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.

Designation: D5259 – 19

Standard Test Method for Isolation and Enumeration of Enterococci from Water by the Membrane Filter Procedure[1]

This standard is issued under the fixed designation D5259; 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 standard. The values given in parentheses after SI units are included for information only and are not considered 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.* For specific hazard statements, see Section 9.

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

D3370 Practices for Sampling Water from Flowing Process Streams

D3870 Practice for Establishing Performance Characteristics for Colony Counting Methods in Microbiology (Withdrawn 2000)[3]

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 *Enterococcus, n*—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.

3.2.1.1 *Discussion*—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

[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] The last approved version of this historical standard is referenced on www.astm.org.

[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*, Vol 69, 1979, pp. 690–696.

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