

IDEXX

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Challenges and advances in laboratory methods for the
detection of *Legionella pneumophila* in on-premise testing

Why should you be concerned with *Legionella*?

- The # of cases of legionellosis have increased 286% over the past 14 years Ο
- 8,000 to 18,000 people contract legionellosis in the U.S. each year (estimated) Ο
- 5-15% of the known cases of legionellosis are fatal 0



Clinical causes of legionellosis – causative agents

Culture-confirmed patient cases of L. pneumophila

Year	n	%
2009	448	99
2010	652	98
2011	600	96
2012	661	98
2013	691	96
2014	777	95

ECDC Legionnaires' disease in Europe, Surveillance Report 2009-2014

Reported culture-confirmed cases of Legionnaires' disease and *Legionella* isolates by species, EU/EEA, 2014

O menian	Culture-confirmed cases		
Species	n	%	
L. pneumophila	777	95	
L. longbeachae	14	2	
L. micdadei	6	1	
L. bozemanii	2	<1	
L. macaechernii	1	<1	
L. sainthelensi	1	<1	
L, other species	6	<1	
L. species unknown	12	1	
Total	819	100	



Outbreaks: potable vs. nonpotable water



Outbreaks and cases

https://www.cdc.gov/mmwr/volumes/65/wr/mm6522e1.htm



Legionella is ubiquitous, but it can be managed

- Up to 70% of all building water systems are contaminated with Legionella
- Potable and nonpotable waters are potential sources of Legionella contamination
- Building owners & managers are responsible for controlling it
- Legionnaires' Disease is a growing public health issue that is preventable



Legionellosis is preventable with tools and guidance:

ASHRAE Standard 188-2015



ANSI/ASHRAE Standard 188-2015

Legionellosis: Risk Management for Building Water Systems

Approved by the ASHRAE Standards Committee on May 27, 2015; by the ASHRAE Board of Directors on June 4, 2015; and by the American National Standards Institute on June 26, 2015.

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CDC Guidelines Version 1.1-2017





How do you reduce health and legal risks?

- Test regularly to confirm risk management practices are working properly
- Perform corrective actions if needed and retest to confirm actions have reduced the risk
- Keep records of your test results and corrective measures!





Testing for Legionella

o The Gold Standard is culture method





Legionella Traditional Culture Methods Examples





Different methods = Different results

But which culture method?

Many protocol options, each contributing to Measurement Uncertainty

- o Concentration
 - Membrane filtration
 - Centrifugation
- Pretreatments (to reduce the background)
 - Acid
 - Heat

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- Media
- Formulations GVPC, PVC, MWY, DGVP, CCVC, etc.
- Manufacturers
- Follow up and confirmation methods
 - Plate media
 - Serotype latex agglutination
 - Direct fluorescence antibody microscopy
 - Sequencing





Contributors to Measurement Uncertainty

- Concentration
 - Membrane filtration
 - Loss of target organism due to vacuum pressure
 - Membrane variability can impact target capture and recovery
 - Impaired growth of target due to reduced contact with media
 - Centrifugation
 - Loss of target organism during the decant
- Pretreatments (to reduce the background)
 - Acid and/or Heat
 - Loss of target organisms that are sensitive to treatment
 - Non-target organisms resistant to treatment and impact target
- o Media
 - Formulations GVPC, PVC, MWY, DGVP, CCVC, etc.
 - Loss of target organisms that are sensitive to formulation
 - Non-target organisms resistant to formulation and impact target
 - Manufacturers
 - Products vary by manufacturer and lot which can negatively impact target recovery and non-target suppression



Contributors to Measurement Uncertainty

- o Results Interpretation
 - Analyst
 - Analyst experience level
 - Method
 - Subjectivity of colony differentiation
 - Interference of non-target growth





Results Interpretation, 7 day results

Plates countable

Some non-*Legionella* interference, plates difficult to read and count Complete non-*Legionella* interference, plates unreadable





Results Interpretation, 7 day results

- One sample was plated 4 times at 3 dilutions
 - The impact of the non-target interference can vary wildly



1. Sample A, 1:2 dilution

2. Sample A, 1:4 dilution

3. Sample A, 1:6 dilution



Cumulative impact of Measurement Uncertainty

The various contributors of measurement uncertainty we have discussed will have a cumulative impact



IDEXX

Routine testing The best decisions come from data that is:

- Accurate
- Highly reproducible so trending is accurate, no matter who is processing your sample
- Specific for disease causing agent



Legiolert[™], the next generation culture test Ideal for routine monitoring and compliance

- Detects all serogroups of *Legionella pneumophila*
- Highly specific, little background interference
- $\circ~$ Simple to use, color reaction similar to Colilert^®
- Reproducible and repeatable
- Can be used for potable and nonpotable matrices
 - Matrix-specific protocols
- Uses most probable number (MPN) to quantify, which is the same quantification as CFU
- Counts of up to 2,272 per test, much higher than petri plates
- Incubates for 7 days to yield a confirmed result



Legiolert™

Unique 100 mL "Quanti-Tray" device

- 6 large wells (overflow)
- 90 small wells (resolution)
- Counts *L. pneumophila;* from 1-2273 MPN/ Quanti-Tray
- Blister pack reagent





Reaction with *L. pneumophila*

Negative Sample



Legiolert™



Legiolert



Quanti-Tray/Legiolert



Quanti-Tray/Legiolert Insert



Quanti-Tray Sealer PLUS



Legiolert[™] – ISO 13842 Method Validation

Performance Validation of Legiolert

ISO/TR 13843:2000(E) Water Quality – Guidance on validation of microbiological methods

Metric	Value
Sensitivity	98%
Specificity	> 99%
False positive rate	< 0.01%
False negative rate	4.20%
Efficiency	> 99%
Repeatability	< 0.01
Reproducibility	< 0.01

Legiolert[™] – Studies completed and in progress

Multiple trials and validations by independent labs with potable and nonpotable samples

Completed to date

- Studies in the US, Canada and Germany
- o 10 independent laboratories
- o 3,570 matched samples analyzed
- All results published, under review or being written up for publication in peer review journals

In progress

• Studies in Italy, UK, Australia, Singapore, US, and Germany

Planning phase

• Studies in France, Portugal and Spain



Questions



