



Designation: D 3863 – 87 (Reapproved 1998)

## Standard Test Method for Retention Characteristics of 0.40 to 0.45- $\mu\text{m}$ Membrane Filters Used in Routine Filtration Procedures for the Evaluation of Microbiological Water Quality<sup>1</sup>

This standard is issued under the fixed designation D 3863; 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.

### 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 membrane filters with pore size rated at 0.40 to 0.45  $\mu\text{m}$ .

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

1.3 *This standard does not purport to address all of the safety problems, 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<sup>2</sup>

D 1193 Specification for Reagent Water<sup>2</sup>

### 3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this method refer to Terminology D 1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *Gram's stain*—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*—for the procedure used, a 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.

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

### 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*, 20 to 25°C.

6.6 *Pinch-Cock Clamps*.

6.7 *Autoclave or Other Sterilizing Equipment*.

6.8 *Appropriate Equipment* for producing reagent grade water.

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.

<sup>1</sup> 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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<sup>2</sup> 1983 Annual Book of ASTM Standards, Vol 11.01.