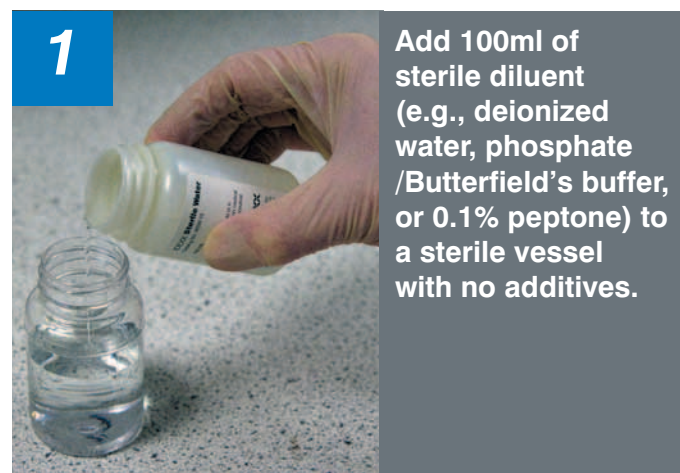


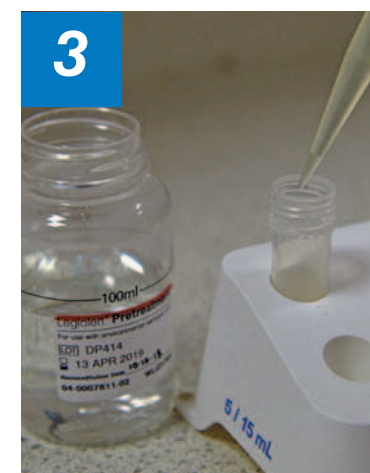
Chlorinated water must be collected in vessels with sodium thiosulfate.



1 Add 100ml of sterile diluent (e.g., deionized water, phosphate /Butterfield's buffer, or 0.1% peptone) to a sterile vessel with no additives.



2 Add contents of Legiolert® blister pack to the vessel containing 100ml sterile diluent and shake until contents are dissolved. The solution may remain cloudy.



3 Add 2.0ml of Legiolert® pre-treatment into a sterile tube.



4 Then add 2.0ml of nonpotable water sample to the same tube.



5a Start a one minute timer then mix the contents. Keep the tube at room temperature for 60 seconds (± 5 seconds). Mix the tube again before 60 seconds has elapsed.



5b



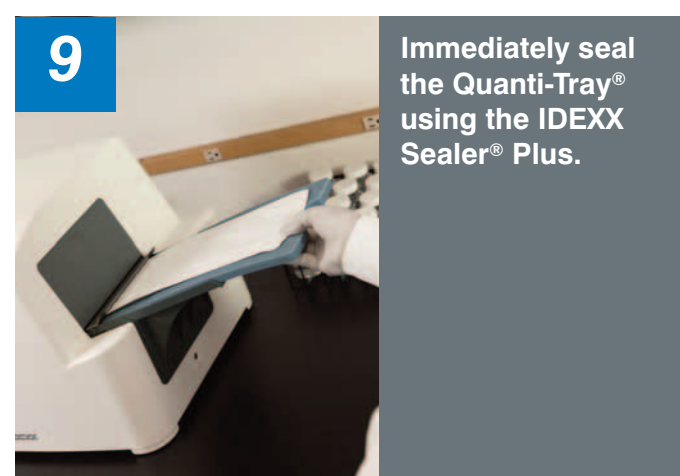
6 At one minute, immediately transfer only 2.0ml of the treated sample to the vessel containing the dissolved Legiolert® reagent as prepared in steps 1 and 2.



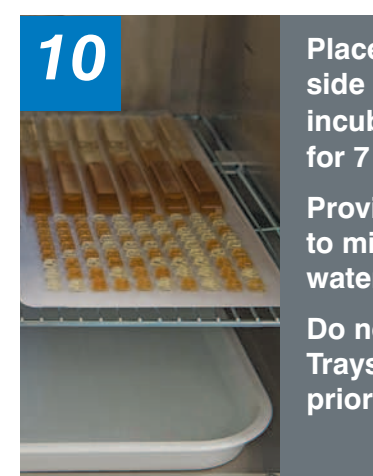
7 Cap the vessel and invert to mix the sample with the reagent. Solution may remain cloudy.



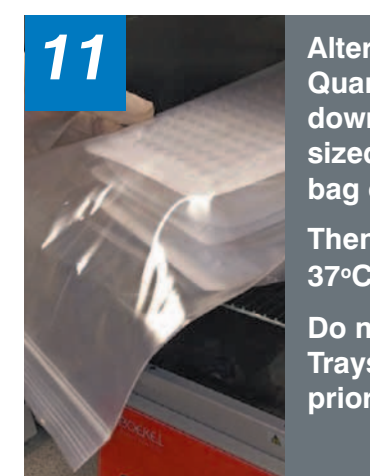
8 Pour sample into Quanti-Tray® Legiolert®. Tap tray to remove any air bubbles from small wells.



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



10 Place Quanti-Trays® paper side down into humidified incubator at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ 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.



11 Alternatively, insert Quanti-Tray®(s) paper side down into an appropriately sized re-sealable plastic bag or box. Then incubate container at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 7 days. Do not remove Quanti-Trays® from the incubator prior to the final read.



12 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.



IDEXX Water 1B & 1C Newmarket Business Park, Studlands Park Avenue, Newmarket, Suffolk, CB8 7ER, UK.
T: +44 (0) 1638 676800 E: waterinfo@idexx.co.uk W: idexx.com/water



ISO 9001:2015 CERTIFIED
ISO 14001:2015 CERTIFIED

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	100 mL Protocol ¹
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	1.0 mL Protocol ¹
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.

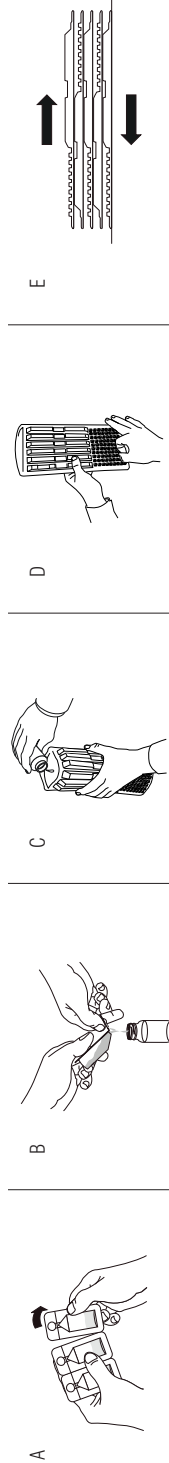
Quanti-Tray® Enumeration Procedure

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 Quanti-Tray/Legiolert tray.^C Tap or flick the tray to remove air bubbles.^D
4. Immediately seal tray in an IDEXX Quanti-Tray Sealer PLUS.
5. Incubate the sealed trays:
 - i. For all potable water or QC and PT samples, incubate at 39±0.5°C for 7 days.
 - ii. For all nonpotable water incubate at 37±0.5°C for 7 days.
6. Quanti-Tray/Legiolert must be incubated paper side down with the wells facing up in an environment that will minimize Quanti-Tray 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	Result
Any brown color (with or without turbidity)	Positive for <i>Legionella pneumophila</i>
Any turbidity (with or without any brown color change)	Positive for <i>Legionella pneumophila</i>
No brown color change and no turbidity	Negative for <i>Legionella pneumophila</i>

- 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*, *Enterococcus 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: *Enterococcus 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 10³ to 10³ cfu for target organisms or 10³ to 10³ cfu for non-target organisms.

2. Follow the Quanti-Tray 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. ARIOR 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-LP 98-0009287-00
5. ATCC American Type Culture Collection 1-800-638-6597 • atcc.org
6. WDCM World Data Centre for Microorganisms • wdcm.org

*Legiolert and Quanti-Tray are trademarks or registered trademarks of IDEXX Laboratories, Inc. or its affiliates in the United States and/or other countries.

Patent information: idexx.com/patents.

© 2019 IDEXX Laboratories, Inc. All rights reserved.