



Designation: D6503 – 19

Standard Test Method for Enterococci in Water Using Enterolert^{1,2}

This standard is issued under the fixed designation D6503; 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 simple procedure for the detection of enterococci in water and wastewater. It is based on IDEXX's patented Defined Substrate Technology (DST).² This product, Enterolert, utilizes a nutrient indicator that fluoresces when metabolized. It can detect these bacteria at one most probable number (MPN)/100 mL within 24 h. The presence of this microorganism in water is an indication of fecal contamination and the possible presence of enteric pathogens.

1.2 This test method can be used successfully with drinking water, source water, recreational (fresh and marine) water, wastewater, and bottled water. It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

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:*³

[D1129 Terminology Relating to Water](#)

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 April 1, 2019. Published May 2019. Originally approved in 1999. Last previous edition approved in 2014 as D6503 – 14. DOI: 10.1520/D6503-19.

² Enterolert and Quanti-Tray are trademarks of IDEXX Laboratories, Inc., in Westbrook, ME.

³ 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.

[D1193 Specification for Reagent Water](#)

[D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water](#)

[D3370 Practices for Sampling Water from Flowing Process Streams](#)

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method, refer to Terminology [D1129](#).

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *enterococci, n*—a gram positive bacteria possessing the enzyme β -D-glucosidase, which cleaves the nutrient indicator and produces fluorescence under a long wave length (365–366 nm) ultraviolet (UV) light.

3.2.2 *most probable number (MPN), n*—a statistical method for determining bacterial density based on the Poisson distribution.

3.2.3 *presence-absence, n*—a term used to indicate if enterococci are present or absent in a water sample.

3.2.3.1 *Discussion*—It is a qualitative value, “yes” or “no” for reporting results.

3.2.4 *Quanti-Tray, n*—a system for the quantification of enterococci.²

3.2.4.1 *Discussion*—It consists of a sealer and trays which have multi-wells and can enumerate up to 2419 MPN/100 mL without dilution using the Quanti-Tray 2000 tray or it can detect up to 200.5 MPN/100 mL using the 51-well Quanti-Tray.

3.2.5 *snap pack, n*—a package containing Enterolert reagent for testing 100-mL sample either in the P/A format or quantitatively, with the Quanti-Tray system.

4. Summary of Test Method

4.1 This test method is used for the detection of enterococci, such as *E. faecium*, *E. faecalis* in drinking water, source water, recreational waters (marine water and fresh), wastewaters, and bottled water. When the reagent is added to the sample and incubated at $41 \pm 0.5^\circ\text{C}$ for 24 h and up to 28 h, Enterolert can detect these bacteria at 1 MPN/100 mL. Fluorescence is produced when enterococci metabolizes the nutrient indicator. Enterolert can be used as a presence-absence test or for quantification (5-tube, 10-tube MPN, 15-tube serial dilution or the Quanti-Tray system).