Sampling & Analysis for Legionella: What an Investigator Should Know Before Starting a Project

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Legionnaires’ Disease Is On The Rise
2000–2014

Legionellosis Risk

- Most cases are the result of exposure to Legionella associated with building water


Legionellosis --- United States, 2000–2009
Sources of Contaminant

• Naturally occurring in water & soil
• Widely distributed in water systems
• Conditions that tend to promote the growth of Legionella include:
  – stagnation
  – temperatures between 20° and 50°C (68° - 122°F) (The optimal growth range is 35° - 46°C [95° - 115°F])
  – pH between 5.0 and 8.5
  – sediment that tends to promote growth of commensal microflora
  – microorganisms including algae, flavobacteria, and Pseudomonas, which supply essential nutrients for growth of Legionella or harbor the organism (amoebae, protozoa)

Legionella in Biofilm

Water Systems to Sample (amplification sites)

• Cooling Tower Mist (down draft)
• Evaporative Condensers, Air Coolers
• Shower Heads and Faucets
• Hot Tubs and Spas
• Architectural Fountains and Waterfalls
• Body Misters
• Cool Mist Humidifiers – use steam vaporizer instead
• Metalworking Fluids
• Nebulizers, Respiratory Therapy Equipment
• Commercial Car Washes (recycled water only)
• Windshield Wiper Fluid – only use commercial preparations
• Plastic injection molding equipment
• Vegetable Misters
• Ice Machines in hotels and hospitals
• Potting Soil
• CPAP - Using tap water vs distilled water
Potential Sampling Areas in Hospitals

- Potable water system
- Incoming water main
- Water softener
- Holding tanks, cisterns
- Water heater tanks (at the inlets and outlets)
- Potable water outlets, especially those in or near patient rooms
- Faucets or taps
- Showers
- Cooling tower, evaporative condenser
- Makeup water (e.g., added to replace water lost because of evaporation, drift, leakage)
- Basin (i.e., area under the tower for collection of cooled water)
- Sump (i.e., section of basin from which cooled water returns to heat source)
- Heat sources (e.g., chillers)
- Humidifiers (e.g., nebulizers)
- Bubblers for oxygen
- Water used for respiratory therapy equipment
- Decorative fountains
- Irrigation equipment
- Fire sprinkler system (if recently used)
- Hot tubs

CDC Legionella Environmental Assessment Form


Quarterly Inspection and Testing of NYC Cooling Towers

- Inspected and tested at least as frequently as every three months during periods of the year such cooling towers are in use.
- Must be performed in accordance with current standard industry protocols including, but not limited to, ANSI/ASHRAE Standard 188-2015.
- Bacteriological (HPC) testing weekly.
- Legionella testing no less than every 90 days.
NY State Regulations
Protection Against Legionella

- Legionella testing at least every 90 days first year then annually
- HPC testing every 30 days
- Immediate Legionella culture sampling and analysis following specified conditions, such as power failure, loss of biocide of sufficient duration to allow for the growth of bacteria, and if the State or local health department determines that one or more cases of legionellosis is or may be associated with the tower.

Sampling Guidelines (OSHA)

- Use Respiratory Protection:
  - half-face respirator equipped with a HEPA or similar filter capable of effectively collecting particles of 1-micrometer.
  - if a significant potential exists for exposure to high concentrations of contaminated aerosols.
  - while inspecting and collecting water samples near an aerosol generating water source, such as an operating spray humidifier, water mister, fountain, or shower, in a facility where there is a confirmed outbreak.
  - Wear gloves & safety goggles
  - Avoid generating aerosols when sampling

Sampling Potable Water

- If possible collect from hot and cold water supplies:
  - First draw - take first 1000 mL directly from the tap because the end section of the water system may be a contaminated site.
  - Second draw - Let water run for 60 seconds or longer then take the second sample.
Sampling Non-Potable Water

- Cooling towers, hot tubs, ornamental fountains - 250 mL
  - inlet and outlet side
  - from any filters
  - cooling tower pack and sump
- Leave air space in the bottle!
- Look for slime or biofilm, take swab sample.

Sampling Soil, Potting Soil, Wastewater or Compost

- If soil, collect at soil/water interface
- Use sterile bottles supplied by lab
  - Fill halfway following aseptic technique.
- Ensure lab performs a heat pre-treatment step
  - kills protozoans to release Legionella

Swab Sampling Biofilm

- Do NOT use cotton-tipped swabs as they inhibit Legionella growth. Use Dacron/polypropylene-tipped swabs.
- Turn on the water for a couple of seconds to moisten the pipe, and then turn it off. Insert the swab deep into the faucet/pipe. Try to get beyond the bend and swab around the inside surface firmly without breaking the swab stem.
- Swab visible biofilm on the inside of the showerhead or faucet aerator
- Place into plastic tube, add 3-5 mL of water from the same faucet. Snap the wooden or plastic swab stem approximately 1 in. from the top of the tube. Add a drop of 0.1N sodium thiosulfate.

Sampling Considerations

- Optimize recovery of bacteria by looking for source
- Water sampling where aerosolization may occur
- Swab sampling for biofilm or slime
- Air Sampling (from OSHA)
  - Air samples collected on special culture plates with an Andersen-type sampler rarely demonstrate the presence of Legionella in the air.
  - Negative results are frequent because of the difficulty in maintaining viability of the organism on the culture plates.
  - Air sampling for Legionella is strongly discouraged as a means of measuring potential exposure because of the high likelihood of false-negative results.

Shipping Concerns

- Sample Preservation
  - Sodium thiosulfate for potable and water that has been chlorinated/brominated
  - STERILE plastic bottles
- Packaging and Shipping
  - Water tight and secure in cooler
  - Ship on ice packs to maintain temp between 40 and < 64 F. Do NOT freeze. Do NOT use dry ice or ice cubes.
  - Recommend overnight shipping
  - Always include Chain of Custody (waterproof ink)

Hold Times

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>DATE</th>
<th>PAGE</th>
<th>DLLN No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Collection: Requirements for Microbiological Testing of Water Samples</td>
<td>9/1/15</td>
<td>1 of 4</td>
<td>245</td>
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</table>

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Analyte</th>
<th>Water Conditioning</th>
<th>Tenta. &lt; 45°C</th>
<th>MB, mL/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooling Water</td>
<td>Trypticase Soy Broth</td>
<td>32°F</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Cooling Water</td>
<td>Trypticase Soy Broth</td>
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<td>5</td>
<td>5</td>
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<tr>
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<td>Trypticase Soy Broth</td>
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<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Cooling Water</td>
<td>Trypticase Soy Broth</td>
<td>32°F</td>
<td>1</td>
<td>1</td>
</tr>
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</table>

* Samples should be transported at less than 10°C but not less than 6°C and be protected from direct sunlight per ISO 11731-2. If sampled the same morning day, transport samples at ambient temperature as soon as possible. After sampling, store samples, identify with 32°F. During transport, store samples in 32°F or colder. Keep samples and its concentrations in 32°F for 2 days and shall not exceed 5 days. The maximum time from sample collection to culture of the concentration is 14 days.
Lab Qualifications Needed

- CDC ELITE – Proficiency program
- AIHA EMLAP – Accreditation to ISO 17025
- NY ELAP – certification required for all NY state samples
  - Does not accept CDC ELITE since it is not NELAC Approved

Choosing a Lab Method

- Conventional culture methods are still the Gold Standard (CDC or ISO Methods)
  - 14 day test
  - DFA stain for most species and 14 serotypes
- IDEXX Legiolert
  - 7 day test
  - P/A or Quantitative for L. pneumophila
- PCR Broad screen for 50 Legionella
  - Capable of same day results
  - Total +/-, no individual identification

Choosing a Lab Method

- qPCR for individual species
  - Legionella maceachrenii, L. micdadei, L. pneumophila, L. sainthelensis/cincinnatiensis
- Whole Genome Sequencing by NGS for comparing isolates
  - More powerful resolution provided by analysis of whole genome sequences allows outbreak isolates to be distinguished from isolates that are temporally and spatially unassociated with the outbreak
  - PFGE and Sequence based typing are less discriminatory
  - Now being used by CDC for investigations
Primary Differences Between CDC and ISO Methods

- CDC
  - Potable water concentrated
  - Acid treat overgrown plates
  - NP is direct plated (LOD of 10 CFU/mL)
  - Acid treat NP

- ISO
  - Both potable and NP samples are concentrated (LOD of 0.2 CFU/mL for NP samples)
  - All samples are acid-treated and heat-treated
  - Heat treatment more effective at decreasing background flora
  - reducing inhibitory growth, such as Pseudomonas aeruginosa, whose presence can result in false negative results.

Legiolert (IDEXX)

- Sample 100 mL
  - 10 mL used for potable water
  - Pre-treatment then 0.1 mL used for non-potable water
- Only 7 day incubation needed
- *L. pneumophila* detection (P/A or Quantitative)

EMLSIDEXX Study

- 290 non-potable samples tested from June to September 2016
- Non-potable samples were made up of the following types:
Data Analysis

- 290 samples tested with 94 positives total (32.4%)
- Excluded data:
  - Sample #113 = no valid result for CDC method due to interference
  - Sample #222 = Legiolert QT all wells positive
  - 91 remaining data pairs for analysis

Conclusions – Sensitivity for *L. pneumophila*

- Legiolert 0.1 mL protocol provides equivalent detection and quantification of *Legionella pneumophila* when compared to the CDC method.
- As designed, Legiolert provides specific detection of *Legionella pneumophila*.
  - All serotype results from Legiolert showed *L. pneumophila*

Interference from non-Legionella on CDC Plates

- Many of the agar plates in the CDC method were either unreadable (light gray bars) or showed potential for interference (medium gray bars) from non-Legionella bacteria.
Legiolert – False Positive Results

- Results from four (4) North American trial sites in 2016 (including EMSL) showed that 18 of 597 positive wells from the Legiolert 0.1 mL protocol did not confirm as *L. pneumophila*, giving a cumulative false positive rate of 3.0%.

Interpreting Lab Results

- None Detected is the goal
  - continue existing maintenance program with periodic sampling
- Escalating course of action based on concentration
  - OSHA Action Levels

OSHA Action Levels

<table>
<thead>
<tr>
<th>OSHA Action Levels</th>
<th>Non-Potable CFU/mL</th>
<th>Potable CFU/mL</th>
<th>Humidifiers CFU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>100</td>
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Action 1: Prompt cleaning and/or biocide treatment of the system
Action 2: Immediate cleaning and/or biocide treatment. Take prompt steps to prevent employee exposure.
Action Levels

Table 2. Cooling Tower Legionella Content, colony-forming units (CFU) per milliliter

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NYC Action Levels

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NY State Regulations

Protection Against Legionella

- Requires that Legionella culture analysis be performed by a laboratory that is approved to perform such analysis by the New York State Environmental Laboratory Approval Program (ELAP)
- Notify the local health department within 24 hours of receipt of a Legionella culture sample result that exceeds 1,000 CFU/mL. The owner must also notify the public of the test result in a manner determined by the local health department.
NY State Regulations
Protection Against Legionella

- No detection (< 20 CFU/mL)
  - Maintain treatment program and Legionella monitoring in accordance with the maintenance program and plan
- For levels at ≥ 20 CFU/mL but <1000 CFU/mL perform the following
  - Review treatment program.
  - Institute immediate decontamination control
  - Retest the water in 3 – 7 days.
  - Continue to retest at the same time interval until one sample retest result is < 20 CFU/mL. With receipt of result < 20 CFU/mL, resume routine maintenance program and plan.

- For levels ≥ 1000 CFU/mL perform the following:
  - Review the treatment program and provide appropriate notifications
  - Institute immediate decontamination controls
  - Retest the water in 3 – 7 days.

NY State Regulations
Protection Against Legionella

- General hospitals and residential health care facilities
  - Annual environmental assessments
  - Legionella culture sampling and management plan for their potable water systems every 90 days
  - Percentage of Positive Legionella Test Sites:
    - ≤ 30%, Maintain environmental assessment and Legionella monitoring in accordance with the sampling and management plan
    - ≥ 30%, Immediately institute short-term control measures and notify the department.
    - The water system shall be re-sampled no sooner than 7 days and no later than 4 weeks after disinfection to determine the efficacy of the treatment
THANK YOU!

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