Sampling and Analyzing for Legionella Bacteria

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General Considerations

- Proper Sampling Methods
- Selecting the proper method of analysis
- Field Sampling QA/QC
- Result Interpretation/OSHA Recommended Action Levels
- Be an Informed Buyer-Choosing an Env. Microbiology Lab
Regulations and Guidelines

- There are currently no Federal or State regulations for monitoring Legionella - Garland, Texas
- There are currently no governmental or professional exposure limits (such as TELs or PELs)
- Professional guidelines exist as well as guidelines in TX, NY, MD, Allegheny County PA, Los Angeles County, Clark County NV (monitoring)
Choosing Sampling Methods

- Optimize Recovery of the bacteria by looking for its source
- Water Sampling where aerosolization may occur
- Swab Sampling look for biofilm or slime
- Air Sampling
  » Not recommended
  » *Legionella* do not survive drying or may be stressed
Importance of Biofilm

- “Slime” that consists of protozoans, amoeba, bacteria, blue green algae
- Offers protection for Legionella
- Concentration of Legionella higher in biofilm than in water
- Emphasizes the Importance of Swab Samples
Sample Collection

- Use personal safety precautions during sampling: high efficiency particulate respirators, gloves, safety goggles
- Take care not to generate aerosols when sampling
- Obtain sterile, preserved bottles from the lab
  - 1000 ml bottles for potable (drinking) water
  - 250 ml bottles for non-potable water
- Leave an air space in the bottle!
Sample Collection – Potable Water

- Collect two if possible from hot and cold water supplies
  - first draw- take first 1000 ml directly from the tap. Let water run for 60 seconds or longer then take the
  - second draw sample
- Aseptically remove aerators and swab inside faucet or showerhead.
- Sample every 6 months for routine monitoring
Sample Collection – Non-Potable Water

- Water from cooling towers, whirlpool spas, ornamental fountains -250 ml
  - inlet and outlet side
  - from any filters
  - cooling tower pack and sump

- Leave air space in the bottle!

- Look for any slime or biofilm and take swab sample

- Sample quarterly for routine monitoring
Sample Shipping

Sample Preservation
› Sodium thiosulfate for potable water *
› STERILE plastic bottles

Packaging and Shipping
› Water tight and secure in cooler
› Ship on freezer packs (no ice cubes or dry ice)
› Delivery priority overnight
› Always include Chain of Custody using waterproof ink.
Choosing the Proper Analysis

- Methods that are selective for *Legionella* bacteria
- Conventional culture methods versus PCR
  - Culture methods-most species and 15 serotypes of *L. pneumophila*
  - PCR What species?
  - GC FAME
Choosing the Proper Analysis

Culture based method – Recommended method worldwide. Quantitative

› Potable water – filtered or centrifuged to concentrate. CFU/1000 ml or CFU/Volume sampled

› Non-potable water – acid or heat pretreated to reduce non-legionella bacteria. CFU/ml

› Quantitation limit varies depending upon concentration, pretreated, and processed.
Choosing the Proper Analysis

- Culture on selective media (BCYE, BCYE with PCV, BCYE with GPCV, BCYE without cysteine)
- Incubate at 35-37°C
- Read after 4, 7, and 10 days. Confirmed negative result after day 10.
- Identify species and serotype
- Pulsed Field Gel Electrophoresis-strain identification to compare clinical samples to env. samples during an outbreak
Choosing the Proper Analysis

- Direct Fluorescence Antibody (DFA)
  - Used to confirm Legionella and identify species from isolated colonies
  - Used to serotype L. pneumophila
  - Should not be used directly on environmental samples to identify Legionella
Choosing the Proper Analysis

- Polymerase Chain Reaction (PCR)
  - Qualitative Test- presence/absence only
  - Measure genetic material in a cell –DNA/RNA
  - Measures all DNA-viable and non-viable
  - Not a measure of infectivity since need entire cell to be infectious
  - Sample matrix effects –false negatives. Lab needs to run positive and negative control
The Analysis and Useful Information

- Type of Analytical Method Determines the Type of Data - Analysis varies from Lab to Lab
- Data Varies from Lab to Lab
  - God is in the Details
  - Reagents Not Standardized
  - Identification Methods Not Standardized
Data Needs Depend on Goal

- Proactive Monitoring
  - Determine Effectiveness of Maintenance Program
  - Present/Absent Result or Enumeration
  - Species Identification Optional
  - Serotype Identification Optional
  - Strain Identification not needed
Data Needs Depend on Goal

- Reactive Monitoring
  - Outbreak or Suspected Case
  - Quantitative Result Critical - Enumeration
  - Species Identification Critical
  - Serotype Identification Critical
  - Strain Identification Often Needed
  - Pure Culture Prep and Long Term Storage
Result Interpretation

- Enumeration is relative
  - Bacterial populations in flux
  - Indicative of sample when we receive it in the lab; not necessarily field conditions
- Not detected result is the goal – continue existing maintenance program with periodic sampling
- Escalating course of action based on concentration – OSHA Action Levels
## OSHA Action Levels

**Performance Level: Non-Detect**

<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>
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<tr>
<td>1</td>
<td>100</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1000</td>
<td>100</td>
<td>10</td>
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</tbody>
</table>
Detection Limit vs Quantitation Limit

- Detection Limit – Smallest amount that can be detected within a certain confidence limit.
  - 1 CFU/ml is conventional expression*
- Quantitation Limit – More meaningful term.
  - Concentration
  - Pretreatment
  - Amount processed and plated
How to Select a Lab

- Goal is Data that is Scientifically Defensible and Without Bias

- Avoid Potential Conflict of Interest
  - Lab Within your Consulting Firm
  - Lab that Also Consults on Legionella
  - Lab as Part of Water Treatment Provider

- Independent Third Party Lab
How to Select a Lab

- Qualified Staff
- Experienced Supervisors and Microbiologists in CDC/ISO method
- Participation in Proficiency Testing
- Laboratory QA/QC Program
- Identify Analysis Available
- Pure Culture Prep and Long Term Storage
- AIHA Env. Micro. Laboratory Proficiency Test for Legionella isn’t included
References

- *Approaches to Prevention and Control of Legionella Infection*, Allegheny County Health Department, Allegheny County, PA January, 1997
References

- Procedures of the Recovery of Legionella from the Environment, US Centers for Disease Control, January, 2005
- Water Quality-Detection and Enumeration of Legionella, ISO 11731 1&2,