



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.

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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.

3.2.2 *Quanti-Tray/Legiolert, n*—a system for the quantification of *Legionella pneumophila*. It consists of a Quanti-Tray Sealer PLUS^{5,6} and 96-multi well Quanti-Trays that express *L. pneumophila* counts as MPN per volume of sample.

3.2.2.1 *Discussion*—For drinking water or similar waters where the volume is typically 100 mL, the system can enumerate up to 2272.6/100 mL or for small sample volumes such as the 0.1 mL protocol for non-potable water the system can enumerate up to 22726/1 mL.

3.2.2.2 *Discussion*—To increase the systems counting range a sample dilution can be utilized, but to obtain the correct MPN value and lower and upper confidence limits, these values must be multiplied by the dilution factor.

3.2.3 *snap pack, n*—a package containing Legiolert reagent for testing 100 mL sample volumes or dilutions thereof, quantitatively by means of the Quanti-Tray/Legiolert⁷ system.

4. Summary of Test Method

4.1 Bacteria are grown in a defined liquid culture medium containing the Legiolert reagent.

4.2 The reagent is added to the sample, sealed in a Quanti-Tray, and then incubated, in the presence of humidity, for 7 days at either at 39 °C ± 0.5 °C or 37 °C ± 0.5 °C for potable or non-potable water samples, respectively.

4.3 *L. pneumophila* is detected if any of the Quanti-Tray wells exhibit growth indicated as a brown color change and/or turbidity.

4.4 Test results are reported as MPN per volume of sample (that is, MPN/mL).

5. Significance and Use

5.1 This test provides an easy and reliable method for the detection of *L. pneumophila* in potable and non-potable waters in 7 days.

5.2 Routine monitoring for *L. pneumophila* determines whether implemented control measures are effective, such as those outlined in a water safety program (2).

5.2.1 Water system management is necessary to maintain *L. pneumophila* concentrations below hazardous levels. Through routine measurement of *L. pneumophila* levels, a monitoring program can ensure that control measures are effective and implemented when necessary in response to increasing levels. Water samples may be examined for *L. pneumophila* during epidemiological investigations as part of local authority, industrial, or hospital programs, or in order to validate treatment control methods. Routine sampling could also be carried

out based on risk assessments or on local, state, or federal requirements or guidelines.

6. Interferences

6.1 Hardness can interfere with the use of 100 mL of potable water sample and the addition of the Legiolert Supplement is required to prevent interference (12.2.1.3).

6.2 For non-potable, the Legiolert Pretreatment reagent is required to be added to the sample (12.2.1.4).

7. Apparatus

7.1 *Incubator*, capable of maintaining a temperature of 39 °C ± 0.5 °C (for potable water samples).

7.2 *Incubator*, capable of maintaining a temperature of 37 °C ± 0.5 °C (for non-potable water samples).

7.3 *Quanti-Tray Sealer PLUS*.

8. Reagents and Materials

8.1 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D1193, Type IV. Sterilize water by either autoclaving or by sterile filtration (0.22-micron filter filtered water).

8.2 *Legiolert Test Kit*:

8.2.1 The Legiolert reagent is a commercially available substrate provided in a snap pack format and is suitable for single samples.

8.3 *Quanti-Tray/Legiolert*:

8.3.1 For MPN results, the Legiolert reagent must be used in conjunction with the Quanti-Tray/Legiolert 96-well trays. Quanti-Tray/Legiolert is commercially available, and one tray is suitable for a single sample.

8.4 *Legiolert Supplement Kit*:

8.4.1 The Legiolert Supplement is a commercially available kit containing of (1) a canister of dip strip tests designed to determine the total calcium/magnesium hardness in a water sample and (2) a 100 mL vessel containing supplement solution powder. The hardness strips are used to determine the total hardness of the sample and the supplement solution, once the powder is reconstituted with 100 mL of sterile deionized water, is added to the sample based on the determined sample's hardness.

8.5 *Legiolert Pretreatment Kit*:

8.5.1 The Legiolert Pretreatment is a commercially available kit containing of four vessels containing a dry powder to be reconstituted in sterile water.

8.6 *Quality Control Strains*:

8.6.1 IDEXX-QC *Legionella pneumophila*⁸ or equivalent.

⁵ Quanti-Tray is a trademark of IDEXX Laboratories, Inc., Westbrook, ME.

⁶ The sole source of supply of Quanti-Tray Sealer PLUS known to the committee at this time is IDEXX Laboratories, Inc. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,² which you may attend.

⁷ The sole source of supply of Quanti-Tray/Legiolert supplies and reagents known to the committee at this time is IDEXX Laboratories, Inc. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,² which you may attend.

⁸ The sole source of supply of IDEXX-QC *Legionella pneumophila*, UN3373-WQC-LP98-009287-00, known to the committee at this time is IDEXX Laboratories, Inc. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,² which you may attend.