



Designation: D8429 – 21

Standard Test Method for *Legionella pneumophila* in Water Samples Using Legiolert^{1,2}

This standard is issued under the fixed designation D8429; 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 describes a simple procedure for the detection of *Legionella pneumophila* (*L. pneumophila*) in potable water and non-potable waters (cooling towers, for example). This procedure describes a liquid culture method based on a bacterial enzyme technology. The detection of *L. pneumophila* is signaled through the utilization of a substrate present in the Legiolert reagent. *L. pneumophila* cells grow rapidly and reproduce using the rich supply of amino acids, vitamins and other nutrients present in the Legiolert reagent. Actively growing strains of *L. pneumophila* use the added substrate to produce a brown color indicator or produce turbid growth with or without brown coloration. Legiolert can detect this bacterial species at the following minimum concentrations based on the protocol employed:

1.1.1 Potable Water:

1.1.1.1 ≥ 1 organism / 100 mL at 7 days for 100 mL potable protocol.

1.1.1.2 ≥ 1 organism / 10 mL at 7 days for 10 mL potable protocol.

1.1.2 Non-potable Water:

1.1.2.1 ≥ 1 organism / 1.0 mL at 7 days for 1.0 mL non-potable protocol.

1.1.2.2 ≥ 1 organism / 0.1 mL at 7 days for 0.1 mL non-potable protocol.

1.1.3 This test method can be used for potable (drinking) waters and non-potable waters such as cooling tower waters (1).³ It is the user's responsibility to ensure the validity of this test method for waters of untested matrices.

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

1.3 *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.

1.4 *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:⁴

D1129 Terminology Relating to Water

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

D5952 Guide for the Inspection of Water Systems for Legionella and the Investigation of Possible Outbreaks of Legionellosis (Legionnaires' Disease or Pontiac Fever)

D6503 Test Method for Enterococci in Water Using Enterolert

3. Terminology

3.1 Definitions:

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

3.1.2 *Legionella*, *n*—a bacterial genus containing over 50 species and at least 71 serogroups; abbreviated to the first initial when used relatedly with a species name, for example, *L. pneumophila*.

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

3.2 Definitions of Terms Specific to This Standard:

3.2.1 *Legionella pneumophila*, *n*—in the context of this method, a pathogenic gram-negative bacteria that produces any degree of brown coloration or turbid growth with or without brown coloration in the presence of the Legiolert reagent.

¹ Legiolert is a trademark of IDEXX Laboratories, Inc., Westbrook, ME.

² 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 Dec. 1, 2021. Published December 2021. DOI: 10.1520/D8429-21.

³ The boldface numbers in parentheses refer to a list of references at the end of this standard.

⁴ 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.