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
- 8,000 to 18,000 people contract legionellosis in the U.S. each year (estimated)
- 5-15% of the known cases of legionellosis are fatal

[Graph showing the increase in cases per 100,000 population from 2000 to 2014]

[Bar graph showing the number of cases by month in 2014]

[Link to CDC report: https://www.cdc.gov/mmwr/volumes/65/wr/mm6522e1.htm]
Clinical causes of legionellosis – causative agents

Culture-confirmed patient cases of *L. pneumophila*

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>448</td>
<td>99</td>
</tr>
<tr>
<td>2010</td>
<td>652</td>
<td>98</td>
</tr>
<tr>
<td>2011</td>
<td>600</td>
<td>96</td>
</tr>
<tr>
<td>2012</td>
<td>661</td>
<td>98</td>
</tr>
<tr>
<td>2013</td>
<td>691</td>
<td>96</td>
</tr>
<tr>
<td>2014</td>
<td>777</td>
<td>95</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Culture-confirmed cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td><em>L. pneumophila</em></td>
<td>777</td>
</tr>
<tr>
<td><em>L. longbeachae</em></td>
<td>14</td>
</tr>
<tr>
<td><em>L. micdadei</em></td>
<td>6</td>
</tr>
<tr>
<td><em>L. bozemanii</em></td>
<td>2</td>
</tr>
<tr>
<td><em>L. maaechernii</em></td>
<td>1</td>
</tr>
<tr>
<td><em>L. sainthelensi</em></td>
<td>1</td>
</tr>
<tr>
<td><em>L. other species</em></td>
<td>6</td>
</tr>
<tr>
<td><em>L. species unknown</em></td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>819</td>
</tr>
</tbody>
</table>
Outbreaks: potable vs. nonpotable water

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

What can be done to reduce the risk of exposure to Legionella bacteria?

Perform a RISK ASSESSMENT of your building
Legionellosis is preventable with tools and guidance:

ASHRAE Standard 188-2015

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!

Risk
Testing for *Legionella*

- The Gold Standard is culture method
**Legionella Traditional Culture Methods Examples**

**ISO 11731-1/2 Method**
- High Counts (Non-potable water)
  - Acid Treat
  - Plate 0.1-0.5 mL
  - Heat Treat
- Low Counts (Potable water)
  - Direct Plate 0.5 mL
  - Membrane Filter

**Confirmation**
- BCYE AND
- Check Plates at 7 and 10 days
- Blood Agar

**CDC Method**
- High Counts (Non-potable water)
  - Transfer 1 mL
  - Acid treat pH 2.2 15 min
  - Plate 0.1 mL
- Moderate Counts (Potable or Non-potable)
  - Membrane Filter
  - GPCV GPCV BCYE
  - Plate 0.1 mL
- Low Counts (Potable water)
  - Transfer 1 mL
  - Vortex to resuspend
  - Plate 0.1 mL

**AFNOR Method Overview**
- Nonpotable
  - Plate 0.2 mL
  - Heat Treat
  - Acid Treat Plate 2 mL
- Potable
  - Membrane Filter
  - Filter 100 mL
  - Plate 0.1 mL

*Different methods = Different results*
But which culture method?

Many protocol options, each contributing to Measurement Uncertainty

- Concentration
  - Membrane filtration
  - Centrifugation

- Pretreatments (to reduce the background)
  - Acid
  - Heat

- 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

- 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

- 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

![Bar chart showing results](image)
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
The various contributors of measurement uncertainty we have discussed will have a cumulative impact.
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
- Simple to use, color reaction similar to Colilert®
- Reproducible andrepeatable
- 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
Performance Validation of Legiolert

<table>
<thead>
<tr>
<th>Metric</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>98%</td>
</tr>
<tr>
<td>Specificity</td>
<td>&gt; 99%</td>
</tr>
<tr>
<td>False positive rate</td>
<td>&lt; 0.01%</td>
</tr>
<tr>
<td>False negative rate</td>
<td>4.20%</td>
</tr>
<tr>
<td>Efficiency</td>
<td>&gt; 99%</td>
</tr>
<tr>
<td>Repeatability</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>&lt; 0.01</td>
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</tbody>
</table>
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
- 10 independent laboratories
- 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