Designation: D3862 – 13 (Reapproved 2019)

Standard Test Method for Retention Characteristics of 0.2-µm Membrane Filters Used in Routine Filtration Procedures for the Evaluation of Microbiological Water Quality[1]

This standard is issued under the fixed designation D3862; 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 covers a procedure to test membrane filters for their ability to retain bacteria whose diameter is equal to or slightly larger than the 0.2-µm pore size of the membrane filter.

1.2 The procedures described are for the use of user laboratories as differentiated from manufacturers' laboratories.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *Gram's stain, n*—a routine bacterial stain that divides bacteria into two categories, depending on whether they can be decolorized with acetone, alcohol, or aniline oil after staining with one of the rosaniline dyes such as crystal violet, methyl violet, or gentian violet and treating with iodine. Those that resist decolorization remain blue or violet and are designated Gram-positive; those that are decolorized and take up the red counterstain, such as neutral red, safranin, or dilute carbol fuchsin are termed Gram-negative.

3.2.2 *vacuum, n—for the procedure used*, source of suction that can produce a reading of 500 to 600 mm Hg on a vacuum gage.

4. Summary of Test Method

4.1 This test method is based on the cultivation of organisms whose diameters are equal to or slightly larger than pores of the membrane filter to be tested and then filtering a specific aliquot containing organisms through the membrane followed by an examination of the filtrate after incubation for sterility. A sterile filtrate indicates complete retention of the organism and validates the ability of the membrane to retain bacteria equal to or slightly larger than the stated pore size.

5. Significance and Use

5.1 Microbiological water testing procedures using membrane filtration are based on the premise that all bacteria within a specific size range will be retained by the membrane filter used. If the membrane filter does not retain these bacteria, false negative results or lowered density estimates may occur that could have serious repercussions due to the presence of unrecognized potential health hazards in the water being tested, especially in drinking water.

5.1.1 This procedure as devised will enable the user to test each membrane filter lot number for its ability to retain all bacterial equal to, or larger than, the stated membrane pore size.

5.2 Since this membrane is often used to sterilize nonautoclavable liquids, it is essential that the retention characteristics of this membrane are stable.

[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.

6. Apparatus

6.1 *Membrane Filtration Units*, six.

6.2 *Vacuum Source*, with trap vessel.

6.3 *Filtering flasks*, 1-L, with vacuum tubing into which a glass tube and a Y-tube have been incorporated as in Fig. 1. The free end of the Y-tube is connected by tubing to a sterile bacterial air vent. The tubing to air vent is clamped shut during filtration and released after filtration.

6.4 *Forceps*, blunt-nosed, and small beaker of 95 % ethanol.

6.5 *Incubator*, 37°C.

6.6 *Pinch-Cock Clamps*.

6.7 *Autoclave or Other Sterilizing Equipment*.

6.8 *Appropriate Equipment* for producing reagent grade waters.

6.9 *Appropriate Laboratory Glassware*.

6.10 *Sterile Rubber Stoppers*, to fit 1-L filtering flask.

6.11 *Expendables*:

6.11.1 *Double-Strength Broth*, 140-mL aliquots.

6.11.2 *Sterile Pipets*, 1 and 10-mL.

6.11.3 *Sterile 0.1 % Peptone*, in 99-mL quantities.

6.11.4 *Sterile 0.1 % Peptone*, as rinse water.

6.11.5 *Broth Culture of Pseudomonas diminuta*, 24 ± 2 h.

6.11.6 *Sterile Membrane Filters*—Test membranes.

6.11.7 *Petri Dishes*, 50-mm, containing 6 to 8 mL of agar.

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.[3] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, reference to water shall be understood to mean reagent water conforming to Specification D1193, Type II, for 0.2-µm membrane filtration. In addition, suitability tests for determining the bactericidal properties of the reagent grade water should be performed.

7.3 *Bacterial Suspension*[4]—Prepare 100 mL of a suspension of a *Pseudomonas diminuta* (ATCC 19146). Add 1.0 mL of a 24 ± 2 h saline lactose broth culture to 99 mL of 0.1 % peptone water. This suspension will contain approximately 106 to 107 organisms per millilitre.

7.4 *Peptone Water (0.1 %)*—Prepare a 10 % stock solution of peptone in water. Dilute a measured volume of the 10 % stock solution to obtain final solution of 0.1 % peptone in required amount. Sterilize at 121°C for 15 min.

7.5 *Test Organism—Pseudomonas diminuta* ATCC strain 19146, also called FDA strain PC1—818.

7.6 *Tryptic Soy Agar and Tryptone Soya Agar* are interchangeable and henceforth referred to as agar medium, formulated, prepared, and dispensed in accordance with the manufacturer's specifications.

[3] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[4] For additional details on growing the challenge suspension, refer to the publication: Leahy, T., and Sullivan, M., "Validation of Bacterial Retention Capabilities of Membrane Filters," *Pharmaceutical Technology*, Vol 2, No. 11, 1978, pp. 65–75.

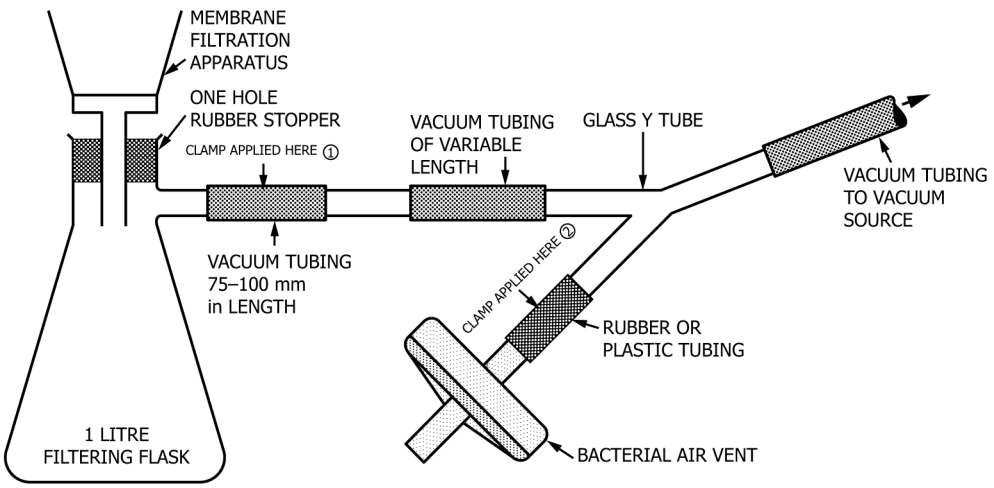

FIG. 1 Apparatus Required for Testing Retention Characteristics of Membrane Filters