

Chlorinated water must be collected in vessels with sodium thiosulfate.

1



Bring sample to room temperature and measure hardness.

2



Add appropriate volume of reconstituted Potable Supplement.

3



Add Legiolert® reagent to water sample.

4



Cap the vessel and shake to dissolve. Sample may remain cloudy but can be added to the Quanti-tray® when no large particles are visible.

5



Agitate and pour sample into Quanti-Tray® Legiolert®* Tap tray to remove any air bubbles from small wells.

6



Immediately seal the Quanti-Tray® using the IDEXX Sealer Plus®.

7a



Place Quanti-Trays® paper side down into humidified incubator at 39°C ± 0.5°C for 7 days. Provide adequate humidity to minimise Quanti-Tray water loss during 7 days. Do not remove Quanti-Trays® from the incubator prior to the final read.

7b



Alternatively, insert Quanti-Tray®(s) paper side down into an appropriately sized re-sealable plastic bag or box. Then incubate container at 39°C ± 0.5°C for 7 days. Do not remove Quanti-Trays® from the incubator prior to the final read.

8



Read results: Any brown and/or turbid wells are positive for *Legionella pneumophila*. Count positive wells and quantify using MPN table.

The process steps above are intended as a guide only. Please refer to the current product insert for detailed instructions.



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Legiolert* Test Kit

Introduction

Legiolert is a test that detects *Legionella pneumophila* in water samples. This test is based on a bacterial enzyme detection technology that signals the presence of *Legionella pneumophila* through utilization of a substrate present in the Legiolert reagent. *Legionella 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 *Legionella pneumophila* use the added substrate to produce a brown color indicator. Legiolert detects *Legionella pneumophila* at 1 organism in 100 mL within 7 days.

Storage

Store at 2–25°C away from direct light and humidity.

Potable Water Sample Preparation (for use with environmental samples only)

10 mL Protocol

1. Add 90 mL of sterile diluent (e.g., deionized water, phosphate/Butterfield's buffer, or 0.1% peptone) to a sterile vessel without sodium thiosulfate.
 2. Add 10 mL water sample.
 3. Bring sample to room temperature.
 4. Add contents of Legiolert blister pack to sample.^{A, B}
 5. Shake until contents are dissolved. Sample may remain cloudy.
1. Collect 100 mL of sample in a sterile vessel.
 2. Bring sample to room temperature.
 3. Using a hardness dip strip (supplied as part of the Legiolert Supplement Kit²), follow the manufacturer's instructions to determine if your sample has low or high hardness. Consider 0–2 pads positive as low; 3–4 pads positive as high.
 4. Add corresponding supplement (supplied as part of the supplement kit) as follows: Briefly agitate the supplement vessel immediately prior to use, then for low hardness samples add 0.33 mL and for high hardness samples add 1.0 mL.
 5. Add contents of Legiolert blister pack to sample.^{A, B}
 6. Shake until contents are dissolved. Sample may remain cloudy.

Nonpotable Water Sample Preparation (for use with environmental samples only)

0.1 mL Protocol

1. Add 100 mL of sterile diluent (e.g., deionized water, phosphate/Butterfield's buffer, or 0.1% peptone) to a sterile vessel without sodium thiosulfate.
 2. Add contents of Legiolert blister pack to vessel.^{A, B}
 3. Shake until contents are dissolved. Solution may remain cloudy.
 4. Add 0.2 mL of Legiolert Pretreatment into a sterile microtube.³
 5. Add 0.2 mL of nonpotable water sample to the same microtube and mix well.
 6. Incubate the microtube at room temperature for 60 seconds (± 5 seconds).
 7. Mix the microtube contents and immediately transfer 0.2 mL to the vessel containing Legiolert and mix well.
1. Add 100 mL of sterile diluent (e.g., deionized water, phosphate/Butterfield's buffer, or 0.1% peptone) to a sterile vessel without sodium thiosulfate.
 2. Add contents of Legiolert blister pack to vessel.^{A, B}
 3. Shake until contents are dissolved. Solution may remain cloudy.
 4. Add 2.0 mL of Legiolert Pretreatment into a sterile tube.³
 5. Add 2.0 mL of nonpotable water sample to the same tube and mix well.
 6. Incubate the tube at room temperature for 60 seconds (± 5 seconds).
 7. Mix the tube contents and immediately transfer 2.0 mL to the vessel containing Legiolert and mix well.

Quantitray* Enumeration Procedure

For Quality Control (QC) and Proficiency Test (PT) samples only:

1. Add contents of Legiolert blister pack to sample.^{A, B}
2. Shake until contents are dissolved. Sample may remain cloudy.

For all test samples:

3. Pour sample/reagent mixture into a Quantitray/Legiolert tray.^C Tap or flick the tray to remove air bubbles.^D
4. Immediately seal tray in an IDEXX Quantitray Sealer PLUS.
5. Incubate the sealed trays:
 - i. For all potable water or QC and PT samples, incubate at $39 \pm 0.5^\circ\text{C}$ for 7 days.
 - ii. For all nonpotable water incubate at $37 \pm 0.5^\circ\text{C}$ for 7 days.
6. Quantitray/Legiolert must be incubated paper side down with the wells facing up in an environment that will minimize Quantitray water loss (either in high humidity or in an enclosure that traps moisture). Stack trays in alternating directions for added stability.^E Do not remove trays completely from incubator prior to final read.
7. Read results according to the Result Interpretation table below. Count the number of positive wells and refer to the Legiolert MPN table provided with the trays to obtain a Most Probable Number. Multiply results by the dilution factor for the final MPN, if applicable.



Result Interpretation

Appearance

Any brown color (with or without turbidity)

Any turbidity (with or without any brown color change)

No brown color change and no turbidity

Positive for *Legionella pneumophila*

Positive for *Legionella pneumophila*

Negative for *Legionella pneumophila*

- Legiolert quantitative results are definitive at 7 days. In addition, positive wells observed before 7 days and negative wells observed after 7 days are also valid.
- Turbidity alone indicates growth of *Legionella pneumophila* when the indicator may not develop as quickly or strongly.

Procedural Notes

- For comparison, an incubated sterile diluent blank containing Legiolert reagent (negative control) can be used when interpreting results.
- Legiolert is a primary water test. Legiolert performance characteristics do not apply to samples altered by pre-enrichment or concentration.
- Aseptic technique should always be followed when using Legiolert. Dispose of materials in accordance with Good Laboratory Practices.

Quality Control Procedures

Note: Quality control procedures must be carried out in the absence of sodium thiosulfate.

1. One of the following quality control procedures is recommended for each lot of Legiolert:

A. IDEXX-QC *Legionella pneumophila*⁴: *Legionella pneumophila*, *Eritrococcus faecalis*.

B. For each of the American Type Culture Collection (ATCC)⁵ or World Data Centre for Microorganisms (WDCM)⁶ bacterial strains:

- i. Target organisms: *Legionella pneumophila* ATCC 33152/WDCM 00107 and/or ATCC 33156/WDCM 00180; streak the culture onto BCYE and incubate at 35°C for 48–72 hours.
- ii. Non-target organism: *Eritrococcus faecalis* ATCC 29212/WDCM 00087; streak onto blood agar plate and incubate at 35°C for 18–24 hours.
- iii. For each bacterial strain inoculate a sterile vessel containing 100 mL of sterile diluent (e.g., deionized water, phosphate/Butterfield's buffer, or 0.1% peptone) with 10^2 to 10^3 cfu for target organisms or 10^3 to 10^4 cfu for non-target organisms.

2. Follow the Quantitray Enumeration Procedure above.

3. Results should match the Result Interpretation table above.

Note: IDEXX internal quality control testing is performed in accordance with ISO 11133:2014. Quality Control Certificates are available at idexx.com/water.

1. AFNOR Certification NF Validation covers the potable 100 mL sample protocol and nonpotable 1.0 mL sample protocol.

2. Legiolert Supplement kit, WLGT-SUP, 98-0005745-00

3. Legiolert Pretreatment, WLGT-PRE, 98-0007740-00

4. IDEXX-QC *Legionella pneumophila*, UN3373-WQC-LF 98-0009287-00

5. ATCC American Type Culture Collection 1-800-638-6597 • atcc.org

6. WDCM World Data Centre for Microorganisms • wdcm.org

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Patent information: idexx.com/patents.

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