Legionella Reference Center Onboarding Documentation

Legionella Reference Center

The *Legionella* Reference Center (LRC) is located at the State Hygienic Laboratory (SHL) at the University of Iowa, Coralville, IA. The LRC was selected through a competitive process to perform quality *Legionella* testing on clinical specimens and isolates, environmental isolates and environmental outbreak samples submitted by governmental public health or environmental laboratories, with support from APHL and CDC.

State Hygienic Laboratory at the University of Iowa

SHL is Iowa's environmental and public health laboratory comprised of approximately 160 professionals led by Director Dr. Michael Pentella, D(ABMM). SHL's highly trained clinical staff provides comprehensive microbiological reference and diagnostic testing services and responds to emerging and reemerging public health threats. SHL offers several tests for the detection and identification of *Legionella* for the LRC, including spread plate culture with enumeration for environmental samples, culture for clinical specimens (isolates identified with MALDI-TOF mass spectrometry) and multiplex PCR. In addition, SHL has been a CDC Environmental *Legionella* Isolation Techniques Evaluation (ELITE) member laboratory since 2009.

Ship samples, specimens and isolates to:

State Hygienic Laboratory at the University of Iowa
U of I Research Park
2490 Crosspark Road,
Coralville, IA 52241-4721
Phone: 319-335-4500

Please ship samples Monday through Thursday only. Do not ship on Fridays or on the eve of holidays. SHL is closed on Saturdays, Sundays and holidays.

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 or Valerie Reeb 319-335-4866 (valerie-reeb@uiowa.edu)

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.

Access all of the latest *Legionella* Reference Center documents on the APHL LRC Enrollee Resources site.

LRC Onboarding & Testing Process

1. **Enroll to become an LRC submitter**

   All US state, local and territorial public health and environmental laboratories are eligible to enroll with the LRC. An enrollment application must be submitted and approved prior to requesting testing assistance for clinical or environmental testing.

   Once enrolled, SHL will enter the submitter laboratory information into the SHL LIMS (OpenELIS) to facilitate future test requests. SHL will send the newly enrolled laboratory a PHL Org ID. Please safeguard your PHL Org ID, it will be requested on the SHL Test Request Forms (TRFs), the APHL Environmental Sample Testing Assistance Request Form and the Order Form for Environmental Legionella Collection Kits.

   Being an enrolled submitter does not guarantee acceptance of samples; clinical specimens, environmental samples and isolates must meet the LRC sampling and shipping requirements, and environmental samples must have preapproval (see Environmental sample testing section).

2. **Create an account on the SHL web portal to receive results**

   To access clinical and environmental testing results, enrolled laboratories need to request an account for the SHL OpenELIS Web Portal. Since access is user-based and not laboratory-based, we ask that each laboratory limit requests to two user accounts maximum. Applicants will receive their user credentials and account information via email, together with a link to the OpenELIS Web Portal and a user guide on how to access results.

3. **Clinical specimen and isolate testing (pages 4-5)**

   Clinical specimens and isolates can be sent directly to SHL by enrolled laboratories (no prior approval necessary).

4. **Environmental isolate testing (page 6)**

   Environmental isolates can be sent directly to SHL by enrolled laboratories (no prior approval necessary).

5. **Environmental sample testing (pages 7-9) and collection kit considerations/procedures (pages 10-12)**

   Testing of environmental samples requires PRIOR APPROVAL from APHL, CDC and LRC. Please complete the APHL Environmental Test Assistance Request Form before sending environmental samples.

   If the submission request is approved, you will receive an email from SHL-LRC with a customized, pre-populated Environmental Sample TRF and a pre-populated Environmental Sample Log Form (examples included in Appendices A and B). Those forms should be used to enter sample information and should be shipped with the samples. Collection kits are available for purchase from the LRC if needed and can be ordered via the LRC Order Form for Environmental Legionella Collection Kits**.**

6. **Testing methodology (page 13) and result reporting and interpretation (pages 14-15)**

   Test results are available from the SHL OpenELIS Web Portal. Test results can be downloaded as either a PDF or spreadsheet. Users can also monitor the status of their tests and sign-up to receive "test results available" and "samples received" email notifications.

LRC Onboarding & Testing Process- Workflow

Governmental public health and environmental laboratories need to follow the steps below to enroll and onboard with the LRC.

ONBOARDING WORKFLOW

PHL enrolls to become an LRC Submitter

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PHL POC receives welcome package from APHL via email

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PHL POC applies for an SHL web portal account

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PHL is ready to submit specimens/isolates to LRC

Clinical specimen/isolate submission	Environmental isolate submission	Environmental sample submission
No prior approval needed Use LRC Clinical TRF	No prior approval needed Use LRC Environmental Isolate TRF	PRIOR APPROVAL REQUIRED Pre-filled LRC TRF will be provided
↓	↓	↓
PHL sends specimens/isolates to LRC One TRF per specimen/isolate If sending more than 5, email first	**PHL sends isolates to LRC** One TRF per isolate If sending more than 5, email first	**PHL POC completes APHL Environmental Testing Assistance Request**
↓	↓	↓
PHL POC accesses results via SHL web portal	**PHL POC accesses results via SHL web portal**	**CDC/APHL/LRC reviews request** PHL notified of decision via email LRC sends pre-filled Environmental Sample TRF and Sample Log Form
		↓
		PHL sends environmental samples to LRC Send with completed Environmental Sample TRF and Sample Log Form
		↓
		PHL POC accesses results via SHL web portal

APHL – Association of Public Health Laboratories
LRC – Legionella Reference Center
PHL – Public health laboratory
POC – Point of contact
SHL – State Hygienic Laboratory at the University of Iowa
TRF – Test request form

Clinical Specimens and Isolates Testing

Clinical specimens and isolates can be sent to the SHL-LRC by enrolled laboratories without prior approval. Clinical specimens submitted to the LRC must originate from an individual with signs and symptoms clinically compatible with Legionnaires' disease (LD) or have an epidemiological link to a suspected or confirmed case(s) or source(s) of *Legionella*.

Although not required, advance notice of shipment to the SHL-LRC would be appreciated. However, if the submitter plans on sending more than 5 specimens/isolates, please send an email to the LRC prior to shipment:

SHL-LRC@iowa.uiowa.edu
Subject "LRC clinical specimens/isolates shipment"
Text: Indicate number of clinical specimens/isolates sent and submitting institution name and PHL Org. ID

Clinical Specimen and Isolate Test Request Form

The LRC Clinical Specimen and Isolate Test Request Form will be sent to submitters as part of their onboarding documents. The electronic form is fillable (blue boxes). Please complete all sections of the form electronically (i.e., patient information, public health laboratory information, sample and test information, requested information for public health purposes), print and ship with specimens/isolates.

The same two patient identifiers are required on both the Clinical Specimen and Isolate Test Request Form and the specimen/isolate tube. Preferred identifiers are patient name (first and last) and date of birth.

Table 1. Acceptable Clinical Specimen/Isolate Types

Sample Type[1]	Sample Source	Minimum Sample Volume	Sample Storage	Shipping Requirements
Clinical Specimens[2]	Sputum Bronchial alveolar lavage Other lower respiratory tract specimens	3 mL	2-8°C	Ship overnight to ensure arrival at the LRC within 24 hours of collection[3]. Ship with frozen ice packs
	Lung tissue	Pea-sized or larger		
Clinical *Legionella* Isolates	BCYE slant (preferred) or culture plate from a single colony	N/A	2-8°C for up to 4 days or frozen (≤ -20°C) in calf serum up to 40 days	Ship overnight at ambient temperature or with frozen ice packs. If frozen in calf serum, send on dry ice

[1]Unacceptable specimen/isolate types include leaking specimens, insufficient quantity of specimen and specimens sent at the wrong temperature (e.g., frozen specimens).
[2]For consultation on specimen types not listed here, or any other questions, call 319-335-4335.
[3]Samples received after 24 hours of collection will still be accepted, however, the final report will indicate that samples were received outside of acceptable limits.

All specimens/isolates should be labelled with a unique identifier for purposes of tracking. This is in addition to the two unique patient identifiers and should be something assigned by the submitting laboratory. It can be the submitting laboratory's accession number or a simple number such as Sample 1, Sample 2, etc. The unique identifier should be entered on the Clinical Specimen and Isolate Test Request Form under "Client Reference #". This number will be used to track the specimen and will be included on reports.

PCR is a very sensitive test and precautions should be taken to not cross-contaminate specimens (i.e., don fresh gloves before collecting specimen. Avoid contact with environmental surfaces). All specimens should be collected using sterile containers.

- **Sputum**: Patient should rinse their mouth with water prior to collection. Instruct the patient not to spit out saliva or postnasal discharge into container. Collect specimen resulting from deep cough (expectorate) in sterile screw-cap container. Satisfactory quality implies the presence of mucoid or mucopurulent material and is of greater significance than volume. Ideally, a sputum specimen should have a volume of 3–5 mL, although smaller quantities are acceptable.
- **Bronchoalveolar lavage (BAL)**: Collect fluid into a sterile container. Minimum volume of 3 mL.
- **Tracheal aspirates**: Minimum volume of 3 mL
- **Bronchoscopy specimens**: Minimum volume of 3 mL
- **Isolate**: Prepare a BCYE slant (preferred) or culture plate from a single colony. If sending frozen isolates, bacterial growth should be resuspended preferably in calf serum prior to freezing.
- **Lung Biopsy specimens**: Transfer a pea-size fragment of the lung into the container and add a small amount of sterile, non-bacteriostatic distilled water to prevent desiccation. DO NOT ADD SALINE, as it may be inhibitory.

Shipping conditions:

Shipping costs are the responsibility of the submitting laboratory and it is the sender's responsibility to minimize risk of shipping infectious substances through proper packaging and compliance with regulations.

- Wrap specimen container in absorbent material and place inside a biohazard bag.
- If sending an isolate plate, tape the plate to keep it closed.
- Ship overnight (avoid Fridays, weekends, and holidays) within 24 hours of collection to:

State Hygienic Laboratory at the University of Iowa
U of I Research Park
2490 Crosspark Road,
Coralville, IA 52241-4721
Phone: 319-335-4500

Environmental Isolate Testing

Environmental isolates can be sent to the SHL-LRC by enrolled laboratories without prior approval. Although not required, advance notice of shipment to the SHL-LRC would be appreciated:

SHL-LRC@iowa.uiowa.edu
Subject "Environmental isolate shipment"
Text: Indicate number of isolates sent and submitting institution name

Environmental Isolate Test Request Form

The LRC Environmental Isolate Test Request Form will be sent to submitters as part of their onboarding documents. This electronic form is fillable (blue boxes). Please complete one form electronically with the proper information for each isolate, print and ship with isolates.

Collection Instructions

All isolates should be labelled with a unique identifier. It can be the submitting laboratory's accession number or a simple number such as Sample 1, Sample 2, etc. The unique identifier should be entered on the Environmental Isolate Test Request Form under "Client Reference #".

Table 2. Acceptable Environmental Isolate Types

Sample Type[1]	Sample Source	Minimum Sample Volume	Sample Storage	Shipping Requirements
Environmental *Legionella* Isolates	BCYE slant (preferred) or culture plate from a single colony.	N/A	2-8°C for up to 4 days or frozen (≤ -20°C) in calf serum up to 40 days	Ship overnight at ambient temperature or with frozen ice packs. If frozen in calf serum, send on dry ice

[1]Unacceptable isolate types include broken plates or tubes.

Shipping conditions:

Shipping costs are the responsibility of the submitting laboratory and it is the sender's responsibility to minimize risk of shipping infectious substances through proper packaging and compliance with regulations.

- Wrap specimen container in absorbent material and place inside a biohazard bag.
- If sending isolate plate, tape the plate to keep it closed.
- Ship overnight (avoid Fridays, weekends, and holidays) to:

State Hygienic Laboratory at the University of Iowa
U of I Research Park
2490 Crosspark Road,
Coralville, IA 52241-4721
Phone: 319-335-4500

Environmental Sample Testing

Only samples associated with an outbreak investigation will be considered for submission to the LRC. Post-remediation samples should not be submitted to the LRC. Instead, post-remediation samples should be submitted to a third-party laboratory/ institution. The LRC's role is to accelerate initial outbreak investigations for jurisdictions that do not have the capability or capacity for outbreak testing, not to provide routine testing.

Environmental Sample Testing Approval Procedure

- Testing of environmental samples requires PRIOR APPROVAL from APHL, CDC and LRC. To request testing of environmental samples, please complete the Environmental Testing Assistance Request Form on the APHL website.
- The requester will receive an automatic email confirming receipt of their environmental sample test request.
- Approval decisions will be communicated with the requester via email.
- If the submission request is approved, the requester will receive an email from SHL-LRC including:
    - The specific request's "APHL Environmental Request ID". Please include this number on any communication regarding that specific request.
    - LRC Environmental Sample Test Request Form and an Environmental Sample Log Form that has been customized and pre-filled for THIS REQUEST ONLY. Please complete both forms and return them with the environmental samples. Please also email the completed forms to SHL-LRC@iowa.uiowa.edu along with FedEx tracking information once samples are shipped.
    - Consultation with the CDC and LRC is available via phone or video chat if needed for development or review of a sampling plan.
    - PHLs that do not have environmental collection kits can purchase them from the LRC by filling out the LRC Order Form for Environmental Legionella Collection Kits.

Environmental Sample Test Request Form and Environmental Sample Log Form

Both the Environmental Sample Test Request Form and Environmental Sample Log Form are prepared specifically for the current testing assistance request. Only forms prepared for a specific testing assistance request should be used for that event. A new Environmental Sample Test Request Form and Environmental Sample Log Form will be provided with each new, approved request.

Pre-populated Environmental Sample Test Request Form

- An Environmental Sample Test Request Form will be sent pre-populated to the submitting laboratory. The form is used to provide general information on the facility where samples are collected. An example Environmental Sample Test Request Form can be found in Appendix A.
- If your testing assistance request includes sampling at multiple facilities, please make a copy of the Environmental Sample Test Request Form for the current event and use one form per facility. For each facility, enter the relevant information on the Environmental Sample Test Request Form.

Pre-populated Environmental Sample Log Form

- A pre-populated Environmental Sample Log Form will be sent to the submitter at the same time as the pre-populated Environmental Sample Test Request Form. An example Environmental Sample Log Form can be found in Appendix B.
- Fill out one Environmental Sample Log Form per facility to accompany the Environmental Sample Test Request Form. Enter information for one sample per row. Up to seven samples can be logged per Environmental Sample Log Form. If you collect more than seven samples within a facility, make a copy of the Environmental Sample Log Form and complete with the additional samples.
  For each sample, record the following:
  - Collection time HH:MM (e.g., 08:29, 15:23) - required
  - Client Reference #: The submitter should assign each sample collected within a facility a unique identifier (e.g., Sample 1, Sample 2, or LIMS #) - required
  - Source location: Indicate from which source the sample was collected (e.g., sink, shower head, water fountains, cooling tower, etc.) - required
  - Collection location: Indicate the building, floor, room number from which the sample was collected – required
  - Sample Type: Drinking Water (DW, e.g., faucets, showers, drinking fountains, ice machine, water heater, water tanks), Non-Drinking Water (NDW, e.g., decorative fountains, water features, spas, hot tubs, pools, cooling towers), Swab (S) and Filter (F) - required
  - Water temperature (°C) at collection time
  - Free and total chlorine concentration in mg/L at collection time
  - pH at collection time

Table 3. Acceptable Environmental Sample Types

Sample Type[1]	Sample Source	Minimum Sample Volume	Sample Storage	Shipping Requirements
Environmental Samples	Bulk water (DW and most NDW)	1 L	2-8°C	Ship overnight within 24 hours of collection (to ensure arrival at the LRC within 2 days of collection)[2]. Ship with frozen ice packs in insulated coolers
	Hot water tank sediment Cooling tower water	120 mL		
	Swabs	N/A		
	Filter Media	300-500 mL		

[1]Unacceptable sample types include broken containers and frozen samples.
[2]Samples received after 48 hours of collection will still be accepted, however, the final report will indicate that samples were received outside of acceptable limits.

Shipping conditions:

Shipping costs are the responsibility of the submitting laboratory and it is the sender's responsibility to minimize risk of shipping infectious substances through proper packaging and compliance with regulations.

- Samples must be transported cool in insulated containers.
- Place the bottles and swabs in the bottom of cooler. Avoid direct contact between sample and ice packs by insulating samples with bubble-wrap or other packing material. Tubes with swabs should be placed together in a Ziplock bag prior to adding to the cooler. Consider taping the cap to prevent leakage.
- If necessary, use multiple boxes to ship a large quantity of bulk water samples.
- Samples should be shipped overnight, preferably the same day as collected, otherwise within 24 hours of collection (avoid Fridays, weekends, and holidays) and received cool but not frozen.
- Shipping address:

  State Hygienic Laboratory at the University of Iowa
  U of I Research Park
  2490 Crosspark Road,
  Coralville, IA 52241-4721
  Phone: 319-335-4500

Environmental Sample Collection Kit Consideration and Procedure

Samples are collected from a variety of sources which may include cooling towers, decorative water fountains, storage tanks, showers, or water taps. All samples should be labeled with a unique identifier. It can be the submitting laboratory's accession number or a unique number such as Sample 1, Sample 2, etc. The unique identifier should be entered on the Environmental Sample Log Form under "Client Reference #". If the supply is chlorinated, a chlorine neutralizer (sodium thiosulfate) should be added to the containers maintaining the following ratio: 5 mL of 2% sodium thiosulfate solution for a 1 L sample.

- **Drinking and non-drinking water:**
  - Use a leak-proof wide-mouth sterile polypropylene plastic bottle of appropriate size and contains sodium thiosulfate to collect sample. Use a separate aliquot of the sample to measure water quality parameters. Record water temperature, pH, chlorine measurements for each sample on your sample log.
  - **Faucets**: Turn on the hot water (or cold-water) tap, immediately collect the first liter of water into a 1 L container. Leave approximately 1 inch headspace for mixing at the laboratory. Be careful not to touch the inside of the lid or sterile bottle.
  - **Hot Water Tanks**: For sediment testing, open drain valve and immediately fill small bottle (120 mL). For bulk hot water tank water, let the water continue to drain a few minutes and then collect one liter of water. Leave approximately 1inch headspace in bottle for mixing. Note: Use caution since water may be very hot.
  - **Cooling Towers**: Fill container with approximately 120 mL of cooling tower water.

- **Swabs** (Surfaces): Use a sterile transport tube containing 5 mL of neutralizing broth, saline or water from the source to be swabbed. Add a drop of sodium thiosulfate to the tube. Remove strainers, screens, diffusers, or shower heads before collection. Moisten the outlet by briefly turning on the hot water. Using a sterile swab (no cotton, no wood) ream out the inside surface of the faucet as far as the swab will reach (four times around the inner circumference). Also swab inside the removed showerhead (rotate over the entire surface of the showerhead four times). Place swab into the tube and label appropriately. It may be necessary to break off the swab shaft to fit it into the tube.

- **Filters**: Wear gloves while collecting filters. Add the appropriate volume of sodium thiosulfate to a 1 L sterile wide-mouth polypropylene bottle to neutralize residual disinfectants. Tighten the bottle top to prevent leakage.
  - **Sand filters**: It is important to collect some sand and enough water from the filter to cover the sand and keep it moist. Collect 300–500 mL of water from the filter chamber into a sterile 1 L bottle. Use the same or a new bottle to scoop sand from the chamber and pour the sand into the bottle making sure that it is completely covered by water.
  - **Cartridge filters**: Cut a portion of the filter to fit inside a 1 L bottle and add enough water from the filter chamber to cover it and keep it moist.
  - **Diatomaceous earth filters**: Collect 300–500 mL of water from the filter chamber into a sterile 1 L bottle and use a sterile swab to scrape diatom powder from the grid. Place the powder into the bottle making sure that it is completely covered by at least 1 inch of water.

Table 4. Examples of Environmental Sample Collection Supplies Used at the LRC:

Purpose	Company	Product	Catalog #
Water - large volume and filter	Fisher scientific	Wide-mouth 1 L polypropylene bottle	50-199-5103
Water - small volume	IDEXX	120 mL sample vessel contains sodium thiosulfate	WV-120SBST-20
Swabs	World BioProducts	PUR BLU swab with D/E neutralizing broth	BLU-DE-P

Measure water parameters:

- This will require a thermometer, pH test kit and chlorine test kit to detect chlorine level below 2 ppm and up to 10 ppm (may need two kits)
- Collect 100–300 mL from the hot- or cold-water tap in a separate plastic sampling bottle. The same bottle can be used for measuring water parameters at every sampling site. Measure temperature, pH, and chlorine level of the sample. Record all measured data on the Sample Log sheet
    - **Temperature**: Use thermometer.
    - **pH**: Follow kit's instructions.
    - **Chlorine**:
        - Free chlorine may be measured when it is known that chlorine is the method of disinfection (as opposed to monochloramine, bromine, or another disinfectant).
        - Otherwise, measure total chlorine.

Please see CDC guidance for environmental sampling procedures and additional CDC resources for environmental assessment and sampling.

Order Environmental Collection Kits from the Legionella Reference Center

PHLs that do not have environmental collection kits can purchase them from the LRC. All costs associated with purchasing and shipping the kits are at the expense of the PHL. The LRC will only send kits for the current investigation associated with the environmental testing request. The kits are not meant to be stocked for future investigations.

Instructions:

- Fill-out the "LRC Order Form for Environmental Legionella Collection Kits" Excel file and indicate the quantity desired for each item, whether coolers and ice packs are needed (Yes/No) and shipping preference (overnight or 2-day delivery).
- Email the excel order form to the LRC (shl-lrc@uiowa.edu) with "LRC collection kits request" in the subject line.
- Based on the request and shipping destination, the LRC will provide a total cost estimate for the order within 24-business hours of request.
- Once the cost estimate has been approved by the PHL, kits will be shipped to the PHL within 24-business hours of approval. The LRC will invoice the PHL.

The LRC and the CDC are available for consultation to help determine which and how many supplies are needed for the current investigation.

Items	Purpose	Unit cost	Detailed description
Collection kits			
1 Liter Bottle with Thiosulfate	Bulk water and filters	$2.56	Fisher Scientific cat# 50-199-5103: wide mouth 1L polypropylene bottle with thiosulfate added for neutralizing residual disinfectants. Intended for bulk water samples (e.g., faucet, shower, water fountains) and filters.
Idexx 120 mL Bottle	Small volume non-drinking water	$0.50	IDEXX cat# WV-120SBST-20: intended for small volume, non-potable water samples (e.g., cooling towers, and hot water tank sediment).
Swab	Environmental surfaces	$1.01	World BioProducts cat# BLU-DE-P: consists of a tube containing a pre-hydrated swab with neutralizing media. The swab possesses a medical grade polyurethane foam tip ideal for sampling surfaces.
Shipping materials			
Styrofoam coolers	Shipment of samples	$18.50	LRC will determine the size and quantity needed depending on the type and number of kits ordered.
Ice packs	Shipment of samples	$1.67	If receiving ice packs from the LRC, they need to be frozen overnight prior to shipping samples.
Shipping and handling			
Order handling	Order preparation	$10.00	There is a flat fee of $10 for handling collection kits.
Shipping cost	Packaging and carrier fees	variable	Shipping costs (packaging and carrier fees) will depend on shipment size and destination.

LRC Testing Methods

Legionella Culture and Identification

Clinical: Culture and identification are CLIA-validated for clinical specimens and isolates. Specimens received are plated on buffered charcoal yeast extract (BCYE) and modified Wadowsky Yee (MWY) media and incubated at 35°C. Sputum and purulent bronchial alveolar lavage (BAL) specimens undergo an acid wash step and are plated on BCYE and MWY media. Plates are inspected for growth every day. When a presumptive *Legionella* colony is detected, the colony is transferred to a new BCYE plate and a blood agar plate (BAP). Isolates growing solely on BCYE will be processed further for characterization using MALDI-TOF which can identify 47 species of *Legionella*. If *Legionella* cannot be isolated after a one-week incubation period, the plate will be transferred to a CO_2 incubator for an additional week.

Environmental: Culture for environmental samples follows CDC ELITE requirements. Samples received are concentrated using membrane filtration and inoculated to BCYE and MWY media using a spread-plate method. In addition, samples also undergo an acid wash step prior to plating on BCYE and MWY. All plates are incubated at 35°C with 5% CO_2. Plates are inspected for growth every day. When a presumptive *Legionella* colony is detected, the colony is transferred to a new BCYE plate and a BAP plate. Isolates growing solely on BCYE will be processed further for characterization using the MALDI-TOF which can identify 47 species of *Legionella*. Once the presumptive colonies' identities are confirmed, bulk water samples are quantified by calculating the number of *Legionella* per milliliter on the spread plate.

Legionella Multiplex PCR

The *Legionella* multiplex real-PCR assay is a laboratory developed assay based on the CDC assay (Benitez and Winchell[1]). It is intended for the simultaneous detection of *Legionella* species (L. spp), *Legionella pneumophila* (Lp), and *Legionella pneumophila* serogroup 1 (Lp1) in clinical specimens collected from patients presenting signs and symptoms of pneumonia and clinical and environmental isolates. Bacterial nucleic acids are extracted from clinical specimens using the bioMérieux NucliSENS® easyMag® and from culture isolates using the Qiagen EZ1 DNA Tissue Kit on an EZ1 instrument. The multiplex real-time PCR assay uses primers and probes targeting sequences from the *ssrA*, *mip*, and *wzm* genes to detect L. spp, Lp and Lp1, respectively.

[1] Benitez AJ, Winchell JM. Clinical application of a multiplex real-time PCR assay for simultaneous detection of Legionella species, Legionella pneumophila, and Legionella pneumophila serogroup 1. J Clin Microbiol. 2013 Jan;51(1):348-51. doi: 10.1128/JCM.02510-12. Epub 2012 Nov 7. Erratum in: J Clin Microbiol. 2014 Feb;52(2):709. PMID: 23135949; PMCID: PMC3536254.

Result Reporting and Interpretation

Table 5. Results Reported

Sample type	Plate count	Culture and Identification	PCR Sample/Specimen	PCR Isolate
Clinical specimen		✓	✓	✓
Clinical isolate				✓
Environmental sample	✓	✓		✓
Environmental isolate				✓

Clinical specimens – culture and identification (see Appendix D)

The "Legionella, Bacterial Culture" test provides the name of the *Legionella* species identified in the specimen. Any positive *Legionella* culture result will be reported upon identification through the SHL OpenELIS web portal. If *Legionella* cannot be isolated after a one-week incubation period, a preliminary report will be generated and made available to the submitter through the web portal. Culture will then be transferred to a CO_2 incubator and a final report will be generated as soon as *Legionella* is isolated or after one week if there is still no growth. *Legionella* species is reported after the MALDI-TOF test has confirmed the isolate.

A positive *Legionella* culture result is indicative of active disease. The most common type of *Legionella* associated with pulmonary disease is *Legionella pneumophila* serogroup 1. *Legionella* culture can detect all species of *Legionella*. In the event that *Legionella* cannot be speciated, "Legionella species" will be reported as the final result. If *Legionella* was not isolated, results will be shown as "No *Legionella* species isolated."

Environmental samples – culture and identification (See Appendix C)

The "Legionella species, SM 9260 J" test provides quantitative (water) and qualitative (swabs) analysis of environmental samples for the presence of *Legionella* species. Results will be provided as a range in Colony Forming Unit [CFU]/mL. The limit of detection is variable and depends on the volume of water filtered; Negative results will be reported as "Not Isolated, below the limit of quantification, <'value'". Positive results will be reported as "Isolated" followed by the range: 0.01-0.99; 1-100; 101-1000, or >1000 CFU/mL. Results from swabs will be reported as "Present" or "Absent".

The "Identification, Culture" test provides the name of the *Legionella* species identified in the sample. If the *Legionella* is unable to be speciated, the results will be reported as "*Legionella* sp." If another bacteria has been isolated, the results will be reported as "No *Legionella* species isolated" and if no bacteria can be identified, the results will be reported as "Unable to identify".

More than one *Legionella* species can be isolated from a sample. In that case, two "Identification, Culture" results will be reported, each with the name of one of the species recovered.

Clinical and Environmental - PCR (see Appendix C and D)

Several PCR results may be reported. For clinical specimens, PCR will be performed on the specimen and any isolate(s) recovered. For environmental samples, PCR will be performed only on the isolate(s) recovered. "Sample type and source" is indicated after the PCR test name to distinguish the different results.

Potential results are: "Legionella species detected", "Legionella pneumophila (not serogroup 1) detected", "Legionella pneumophila serogroup 1 detected", "None Detected" and "Indeterminate".

A result of "Legionella species detected" means any other species of *Legionella* that is not *L. pneumophila* or *L. pneumophila serogroup 1*. "Indeterminate" results can be caused by several factors including but not limited to improper collection, improper storage/shipping conditions, low quantity of target organism near limit of detection or inhibition in the sample.

Presence of *Legionella* DNA in clinical specimens indicates likely infection. A negative result does not rule out the presence of PCR inhibitors in the patient specimen or test-specific nucleic acid in concentrations below the level of detection by the test.

Culture negative and PCR positive specimens are not unexpected. *Legionella* is an extremely fastidious organism. It is possible that a specimen with a low quantity of bacteria would not be detectable by culture but is detectable by PCR. In addition, dead *Legionella* cells or free nucleic acids present in a sample could be detected by PCR but are not viable, and therefore would not grow in culture.

This test was developed, and its performance characteristics determined by the State Hygienic Laboratory. The U.S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

Funding and Disclaimer

This project was 100% funded with federal funds from a federal program of $613,445. This publication was supported by Cooperative Agreement #NU60OE000104, funded by the US Centers for Disease Control and Prevention (CDC). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of CDC, the Department of Health and Human Services, or the US Government.

Appendices

- Appendix A – LRC Environmental Sample Test Request Form example (do not use)
- Appendix B – LRC Environmental Sample Log Form example (do not use)
- Appendix C – LRC Environmental Test Result Report Annotated Example
- Appendix D – LRC Clinical Test Result Report Annotated Example

APPENDIX A: EXAMPLE ENVIRONMENTAL SAMPLE TEST REQUEST FORM (DO NOT USE)

Order #: 00000

Pages in Order: 1 of 1

Containers in Order: 1

REPORT TO:

1

NAME _____
ADDRESS _____
ADDRESS _____
CITY _____, 00000

BILL TO:

22742

SHL LEGIONELLA REFERENCE CENTER
UNIVERSITY OF IOWA RESEARCH PK
2490 CROSSPARK RD
CORALVILLE, IA 52241

Environmental
Sample Collection Form

State Hygienic Laboratory

Lakeside Laboratory
1838 Highway 86
Milford, IA 51351-7267
Phone # 712-337-3669

Ankeny Laboratory
2220 S. Ankeny Blvd.
Ankeny, IA 50023-9093
Phone # 515-725-1600

http://www.shl.uiowa.edu

U of I Research Park
2490 Crosspark Road
Coralville, IA 52241-4721
Phone # 319-335-4500 or
800-421-IOWA

EN 062015

Requested Analyses/Tests

LEGIONELLA REFERENCE CENTER ENVIRONMENTAL SAMPLE TEST REQUEST FORM

"lrc env sample panel" (Legionella species, SM 9260 J, CV)

Complete or correct the following information

Collected Date: _____ (yyyy-mm-dd)

Project Name: lrc (Laboratory approved projects only)

APHL Environmental Request ID: _____ (automatically assigned by APHL)

Collection City: _____ (city where sample was collected)

Collection Facility: _____ (name of facility where sample was collected)

Submitting PHL Contact Name: _____

Submitting PHL Contact Phone: _____

Submitting PHL Contact Fax: _____

Submitting PHL Contact Email: _____

Submitting Epi Contact Name: _____

Submitting Epi Contact Phone: _____

Submitting Epi Contact Email: _____

Chain of Custody/Tracking Signatures

Relinquished By: _____ Date/Time: ____ / ____ / ____ : ____ (year / mm / dd / Military Time)

Received By: _____ Date/Time: ____ / ____ / ____ : ____ (year / mm / dd / Military Time)

Relinquished By: _____ Date/Time: ____ / ____ / ____ : ____ (year / mm / dd / Military Time)

Received By: _____ Date/Time: ____ / ____ / ____ : ____ (year / mm / dd / Military Time)

For SHL Use Only -- Please do not write below this line

Received By: _____ pH: _____

Evidence of Tampering: ☐ Yes ☐ No

Evidence of Cooling: ☐ Yes ☐ No

Date Printed: 2024-05-20

Temperature (Celsius): _____

Bottles Received: _____

Thermometer ID: _____

FOR INTERNAL USE ONLY

FOR INTERNAL USE ONLY

FOR INTERNAL USE ONLY

APPENDIX B: EXAMPLE ENVIRONMENTAL SAMPLE LOG FORM (DO NOT USE)

IOWA State Hygienic Laboratory

Sample Log - Fill out all information requested	
SHL Order No.:	**Collector Comments**
REPORT TO:	**Test:**
PROJECT NAME:	**Collector Name:**
BILL TO:	**Collector Phone:**
Sample Type: DW=Drinking Water[¶]; NDW=Non-Drinking Water[§]; S=Swab, F=Filter	**Collected Date:**

Collected Time (HH:MM)*	Client Reference # (unique identifier)*	Description (Source Location): sink, shower, water fountain, cooling tower, etc.*	Location (Collection Location): Room#, Bldg#, Floor#* (additional comments if any)	Select Sample Type*	Water at collection: Temp (°C)	Water at collection: Free Cl_2 (mg/L)	Water at collection: Total Cl_2 (mg/L)	Water at collection: pH	Sample Labels - SHL use only
				☐ DW ☐ NDW ☐ S ☐ F					
				☐ DW ☐ NDW ☐ S ☐ F					
				☐ DW ☐ NDW ☐ S ☐ F					
				☐ DW ☐ NDW ☐ S ☐ F					
				☐ DW ☐ NDW ☐ S ☐ F					
				☐ DW ☐ NDW ☐ S ☐ F					
				☐ DW ☐ NDW ☐ S ☐ F					

State Hygienic Laboratory
2490 Crosspark Rd
Coralville, IA 52241
319-335-4500

State Hygienic Laboratory
2220 S. Ankeny Blvd.
Ankeny, IA 50021
515-725-1600

Lakeside Lab
1838 Hwy 86
Milford, IA 51351
712-337-3669 ext. 6

Examples of source location for: [¶]DW - faucets, showers, drinking fountains, ice machine, water heater, water tank; and **[§]NDW -** decorative fountains, water features, spas, hot tubs, pools, cooling towers

* Required field

Version:241023.1

APPENDIX C: EXAMPLE ENVIRONMENTAL REPORT (ANNOTATED)

THE UNIVERSITY OF IOWA | **STATE HYGIENIC LABORATORY**

ANALYTICAL REPORT **1-800-421-IOWA (4692)**

Collection Location	Collector and Phone	Client Reference	Accession #
roof	Lastname Firstname xxx-xxx-xxxx	Collection1	1234567
	Collected	**Received**	**Project**
	2023-10-23 13:25	2023-10-24 09:45	lrc

Report To

DEPARTMENT OF HEALTH
LABORATORY # STREETNAME
CITY, ST 22222

Sample Description
Cooling tower

Sample Type
Non-Drinking Water

Sample Source

Sample Note(s)
1

ADDITIONAL SAMPLE INFORMATION

APHL Environmental Request ID: xx
Collection City: Cityname
Collection Facility: Facilityname
Submitting PHL Contact Name: Firstname Lastname
Submitting PHL Contact Phone: xxx-xxx-xxxx
Submitting PHL Contact Fax: xxx-xxx-xxxx
Submitting PHL Contact Email: name@ gmail.com
Submitting Epi Contact Name: Firstname Lastname
Submitting Epi Contact Phone: xxx-xxx-xxxx
Epi Contact Email: name@ gmail.com

RESULTS OF ANALYSIS - FINAL REPORT

TEST	RESULT	ANALYSIS NOTE(S)
Legionella Multiplex, Polymerase Chain Reaction (PCR), Sample Type and Source: Bacterial isolate specimen, M2		*4*
Legionella PCR Result	**Legionella species detected**	
Legionella Multiplex, Polymerase Chain Reaction (PCR), Sample Type and Source: Bacterial isolate specimen, BAW1		*4*
Legionella PCR Result	**L. pneumophila (not serogroup 1) detected**	

TEST	RESULT ([CFU]/mL)	ANALYSIS NOTE(S)
Legionella species, SM 9260 J		*2*
Legionella species	**Isolated, 101-1000**	

TEST	RESULT	ANALYSIS NOTE(S)
Identification, Culture		*3*
Culture Identification	**Legionella anisa**	
Identification, Culture		*3*
Culture Identification	**Legionella pneumophila**	

Annotated Notes

Five tests were performed on this sample:

Multiplex PCR on the bacterial isolate in the M2 medium plate identified *Legionella* species.

Multiplex PCR on the bacterial isolate in the BAW1 medium plate identified *L. pneumophila* not sg 1.

Culture on the sample isolated *Legionella* in a range of 101-1000 CFU/mL. The range includes both *L. pneumophila*, not serogroup 1 and *L. anisa* (as identified by MALDI-TOF).

Subculture and identification (MALDI-TOF) was performed on two isolates from the environmental sample and identified *L. anisa* and *L. pneumophila.*

SAMPLE AND ANALYSIS NOTES

1. Unless otherwise noted, the sample met container and preservation requirements for the analysis requested. Please review carefully your sample results for additional analyte comments or method exceptions.

2. Two morphological types of legionellae isolated:
   Legionella pneumophila, Not Serogroup 1: 500 CFU/mL
   Legionella anisa: 70. CFU/mL

3. Isolate identification obtained using MALDI-TOF mass spectrometry.

THE UNIVERSITY OF IOWA | **STATE HYGIENIC LABORATORY**

ANALYTICAL REPORT **1-800-421-IOWA (4692)**

Collection Location	Collector	Client Reference	Accession #
roof	Lastname Firstname	Collection1	1234567

4. For a clinical specimen, PCR will be performed on the specimen and any isolates recovered. For an environmental sample, PCR will be performed only on the isolates recovered.

Culture negative and PCR positive samples are not unexpected. Legionella is an extremely fastidious organism to grow. It is likely that a specimen with a low quantity of bacteria would not be detectable by culture but is detectable by PCR. In addition, dead Legionella cells or free nucleic acids present in a sample could be detected by PCR but are not viable, and therefore would not grow in culture.

A result of "Legionella species detected" means any other species of Legionella that is not L. pneumophila or L. pneumophila serogroup 1.

This test was developed and its performance characteristics determined by the State Hygienic Laboratory. The U.S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

ANALYSIS INFORMATION

TEST	ANALYZED	SITE	RELEASED	ANALYSIS PREP
1. Legionella Multiplex, Polymerase Chain Reaction (PCR)	2023-10-31 11:08 AMY	3200	2023-11-01 09:18 KTE	
2. Legionella Multiplex, Polymerase Chain Reaction (PCR)	2023-10-30 13:51 AMY	3200	2023-10-31 10:40 KTE	
3. Legionella species, SM 9260 J	2023-10-24 12:04 JAS, CLL	3200	2023-11-07 09:49 CAL	
4. Identification, Culture	2023-10-30 14:13 JAS, CLL	3200	2023-11-07 09:49 CAL	
5. Identification, Culture	2023-10-28 14:13 JAS, CLL	3200	2023-11-07 09:49 CAL	

DESCRIPTION OF UNITS

[CFU]/mL = Colony Forming Units per Milliliter

SITE(S) PERFORMING TESTING

3200 STATE HYGIENIC LABORATORY CORALVILLE, UNIVERSITY OF IOWA RESEARCH PK, 2490 CROSSPARK RD, CORALVILLE, IA 52241; Phone 319/335-4500; Fax 319/335-4555; Michael D. Schueller, M.S., Associate Director; Michael A. Pentella, Ph.D., D(ABMM), Director; IOWA ENVIRONMENTAL LAB ID #027

The result(s) of this report relate only to the items analyzed. Where the laboratory has not been responsible for the sampling stage the results apply only to the sample as received. This report shall not be reproduced except in full without the written approval of the laboratory. If you have any questions, please call Client Services at 800/421-IOWA (4692) or 319/335-4500.

APPENDIX D: EXAMPLE CLINICAL REPORT (ANNOTATED)

THE UNIVERSITY OF IOWA | **STATE HYGIENIC LABORATORY**

ANALYTICAL REPORT **1-800-421-IOWA (4692)**

Patient	Birth Date and Gender	Client Reference	Accession #
DOE, JOHN P # STREETNAME CITY, ST 00000	1967-01-01 Male	Patient1	2222001
	Collected	**Received**	**Project**
	2024-01-10	2024-01-15 09:20	Irc

Report To

DEPARTMENT OF HEALTH LABORATORY
STREETNAME
CITY, ST 22222

Provider and Phone Number

Sample Type and Source
Sputum specimen

Sample Note(s)

ADDITIONAL SAMPLE INFORMATION

Clinical Diagnosis: Not Provided
Date of Onset: Not Provided
Previous urinary antigen test result: Not Provided
Previous PCR test result: Not Provided
Previous culture-based test result: Not Provided
PCR Performed?: Not Provided
PCR manufacturer/method: Not Provided
PCR Target 1 detected name: Not Provided
Cycle Threshold Value 1: Not Provided
PCR Target 2 detected name: Not Provided
Cycle Threshold Value 2: Not Provided
PCR Target 3 detected name: Not Provided
Cycle Threshold Value 3: Not Provided
Resident in a congregate care setting: Not Provided
Hospitalized (inpatient/admitted): Not Provided
Collection Facility: Not Provided
Collection City: Not Provided
Antibiotics Used: Not Provided
Antibiotics Start Date: Not Provided
Submitting PHL Contact Name: Firstname Lastname
Submitting PHL Contact Phone: xxx-xxx-xxxx
Submitting PHL Contact Fax: xxx-xxx-xxxx
Submitting PHL Contact Email: name@ gmail.com
Submitting Epi Contact Name: Firstname Lastname
Submitting Epi Contact Phone: xxx-xxx-xxxx
Submitting Epi Contact Email: name@ gmail.com

RESULTS OF ANALYSIS - FINAL REPORT

TEST	RESULT	ANALYSIS NOTE(S)
Legionella, Bacterial Culture, Sample Type and Source: Sputum specimen		
Legionella Culture Identification	**Legionella pneumophila**	
Legionella Multiplex, Polymerase Chain Reaction (PCR), Sample Type and Source: Bacterial isolate specimen		*1*
Legionella PCR Result	**L. pneumophila serogroup 1 detected**	
Legionella Multiplex, Polymerase Chain Reaction (PCR), Sample Type and Source: Sputum specimen		*1*
Legionella PCR Result	**L. pneumophila serogroup 1 detected**	
Legionella, Bacterial Culture - Preliminary, Sample Type and Source: Sputum specimen		
Legionella Culture Identification	**No Legionella pneumophila isolated**	

Annotated Notes

Four tests were performed on this clinical specimen (sputum).

Culture (final report) of the sputum isolated *L. pneumophila.*

Multiplex PCR on the bacterial isolate detected *L. pneumophila* sg 1.

Multiplex PCR on the sputum specimen detected *L. pneumophila* sg 1.

Culture (preliminary report) detected no *Legionella* growth after a one week incubation. A preliminary report was sent to the client and the culture plate was transfered to a CO_2 incubator for another week. Final reports are generated as soon as *Legionella* is isolated or after one week if there is still no growth.

THE UNIVERSITY OF IOWA | **STATE HYGIENIC LABORATORY**

ANALYTICAL REPORT **1-800-421-IOWA (4692)**

Patient Name	Birth Date	Client Reference	Accession #
DOE, JOHN P	1967-01-01	Patient1	2222001

SAMPLE AND ANALYSIS NOTES

1. For a clinical specimen, PCR will be performed on the specimen and any isolates recovered. For an environmental sample,PCR will be performed only on the isolates recovered.

Culture negative and PCR positive samples are not unexpected. Legionella is an extremely fastidious organism to grow. It is likely that a specimen with a low quantity of bacteria would not be detectable by culture but is detectable by PCR. In addition, dead Legionella cells or free nucleic acids present in a sample could be detected by PCR but are not viable, and therefore would not grow in culture.

A result of "Legionella species detected" means any other species of Legionella that is not L. pneumophila or L. pneumophila serogroup 1.

This test was developed and its performance characteristics determined by the State Hygienic Laboratory. The U.S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use. The results are not intended to be used as the sole means for clinical diagnosis or patient management decisions.

ANALYSIS INFORMATION

TEST	ANALYZED	SITE	RELEASED
1. Legionella, Bacterial Culture	2024-01-25 11:01 LC	3200	2024-01-29 15:42 MMK
2. Legionella Multiplex, Polymerase Chain Reaction (PCR)	2024-01-29 11:19 AMY	3200	2024-01-29 15:31 KTE
3. Legionella Multiplex, Polymerase Chain Reaction (PCR)	2024-01-26 09:02 AMY	3200	2024-01-27 08:16 KTE
4. Legionella, Bacterial Culture - Preliminary	2024-01-15 16:14 MNS	3200	2024-01-25 12:52 KH

SITE(S) PERFORMING TESTING

3200 STATE HYGIENIC LABORATORY CORALVILLE, UNIVERSITY OF IOWA RESEARCH PK, 2490 CROSSPARK RD, CORALVILLE, IA 52241; Phone 319/335-4500; Fax 319/335-4555; Michael D. Schueller, M.S., Associate Director; Michael A. Pentella, Ph.D., D(ABMM), Director; IOWA ENVIRONMENTAL LAB ID #027

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