



Standard Test Method for Isolation and Enumeration of Enterococci from Water by the Membrane Filter Procedure¹

This standard is issued under the fixed designation D 5259; 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 membrane filter (MF) procedure for the detection and enumeration of the enterococci bacteria in water. The enterococci, which include *Enterococcus faecalis* (*E. faecalis*), *E. faecium*, and their varieties are commonly found in the feces of humans and other warm-blooded animals. Although some strains are ubiquitous and not related to fecal pollution, enterococci in water are an indication of fecal pollution and the possible presence of enteric pathogens. These bacteria are found in water and wastewater in a wide range of densities. The detection limit is one colony forming unit (CFU)/volume filtered.

1.2 This test method has been used successfully with temperate fresh and marine ambient waters, and wastewaters. It is the user's responsibility to ensure the validity of this test method for waters of untested types.

1.3 The values stated in SI units are to be regarded as the 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 and health practices and determine the applicability of regulatory limitations prior to use.* For specific hazard statements, see Section 9.

2. Referenced Documents

2.1 ASTM Standards:

D 1129 Terminology Relating to Water²

D 1193 Specification for Reagent Water²

D 3370 Practices for Sampling Water from Closed Conduits²

D 3870 Practice for Establishing Performance Characteristics for Colony Counting Methods in Microbiology³

3. Terminology

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

3.2 Definitions of Terms Specific to This Standard:

3.2.1 *Enterococcus*—In this test method, *Enterococcus* species are those bacteria that produce red to maroon colonies with black or reddish-brown precipitate on underside, after incubation on mE agar and subsequent transfer to EIA medium. Enterococci include *E. faecalis*, *E. faecium*, *E. avium*, and their variants.

4. Summary of Test Method

4.1 The procedure given in this test method provides a direct count of bacteria in water based on the development of colonies on the surface of the membrane filter.⁴ A water sample is filtered through the membrane that retains the bacteria. Following filtration, the membrane containing the bacterial cells is placed on a selective, medium, mE agar, and incubated for 48 h at 41°C, then transferred to EIA agar and held at 41°C for 20 min. Enterococci develop as red to maroon colonies with black or reddish-brown precipitate on the underside of the filter.

5. Significance and Use

5.1 The enterococci are indicators of the bacteriological quality for potable water, shellfish growing waters, ambient, and recreational waters. A direct relationship between swimming, associated gastroenteritis, and enterococci has been established through epidemiological studies and marine and fresh water bathing beaches. These studies have led to the development of criteria that can be used to establish bathing water standards based on established health-water quality relationships.

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. Current edition approved June 15, 1992. Published October 1992.

² *Annual Book of ASTM Standards*, Vol 11.01.

³ *Annual Book of ASTM Standards*, Vol 11.02.

⁴ Cabelli, V. J., Dufour, A. P., Levin, M. A., McCabe, L. J., and Haberman, P. W., "Relationship of Microbial Indicators to Health Effects at Marine Bathing Beaches," *American Journal of Public Health*, 69, 1979, pp. 690–696.

