigenous to the process, may then be utilized to determine the probability of obtaining a sterile filtrate. Conversely, the numerical value of the filtration efficiency may be used when one must meet a specified probability of sterility assurance to calculate the volume of fluid that may be filtered in order to maintain that level of assurance.

6. Apparatus

6.1 Assemble the apparatus described below as in **Fig. 1**:

6.1.1 *Stainless Steel Pressure Vessel*, 12-L capacity (or larger), fitted with a 0 to 50-psi (0 to 350-kPa) pressure gage.

6.1.2 *Air Regulator*.

6.1.3 *142-mm Disc Filter Assemblies*, two or more, with hose connections.

6.1.4 *Diaphragm-Protected 0 to 50-psi Pressure Gage* (0 to 350-kPa), for upstream pressure reading. A second equivalent gauge for downstream pressure reading is optional.

6.1.5 *Manifold*, with valves (autoclavable) and hose connections.

6.1.6 *Autoclavable Tubing*, (must be able to withstand a pressure of 50 psi (350 kPa)).

6.1.7 *Filter Housing*, with hose connections.

6.1.8 *Hose Clamps*.

¹ This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.24 on Water Microbiology.

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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 Available from American Type Culture Collection, 21301 Parklawn Dr., Brookeville, MD 20833.

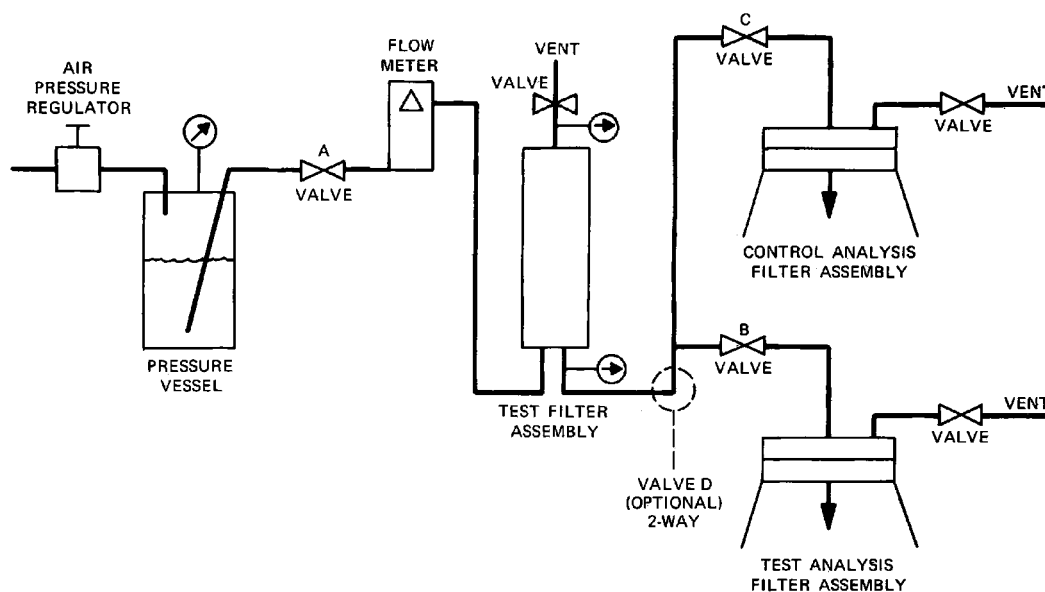


FIG. 1 Test Set-Up for Bacteria Retention Testing

- 6.1.9 Incubator, $30 \pm 2^\circ\text{C}$.
- 6.1.10 Laminar Flow Bench.
- 6.1.11 Smooth-Tip Forceps.

7. Purity of Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used. Unless otherwise indicated, all reagents shall conform to the specifications of the American Chemical Society, where such specifications are available.⁴

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall mean reagent water, Type IV as defined in Specification D1193.

7.2.1 Additionally, any water used in this test method must conform to the requirements for non-bacteriostatic water specified in the current edition of *Standard Methods for the Examination of Water and Wastewater*.⁵

8. Reagents and Materials

8.1 Saline Lactose Broth Medium :

8.1.1 *Lactose Broth*—Dissolve 1.3 g of dehydrated lactose broth medium in 100 mL of water.

8.1.2 *Sodium Chloride Solution*—Dissolve 7.6 g of sodium chloride (NaCl) in 970 mL of water in a 2-L flask with an appropriate closure.

8.1.3 Add 30 mL of lactose broth (8.1.1) to 970 mL of sodium chloride solution. Autoclave at 121°C for 15 min.

8.2 Frozen Cell Paste Method:

8.2.1 *Growth Medium A*—Dissolve in water and dilute to 1 L. Autoclave at 121°C for 15 min (pH 6.8 to 7.0).

Trypticase Peptone (or Casitone)	7.5 g
Yeast Extract	2.5 g
Sodium Chloride (NaCl)	0.5 g
Magnesium Sulfate ($\text{MgSO}_4 \cdot 3\text{H}_2\text{O}$)	0.35 g

8.2.2 *Harvesting Buffer*—Dissolve 0.790 g of monobasic potassium phosphate (KH_2PO_4) and 1.0 g of K_2HPO_4 in 100 mL of glycerol ($\text{C}_3\text{H}_8\text{O}_3$). Adjust to pH 7.2 with 0.1 N potassium hydroxide solution. Dilute to 1 L with water and sterilize at 121°C for 15 min.

8.2.3 *Potassium Hydroxide Solution (0.1 N)*—Dissolve 5.61 g of potassium hydroxide (KOH) in water and dilute to 1 L in a volumetric flask.

8.2.4 *Trypticase Soy Agar*—Prepare according to manufacturer's instructions.

8.2.5 *Trypticase Soy Broth*—Prepare according to manufacturer's instructions.

8.3 Analytical Reagents and Materials :

8.3.1 *M-Plate Count Agar*—Prepare according to manufacturer's instructions.

8.3.2 *Peptone Water (1 g/L)*—Dissolve the peptone in water. Dispense suitable volumes, for preparing decimal dilutions, into screw-cap containers. Autoclave at 121°C for 15 min.

8.4 *Pseudomonas diminuta* (ATCC 19146).

8.5 *Analytical Membrane Filters*, 142-mm diameter, 0.45 μm pore size, 130 to 160 μm thick.

8.6 *Petri Dishes*, 150-mm diameter.

9. Methods for Preparation of Bacterial Challenge Stock Suspension

9.1 *General*—The following two methods have been used extensively for the preparation of *P. diminuta* challenge suspensions. The presentation of these methods is not meant to exclude other equally valid methods for the preparation of *P. diminuta*. It is important, however, that the methods be followed as written.

⁴ "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC.

⁵ Available from the American Public Health Assoc., 1015 18th St., N.W. Washington, DC 20036.