Legionella

- Aerobic, rod-shaped, Gram negative bacteria
- Commonly found in natural and man-made freshwater environments
- Identified and named during the investigation into a pneumonia outbreak among attendees of the American Legion annual convention in Philadelphia, PA in 1976
Legionellosis

**Legionnaires Disease (LD)**
- Pneumonia
- Should be treated by antibiotics
- Fatality rate is approximately 10%
- Hospitalization is common

**Pontiac Fever**
- Flu-like illness
- Self limited
- Not fatal
- Hospitalization is uncommon
Most LD is Associated with *L. pneumophila* serogroup 1

- >60 *Legionella* species
- >90% of LD cases are due to *L. pneumophila*
- >70% of LD cases are due to *L. pneumophila* serogroup 1 (Lp1)

Other clinically relevant *Legionella*
- *L. pneumophila* sg 3 & 6
- *L. micdadei*
- *L. bozemanii* *
- *L. dumoffii* *
- *L. longbeachae*

* blue-white fluorescent legionellae
Legionnaires’ Disease Rates are Increasing

Investigations are Important for Linking Cases and Identifying Likely Sources
Legionnaires’ disease case and outbreak investigations should include epidemiologic, environmental health, and laboratory support.
Laboratory Support is Important for LD Investigations, but Barriers Exist

• Ideally, testing is conducted locally
  • Many jurisdictions do not have in-house testing capacity
  • Commercial laboratory support can be expensive and complicated

• CDC provides laboratory testing
  • Clinical testing
  • Environmental testing in association with an Epi-Aid or official technical assistance request
APHL Conducted an Assessment of Laboratory Needs in the Context of an LD Investigation

- Goal: assess stakeholder capabilities, capacities, needs, and challenges related to Legionella outbreak investigations
- Method: conduct one-on-one and group interviews with CDC and public health laboratory and epidemiology staff
- Outcome: desire to establish a Legionella Reference Center (LRC) providing a standard test menu to jurisdictions with limited in-house testing capability

After a competitive application process, the State Hygienic Laboratory at The University of Iowa was selected as the first LRC.
Steps for Using the LRC

- Enroll
- Submit samples
- Testing
- Receive Results

Preapproval required for environmental samples
Collaboration Between Submitters, the LRC, and CDC will Strengthen Investigations

Consultation including laboratory, epidemiology, and environmental health specialists as appropriate (topics include sampling, submission, result interpretation, next steps)

Slide courtesy of APHL
Please type your questions in the chat. Thank you!

If you think of something later, feel free to contact us:
Melisa Willby, PhD           ghx9@cdc.gov
Elizabeth Hannapel, MPH       qlr0@cdc.gov
Legionella Reference Center Enrollment
Via APHL.org
Enrollment

• **Who**: Any governmental public health or environmental laboratory (PHLs)

• **When**: Enrollment is accepted on a rolling basis

• **Why**: Only enrolled PHLs can send samples to the reference center. Enrolling now ensures you have all the information you need to send samples in the future.

• **How**: The PHL point of contact fills out a brief form on APHL’s website. Upon review and approval, the PHL will receive onboarding documents.

https://www.aphl.org/programs/infectious_disease/Pages/Legionella.aspx
Enrollment & Onboarding Process Overview

1. PHL fills out enrollment application
2. APHL sends onboarding documents to PHL
3. PHL applies for an LRC lab information system web portal account to receive results
4. PHL is ready to submit specimens/isolates
Enrollment form

Fill in basic information about the PHL and lab and epi points of contact.

Legionella Reference Center Submitter Enrollment Application

Complete this application with the requested information. APHL will review your application. Upon approval, you will be sent enrollment materials.

If you have any questions about this application or the LRC, please email Liz Toure (elizabeth.toure@aphl.org) and CC infectious.diseases@aphl.org

<table>
<thead>
<tr>
<th>Laboratory Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Name</td>
</tr>
<tr>
<td>Street</td>
</tr>
<tr>
<td>City</td>
</tr>
<tr>
<td>State</td>
</tr>
</tbody>
</table>
Enrollment form

# Legionellosis cases in most recent year

Submitter type (clinical/environmental/both)

Current capabilities

Sample types you anticipate sending to the LRC
Enrollment Approval

• Upon approval, the lab and epi POC from the PHL will receive an email from APHL with the following:
  
  • Legionella Reference Center Onboarding document
    • Acceptable sample types and sample shipping instructions
    • Instructions for accessing results via the State Hygienic Laboratory’s web portal
    • Description of test methodology and results
  
  • Test Request Forms
    • Clinical specimen/isolate
    • Environmental isolate
Environmental Sample Test Requests

- Testing of environmental samples must be pre-approved by APHL, CDC and the LRC.

- Enrolled PHLs must submit an Environmental Testing Assistance Request Form if they would like to send environmental samples to the LRC.

- Submission of this form is required for each investigation **before** collecting and shipping samples. Approval decisions will be considered based on the characteristics of the outbreak, availability of resources and additional relevant factors.

- Consultation with the CDC and LRC is available if needed for development or review of a sampling plan.

- Environmental samples **must be collected as part of an outbreak investigation**. Testing for routine monitoring or remediation purposes is not offered at the LRC.
Environmental Sample Test Request Process Overview

1. PHL fills out environmental test assistance request form
2. APHL, LRC and CDC Review
3. If approved, LRC sends pre-filled test request form to PHL
4. PHL is ready to collect and send samples
Environmental Sample Test Assistance Request Form

Fill in basic information about the PHL and lab and epi points of contact

<table>
<thead>
<tr>
<th>Laboratory Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory Name</td>
</tr>
<tr>
<td>Laboratory State</td>
</tr>
<tr>
<td>PHL Org ID:</td>
</tr>
</tbody>
</table>

Your PHL Org ID should have been provided to you when you enrolled with the LRC. It is a numerical ID up to five digits long. Leave blank if you do not have it.

Is your laboratory already enrolled in the Legionella Reference Center?  

If no, please submit an enrollment application before submitting this form
Environmental Sample Test Assistance Request Form

- Describe the nature of the investigation
- Describe environmental sampling plan
- Indicate if you would like a consult with CDC/LRC

*A consult may be required depending on the nature of the investigation

Providing as much detail as possible can help expedite the request
Upon approval, the lab and epi POC from the PHL will receive an email from the LRC with the following:

- Pre-populated Environmental Sample Test Request Form
  - Used to provide general info on the facility where samples are collected
- Pre-populated Sample Log Form
  - Used to record sample-specific information (e.g., collection, time, location, source, sample type, etc.)
Thank you
# Tests Offered for the LRC

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Plate count</th>
<th>Culture and Identification</th>
<th>Multiplex PCR Specimen</th>
<th>Multiplex PCR Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical specimen</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Clinical isolate</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Environmental sample</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Environmental isolate</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

- Clinical tests CLIA validated
- Environmental culture and plate count follow CDC ELITE
- WGS in the near future
Clinical specimen/isolate submission

No prior approval needed

PHL sends specimens/isolates to LRC
If sending more than 5, email first
SHL-LRC@iowa.uiowa.edu

PHL access results
SHL OpenELIS web portal
Clinical specimen and isolate TRF

- **Patient info**
- **PHL address and Org. ID**
- **Sample information**
- **Information for Public health purposes and submitter info**

One TRF per specimen/isolate

TRF received with welcome package
Or ask **SHL-LRC@iowa.uiowa.edu**
Clinical specimen/isolate requirements

<table>
<thead>
<tr>
<th>Sample Type¹</th>
<th>Sample Source</th>
<th>Minimum Sample Volume</th>
<th>Sample Storage</th>
<th>Shipping Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Specimens</td>
<td>Sputum</td>
<td>3 mL</td>
<td>2-8°C</td>
<td>Ship overnight within 24 hours of collection. Ship with frozen ice packs</td>
</tr>
<tr>
<td></td>
<td>Bronchial alveolar lavage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other lower respiratory tract specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung tissue</td>
<td>Pea-sized or larger</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical Legionella Isolates</td>
<td>On BCYE slant (preferred) or culture plate from a single colony</td>
<td>N/A</td>
<td>2-8°C for up to 4 days or frozen (≤ -20°C) in calf serum up to 40 days</td>
<td>Ship overnight at ambient temperature or with frozen ice packs. If frozen in calf serum, send on dry ice</td>
</tr>
</tbody>
</table>

¹ Unacceptable specimen/isolate: leaking specimens, insufficient quantity, ship at wrong temperature. For other specimen types or any questions, call 319-335-4335
Clinical specimen/isolate results

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Plate count</th>
<th>Culture and Identification</th>
<th>Multiplex PCR Specimen</th>
<th>Multiplex PCR Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical specimen</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Clinical isolate</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

**Culture and Identification**
- Results: *Legionella* species based on MALDI-TOF results

**Multiplex PCR**
- Results: L. species, *L. pneumophila* (not sg1), *L. pneumophila* sg1
Environmental isolate submission

No prior approval needed

↓

PHL sends isolates to LRC
If sending more than 5, email first
SHL-LRC@iowa.uiowa.edu

↓

PHL access results
SHL OpenELIS web portal
Environmental isolate TRF

One TRF per isolate

PHL address and Org. ID

Sample collection information

Submitter information

TRF received with welcome package
Or ask SHL-LRC@iowa.uiowa.edu
# Environmental isolate requirements

<table>
<thead>
<tr>
<th>Sample Type¹</th>
<th>Sample Source</th>
<th>Minimum Sample Volume</th>
<th>Sample Storage</th>
<th>Shipping Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Environmental Legionella Isolates</strong></td>
<td>On BCYE slant (preferred) or culture plate from a single colony</td>
<td>N/A</td>
<td>2-8°C for up to 4 days or frozen (≤ -20°C) in calf serum up to 40 days</td>
<td>Ship overnight at ambient temperature or with frozen ice packs. If frozen in calf serum, send on dry ice</td>
</tr>
</tbody>
</table>

¹ Unacceptable isolate types: broken plates or tubes. For other specimen types or any questions, call 319-335-4366
## Environmental isolate results

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Plate count</th>
<th>Culture and Identification</th>
<th>Multiplex PCR Specimen</th>
<th>Multiplex PCR Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental isolate</td>
<td></td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

→ Multiplex PCR
  - Results: L. species, *L. pneumophila* (not sg1), *L. pneumophila* sg1
Environmental sample submission

PRIOR APPROVAL REQUIRED

PHL complete APHL env. test request

CDC/APHL/LRC review request
PHL notify of decision via email
LRC send pre-filled TRF and sample log to point of contact

PHL complete TRF and sample log
send to LRC with samples

PHL access results
SHL OpenELIS web portal
Pre-filled environmental sample TRF

One TRF per collection facility
General info on facility where samples are collected

Prefilled PHL address / Org. ID

Collection information

Pre-filled request ID / submittter information

TRF received pre-filled for the request
Or ask SHL-LRC@iowa.uiowa.edu

IOWA State Hygienic Laboratory
Pre-Filled Environmental Sample Log Form

Pre-filled sample log form accompany the TRF

Record sample-specific information (e.g., collection time, unique identifier, location, source, sample type, etc.)

One sample per line (print additional sample log form for over 7 samples)

<table>
<thead>
<tr>
<th>Collected Time</th>
<th>Client Reference # (unique identifier)</th>
<th>Description (Source Location): sink, shower, water fountain, cooling tower, etc.</th>
<th>Location (Collection Location): Room#, Bldg#, Floor#</th>
<th>Sample Type</th>
<th>Temp (°C)</th>
<th>Free Cl. (mg/L)</th>
<th>Total Cl. (mg/L)</th>
<th>pH</th>
<th>Sample Labels - SHL use only</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

Sample Log Form received pre-filled for the request
Or ask [SHL-LRC@iowa.uiowa.edu](mailto:SHL-LRC@iowa.uiowa.edu)
## Environmental sample requirements

<table>
<thead>
<tr>
<th>Sample Type¹</th>
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<th>Minimum Sample Volume</th>
<th>Sample Storage</th>
<th>Shipping Requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental Samples</td>
<td>Bulk water</td>
<td>1 L</td>
<td>2-8°C</td>
<td>Ship overnight within 24 hours of collection. Ship with frozen ice packs in insulated coolers²</td>
</tr>
<tr>
<td></td>
<td>Hot water tank sediment</td>
<td>120 mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cooling tower water</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Swabs</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Filter Media</td>
<td>300-500 mL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹Unacceptable sample types include broken containers and frozen samples. For other specimen types or any questions, call 319-335-4366
# Environmental sample results

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Plate count</th>
<th>Culture and Identification</th>
<th>Multiplex PCR Specimen</th>
<th>Multiplex PCR Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental sample</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

- **Plate count**
  - Results: Range for CFU/mL

- **Culture and Identification**
  - Results: *Legionella* species based on Maldi-TOF

- **Multiplex PCR**
  - Results: L. species, *L. pneumophila* (not sg1), *L. pneumophila* sg1
Results via SHL OpenELIS Web Portal

→ Test results available from LRC lab information system (SHL OpenELIS) Web Portal

→ New enrollee needs to apply for an SHL OpenELIS Web Portal account
  • [https://www.shl.uiowa.edu/openelisweb/OpenELIS.html](https://www.shl.uiowa.edu/openelisweb/OpenELIS.html)

→ Set-up email notifications when samples are received, and test results are ready

→ Results downloadable as PDF or spreadsheet
Contact information

→ LRC shipping address (ship Monday through Thursday)
  State Hygienic Laboratory at the University of Iowa
  U of I Research Park
  2490 Crosspark Road,
  Coralville, IA 52241-4721
  Phone: 319-335-4500

→ LRC Technical Point of Contact:
  • Clinical specimen submission lab phone #: 319-335-4335
  • Environmental sample submission lab phone #: 319-335-4366
  • General LRC:
    ▪ SHL-LRC@iowa.uiowa.edu
    ▪ Valerie Reeb: 319-335-4866 or valerie-reeb@uiowa.edu

→ SHL customer services:
  • SHL Customer Service Phone: 319-335-4500
  • Operating hours: 8 am to 5 pm Central Time
  • After hours calls to SHL customer service phone will be directed to the duty officer.
Legionella Reference Center
State Hygienic Laboratory at the University of Iowa

Michael Pentella, Ph.D., D(ABMM)
Laboratory Director

Cynthia Cass, Ph.D.
Assistant Director
LRC Co-PI

Valérie Reeb, Ph.D.
AMD NGS Lead
LRC Co-PI

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CLIA Director

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M.B. (ASCP)CM
Molecular Supervisor

Megan Nelson, M.S.
Microbiology Supervisor

Ryan Jepson, M(ASCP)CM
Environmental Microbiology Manager

Nancy Hall, MT(ASCP)
Former Environmental Microbiology Manager
**Legionella Reference Center FAQ**

- Can clinical laboratories enroll as submitters with the Legionella Reference Center (LRC)?
  - No, only public health laboratories (PHLs) can enroll as submitters.

- If my PHL already has the capability to test for *Legionella*, can we still enroll?
  - Yes; the LRC can provide surge support for PHLs that already have testing capability. If your PHL has existing *Legionella* testing capability, please continue to maintain that. LRC services are intended to supplement, not replace, *Legionella* testing at PHLs.

- If I enroll as an environmental submitter but later want to send clinical specimens, is that OK?
  - Yes, the ‘submitter type’ question on the enrollment form is just to give us a sense of your current needs. However, if you are enrolled, you can submit both clinical and environmental samples and isolates.

- Is there a cost associated with using the LRC?
  - Testing is provided free of charge, however the submitter is responsible for shipping costs.

- Does the LRC offer urine antigen testing (UAT)?
  - No.
Legionella Reference Center FAQ continued

• How are test results reported?
  • The submitting PHL can access the result reports online from the Iowa State Hygienic Laboratory’s OpenELIS web portal.

• Will the LRC accept environmental samples related to a single clinical case?
  • Yes, the LRC may accept environmental samples for investigations linked to one or more cases. Pre-approval is still required prior to collecting and shipping any environmental samples.

• What if a local health department would like testing assistance for an outbreak?
  • The health department should work with their local or state PHL to coordinate testing assistance. The local/state PHL can enroll as an LRC submitter and can forward samples collected by the local health department to the LRC.
Q&A