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Developing a Legionnaires' Disease Laboratory Response Plan

A PRACTICAL GUIDE FOR STATE AND LOCAL PUBLIC HEALTH LABORATORIES

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

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Toolkit Overview

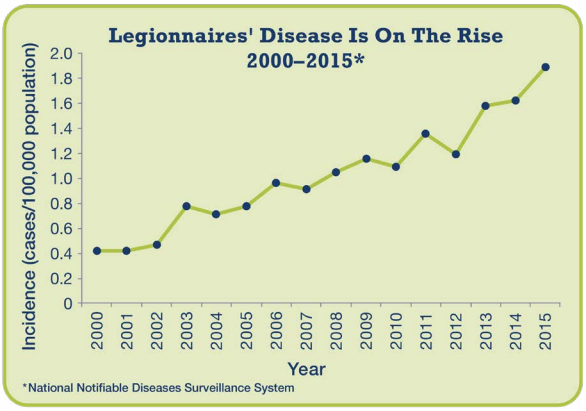
What is Legionnaires' disease?

Legionnaires' disease is a serious type of pneumonia caused by the bacterium *Legionella*. This bacterium can also cause a milder, self-limited, influenza-like illness called Pontiac fever. The term legionellosis refers to either Legionnaires' disease or Pontiac fever. *Legionella* is the leading cause of waterborne pneumonia outbreaks in the United States.[1] The rate of reported cases of legionellosis in the United States increased nearly four and a half times from 2000-2015.[2,3] Those at increased risk for Legionnaires' disease include adults 50 years or older, people with a history of smoking or chronic lung disease, and people who are immunocompromised. *Legionella* lives naturally in soil and freshwater environments, such as lakes and streams, but is spread primarily via inhalation of contaminated aerosols from human-made water systems that are not adequately managed.

What are the challenges of a Legionnaires' disease laboratory response?

Comparing *Legionella* isolates from both clinical respiratory specimens and environmental samples using molecular techniques can be vital for determining the source of a Legionnaires' disease case, cluster, or outbreak. Obtaining isolates can be complicated by many factors, including:

- Limited availability of respiratory specimens for culture since the urinary antigen test (UAT) is the most common clinical diagnostic assay
- *Legionella's* fastidious growth requirements
- Limited commercial availability of specialized media (Buffered Charcoal Yeast Extract [BCYE] agar)
- Challenges related to proper collection, transportation, and processing of environmental samples



In the United States, reported cases of Legionnaires' disease have increased by nearly four and a half times since 2000. More than 6,000 cases of Legionnaires' disease were reported in 2015, but this number is likely an underestimate as the illness is thought to be underdiagnosed. More illness occurs in the summer and early fall but can happen any time of year.

How can laboratories better prepare for a Legionnaires' disease response?

Laboratories can increase their preparedness by developing a Legionnaires' disease Laboratory Response Plan (LDLRP). A completed LDLRP contains important information related to the Response Team, considerations regarding testing that will be performed in-house, and a plan for any tests that will be referred to outside laboratories. Having an LDLRP in place helps laboratories strengthen their Legionnaires' disease response capabilities and prepare for potential cluster or outbreak investigations with confidence.

Who can benefit from an LDLRP?

Every state and local public health laboratory (PHL) can benefit from developing an LDLRP. During a Legionnaires' disease cluster or outbreak response, laboratories may suddenly be faced with processing hundreds of samples. Preparing an LDLRP can help PHLs develop a strategy before a cluster or outbreak occurs, regardless of current *Legionella* testing capacity.

1 Hilborn ED, Wade TJ, Hicks LA, et al. Surveillance for waterborne disease outbreaks associated with drinking water and other nonrecreational water—United States, 2009–2010. *MMWR Morb Mortal Wkly Rep.* 2013;62(35):714–20.

2 Adams D, Fullerton K, Jajosky R, et al. Summary of notifiable infectious diseases and conditions—United States, 2013. *MMWR Morb Mortal Wkly Rep.* 2015;62:1–122.

3 CDC. Notice to readers: final 2014 reports of nationally notifiable infectious diseases. *MMWR Morb Mortal Wkly Rep.* 2015;64:1019–33.

How complicated is it to develop an LDLRP?

Developing an LDLRP may be as simple as vetting and establishing a point of contact with an outside certified laboratory or as complex as creating workflow algorithms for processing clinical specimens and environmental samples. It depends on your laboratory's capacity. This toolkit will walk you through all of the steps that you need to consider in a response.

How to use this toolkit

The toolkit is divided into three parts:

Part One: LDLRP Template. The template can be edited as needed to reflect your laboratory's current capacity and protocols. In addition to outlining the in-house and referral laboratory testing plans, an important component of a complete LDLRP is to identify and solicit input from your health department's epidemiology, environmental health, and communications divisions.

Part Two: Example Response Plan and Scenario. The example LDLRP demonstrates how to develop a plan. The example laboratory response scenario illustrates example workflows and work calendars. The scenario in this example is hypothetical and based on a typical Legionnaires' disease response. It is not intended to be a recommendation for workflows or turn-around times. Every PHL's plan will be different and should reflect current capacity and protocols.

Part Three: Resources. This section provides additional resources for laboratory, epidemiological, and environmental health aspects of a Legionnaires' disease response as well as example laboratory workflow documents that may be adapted for use in your laboratory.

Legionnaires' Disease Laboratory Response Plan Template

Overview

Establishing an LDLRP *before* an outbreak or cluster occurs provides a roadmap for your laboratory's activities and expedites the timeline for results. In the event of an outbreak or cluster, having an established Legionnaires' disease Response Team and LDLRP will facilitate a smooth investigation.

The LDLRP has four sections:

- **Checklist.** Determines which steps of a response will be completed in-house and which will be completed by a referral laboratory.
- **Response Team.** Identifies and establishes consensus among those within the laboratory and the health department's epidemiology, environmental health, and communications teams who will be involved in a Legionnaires' disease response.
- **In-house Testing Plan.** Outlines all of the steps and considerations for performing *Legionella* testing in-house. If your laboratory does not perform any *Legionella* testing in-house, skip this section and proceed to section 4 (Referral Laboratory Plan).
- **Referral Laboratory Plan.** Outlines all of the steps and considerations for formalizing a relationship with a referral laboratory. If your laboratory anticipates performing all steps of a *Legionella* investigation, you may choose to skip this section. However, it may be useful to consider scenarios in which support may be required or needed and establish communication with laboratories that might provide such assistance.

The completed plan should be reviewed and updated annually or with any staff changes to ensure accuracy.

Response Plan Checklist

The checklist, Table A (below), reflects the steps that are *required* in a complete investigation, though additional steps may be performed. Download Appendix A to customize tables as needed.

- Check "In-house" for any steps that your lab currently performs.
- Check "Referral" for any steps that you would need to refer to another laboratory.

Specific *Legionella* tests performed by your laboratory are recorded in Table D.

Table A: Checklist

Laboratory Response Step	In-house	Referral	Notes
Legionella Isolation			
Clinical Specimens	☐	☐	
Environmental Samples	☐	☐	
Isolate Characterization (e.g., qPCR/MALDI-TOF/Antibody-based)			
Species	☐	☐	
L. pneumophila Serogroup	☐	☐	
Molecular Typing (e.g., PFGE/SBT/WGS)			
Legionella Isolate Comparison	☐	☐	

Response Team Plan

Identify and record contact information for primary responders within your public health department who would be involved in a Legionnaires' disease investigation in Table B (below). This team should include representatives from your laboratory and the state or local public health department's epidemiology, environmental health, and communications divisions with whom you will coordinate activities in the event of a response. Response Team information should be reviewed and updated annually or with any staff changes to ensure accuracy. Download Appendix A to customize tables as needed.

Table B: Response Team Contact Information

		Name	Email Address	Office Phone Number	Mobile Phone Number
Laboratory	Primary Contact				
	Secondary Contact				
Epidemiology	Primary Contact				
	Secondary Contact				
Environmental Health	Primary Contact				
	Secondary Contact				
Communications	Primary Contact				
	Secondary Contact				

Response Team Plan

Complete the plan in Table C (below) in coordination with Response Team representatives. This plan allows the team to establish a framework for key activities that will be coordinated across health department epidemiology, laboratory, environmental health, and communications staff in the event of a response. In addition, the completed LDLRP can be shared with the Response Team to inform expectations of laboratory requirements, processes, and turn-around times. The end of this section provides signature lines for documentation of Response Plan agreement. Download Appendix A to customize tables as needed.

Table C: Response Team Plan

Response Communications	
Who will contact the laboratory in the event of an investigation?	
Which laboratorian should be contacted first?	
Which laboratorian will report results?	
Who should receive laboratory reports?	
How will laboratory results be reported?	
Environmental Samples Handling	
What types of environmental samples (e.g., potable water, swabs, cooling tower water) might be collected in an investigation?	
Who will collect environmental samples? *Note: Include the lead person responsible, a back-up, and all of their contact information.*	
What protocols will be followed for environmental sample collection? *See the resources section of this toolkit for links to instructional videos and detailed information regarding environmental sample collection for* Legionella *investigations.*	

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Who will provide environmental sample collection materials (e.g., sterile watertight containers, swabs)? *See the resources section of this toolkit for links to instructional videos and detailed information regarding environmental sample collection for* Legionella *investigations.*	
What are acceptable methods of environmental sample delivery and storage? *See sample requirements in Table F.*	
Clinical Specimens Handling	
What sources (e.g., hospital laboratories) might send clinical specimens (e.g., sputum, lung tissue, bronchoalveolar lavage fluid)?	
Do sources know to collect respiratory specimens during a Legionnaires' disease response? **If not,** how will sources be made aware?	
Do you have specimen collection requirements and shipping instructions that can be shared with labs/providers that are shipping you samples? *See specimen requirements in Table F and example shipping requirements in the resources section of this toolkit.*	

Response Team Signatures

Use Table D (below) to document the LDLRP agreement among your health department's Legionnaires' disease Response Team members. Download Appendix A to customize tables as needed.

Table D: LDLRP Team Signatures

Title	Name	Signature	Date
Laboratory Primary Contact			
Epidemiology Primary Contact			
Environmental Health Primary Contact			
Communications Primary Contact			

In-house Testing Plan

The In-house Testing Plan outlines your laboratory's Legionnaires' disease response workflow. Establishing the processes and tests to be performed, who will be performing them, and when to expect results will increase your laboratory's efficiency and manage expectations for obtaining results during a response.

Use Table E (below) to designate test protocols that will be performed in-house, who is trained and responsible for performing these tests, what results will be generated, and the turn-around time for results. Non-culture methods of *Legionella* detection directly from specimens or samples have also been included below. However, this step is not required for a complete *Legionella* investigation. The In-house Testing Plan should be reviewed and updated annually or with any staff changes to ensure accuracy. Download Appendix A to customize tables as needed.

Table E: In-house Testing Responsibilities

In-house Process Step	**In-house Protocol Name(s)**	**Staff Member Responsible** (multiple names may be listed)	**Results Reported** (genus, spp, serogroup, etc.)	**Turn-around Time**
Legionella Detection (e.g., qPCR/DFA/UAT)				
Clinical Specimens				
Environmental Samples				
Legionella Isolation				
Clinical Specimens				
Environmental Samples				
Isolate Characterization (e.g., qPCR/MALDI-TOF/DFA/Latex agglutination)				
Species				
L. pneumophila Serogroup				
Molecular Typing (e.g., PFGE/SBT/WGS)				
Legionella Isolate Comparison				

Specimen and Sample Requirements and Storage Policies

Table F (below) provides a space to document the minimum requirements or acceptance criteria for:

- Clinical specimens or environmental samples that may be processed during a response
- Short- and long-term storage conditions
- Locations for each material
- How long each material will be retained by your laboratory

Examples are listed. Download Appendix A to customize tables as needed.

Note: Distribute information recorded in the table below related to acceptance criteria, pre-shipment storage instructions, and shipping requirements to entities that may send clinical specimens or environmental samples to your laboratory for processing. Having a pre-made document helps ensure the integrity of specimens and samples received and reduce shipping delays. An example shipping document is available in the resources section of this toolkit.

Table F: Specimen and Sample Requirements and Storage Polices

Accepted Specimens/ Samples	**Specimen/ Sample Minimum Requirements** (volume, other specifications, etc.)	**Storage Before Shipment** (temperature, duration, etc.)	**Shipping Conditions** (temperature, cold packs, dry ice, etc.)	**Length of Retention After Testing**
Clinical Specimens				
Urine				
Sputum				
Tissue				
Bronchoalveolar Lavage				
Other				
Environmental Samples				
Potable Water				
Cooling Tower Water				
Swabs				
Filters				
Other				
Legionella Isolates				

In-house Testing Plan

Table G (below) contains additional questions to consider regarding in-house testing. Space is provided for responses or notes related to each question. Download Appendix A to customize tables as needed.

Table G: In-house Testing Plan

Documentation	
Which documents will be used to record the chain of custody of clinical specimens or environmental samples?	
Which documents will be used to record results from each in-house laboratory process or test that will be performed on clinical specimens or environmental samples?	
Which documents will be used for reporting results from clinical specimens or environmental samples?	
How will you use your document management system to help prepare for information requests related to a potential legal investigation?	
Clinical Specimens	
Most legionellosis cases are identified by UAT, but respiratory specimens are important for linking clinical isolates to environmental sources. How will clinicians be asked to obtain respiratory samples?	
Do you have a document with shipping instructions for labs/providers?	
Have you successfully completed proficiency testing or an alternative assessment for each of your tests?	
Environmental Samples	
Do you plan to screen environmental samples for the presence of *Legionella* DNA with qPCR? **If yes,** does this qPCR include an inhibition control? *This control is required due to the composition of environmental water samples.* **If yes,** does your laboratory retain qPCR-negative environmental samples to attempt isolation if needed? **If yes,** have your tests been validated within your laboratory? *If qPCR is used with environmental samples, CDC recommends that it be implemented primarily to screen and prioritize processing of large numbers of samples for culture.*	
Have you successfully completed proficiency testing or an alternative assessment for each of your tests? *Information related to the Environmental* Legionella *Isolation Techniques Evaluation (ELITE) alternative assessment program can be found here: https://wwwn.cdc.gov/elite/public/elite*	

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Workforce Capacity	
What is the maximum test volume that may be processed by your laboratory using currently trained and competent staff?	
List additional staff that can be trained if a large scale outbreak occurs.	
List any laboratory activities or testing that may be affected by redirecting staff.	
Are staff available to work weekend or holiday hours in the event of a high-priority response? *If staff are not available to work extended hours during an investigation, the Response Team should be informed at the onset and expectations about the timeline for results adjusted accordingly.*	
Culture	
Will your laboratory make or order BCYE agar plates? *BCYE agar is a specialized media specific for* Legionella *growth. This media has an extensive ingredient list and can be challenging to make correctly. BCYE agar is available commercially, but can be expensive and is often back-ordered in the summer months, the most common time of year for Legionnaires' disease outbreaks. See the resources section of this toolkit for links to detailed protocols.*	
How many BCYE plates does your laboratory normally keep in supply? *While actual plate requirements will vary by investigation and laboratory protocols, a mid-size investigation with 25 environmental samples and 2 clinical specimens, for example, could require 250 or more BCYE agar plates total. See the Example Laboratory Response Scenario for more details.*	
What types of selective BCYE agar will you use? *CDC recommends the use of selective BCYE agar plates containing the following types of selection:* 1. *Polymyxin, vancomycin, and cyclohexamide* 2. *Polymyxin, vancomycin, cyclohexamide, and glycine* ***Note:*** *Other antibiotics may be substituted. See the resources section of this toolkit for links to detailed protocols.*	
***Legionella* Identification**	
How many colonies do you pick per plate from original specimen/sample plates to screen for cysteine auxotrophy? *This informs the number of downstream isolates to be processed and BCYE agar plates used. Weigh increased workload vs. sensitivity of detection.*	

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Storage	
Do you have overflow storage space at required temperatures to accommodate environmental samples and related culture plates that can be used during a response? **If yes,** what is the maximum capacity?	
Referral Laboratory	
How many environmental samples can your lab handle before surge assistance is required?	
What other factors will determine if you need support from a referral laboratory? *(i.e., type of isolates recovered, need for molecular typing)*	
Administration	
What are the funding mechanisms for a Legionnaires' disease response? *A Legionnaires' disease response often requires increased use of lab supplies, test reagents, and personnel overtime.*	
Post-response Activities	
Will clinical specimens be retained? **If yes,** for how long?	
Will environmental samples be retained? **If yes,** for how long?	
Will any *Legionella* isolates from clinical specimens or environmental samples be stored? **If yes,** for how long?	
If indicated, will there be remediation sampling and in-house laboratory testing?	

Related Documents

List any laboratory documents (policies, processes, technical procedures, or protocols) related to the In-house Testing Plan in Table H (below). Download Appendix A to customize tables as needed.

Table H: Related Documents

Document Title	Document Number/Location

In-house Testing Plan Notes

Record any additional information or notes related to your In-house Testing Plan in the space below. Download Appendix A to customize tables as needed.

Referral Laboratory Plan

Establishing a Referral Laboratory Plan will help eliminate last-minute requests for support or identification of a qualified referral laboratory. Taking time to identify and establish a relationship with a referral laboratory or laboratories before an investigation can help save time, money, and stress in the event of a Legionnaires' disease response.

There are several options available if you are unsure of which referral laboratory to use. **Local PHLs** should contact your state PHL regarding their *Legionella* testing capacity. **State PHLs** may contact the CDC *Legionella* laboratory (legionellalab@cdc.gov) for recommendations.

If you are referring environmental samples for processing, laboratories in your region enrolled in the alternative assessment Environmental *Legionella* Isolation Techniques Evaluation (ELITE) program can be found on the ELITE member page: wwwn.cdc.gov/elite/Public/MemberList.aspx

Establishing both a primary and a secondary referral laboratory can be helpful in the case that the primary laboratory is unable to provide the required assistance at the time of your response.

If your laboratory does not foresee a possible scenario that would require use of a referral laboratory, you may delete this section of your LDLRP.

Note: CDC typically only processes environmental samples when invited by the state public health department via an Epi-Aid request. See the resources section of this toolkit for more information.

The Referral Laboratory Plan should be reviewed and updated annually or with any staff changes to ensure accuracy.

Referral Laboratory Contact Information

List the contact information for the referral laboratories you have established in Table I (below). Download Appendix A to customize tables as needed.

Table I: Referral Laboratory Contact Information

	Primary Referral Laboratory	Secondary Referral Laboratory
Referral Laboratory Name		
Tests Requested		
Primary Point of Contact		
Name		
Phone Number		
Email		
Secondary Point of Contact		
Name		
Phone Number		
Email		
Who is responsible for contacting the referral laboratory?		
At what point in response should the referral laboratory be contacted?		
Additional Notes		

Referral Laboratory Plan Overview

Document expectations of the referral laboratory in advance of a response in Table J (below). Record each type of specimen or sample, requested tests, anticipated cost, resulting information, and expected turn-around time. In the case of more than one referral laboratory, adding a "Referral Laboratory Name" column may be helpful. Download Appendix A to customize tables as needed.

Table J: Referral Laboratory Plan

Referral Laboratory Process Step	**Materials to Be Shipped** (specimen/ sample/ isolate)	**Tests Requested**	**Cost** (per specimen, sample, or isolate)	**Results Reported** (genus, spp, serogroup, molecular typing)	**Turn-Around Time**
Legionella Isolation					
Clinical Specimens					
Environmental Samples					
Isolate Characterization (e.g., qPCR/MALDI-TOF/Antibody-based)					
Species					
L. pneumophila Serogroup					
Molecular Typing (e.g., PFGE/SBT/WGS)					
Legionella Isolates Comparison					

Referral Laboratory Storage and Shipping Requirements

Complete Table K (below) by contacting the referral laboratory to determine specific requirements for storing and shipping specimens or samples. This information is essential for ensuring materials being shipped will meet the referral laboratory's acceptance criteria and that the integrity of the materials remain uncompromised. Example specimen and sample types are listed. Download Appendix A to customize tables as needed.

Table K: Referral Laboratory Storage and Shipping Requirements

Referred Specimens/ Samples	**Referral Lab Minimum Requirements** (volume, other specifications)	**Referral Lab Storage Requirements Before Shipment** (temperature, duration)	**Referral Lab Shipping Specifications** (temperature, packaging, delivery service, acceptable dates)
Clinical Specimens			
Sputum			
Respiratory Tissue			
Bronchoalveolar Lavage (BAL)			
Other			
Environmental Samples			
Potable Water			
Cooling Tower Water			
Swabs			
Filters			
Other			
Legionella Isolates			

Referral Laboratory Plan Notes

Record any additional information or notes related to your Referral Laboratory Plan in the space below. Download Appendix A to customize tables as needed.

Example LDLRP and Laboratory Response Scenario

Below is an example LDLRP and laboratory response scenario involving PHL X, a fictional local PHL. The response scenario is illustrated by two accompanying graphics:

- Example Response Workflow
- Example Response Calendar

The example materials included in this section demonstrate the development and implementation of an LDLRP. The example scenario, workflow, and calendar illustrate the scope, time, and processes involved in a typical Legionnaires' disease response. These materials are based on a hypothetical scenario and not intended to be recommendations for workflows or turn-around times. Every PHL's plan will be different and should reflect current capacity and protocols.

Example LDLRP

Checklist

PHL X decided to increase their Legionnaires' disease response preparedness by creating an LDLRP. They began by completing the LDLRP checklist to determine which processes of *Legionella* isolation, characterization, and typing they could perform in-house and which would need to be referred to an outside laboratory.

They determined that isolation and some isolate characterization would be performed in-house. PHL X routinely stocks BCYE agar plates, has protocols for *Legionella* isolation from both clinical specimens and environmental samples, and has a validated qPCR test that can detect DNA from *Legionella* genus, *L. pneumophila,* and *L. pneumophilia* serogroup 1 (Lp1).

Testing for any of the other 60+ known non-*L. pneumophila* species (spp) or *L. pneumophila* 2-14 serogroups (sg) would need to be referred. The final step of a Legionnaires' disease response—molecular typing of *Legionella* isolates—would also need to be referred to an outside laboratory.

PHL X Checklist

Laboratory Response Step	In-house	Referral	Notes
Legionella Isolation			
Clinical Specimens	☑	☐	
Environmental Samples	☑	☐	
Isolate Characterization			
Species	☑	☑	*PCR can detect* L. pneumophila, *other* Legionella *spp. would need to be referred*
L. pneumophila Serogroup	☑	☑	*Lp1 only, other serogroups would need to be referred*
Molecular Typing			
Legionella Isolate Comparison	☐	☑	

Identifying the Response Team

Next, PHL X identified the laboratorians, epidemiologists, environmental health scientists, and communications experts within their public health department who would be involved in a Legionnaires' disease response. They worked with these stakeholders to complete the LDLRP so that those involved in a Legionnaires' disease cluster or outbreak response would begin an investigation with a shared understanding of responsibilities, protocols, and results timelines.

In-house Testing Plan

PHL X then completed the In-house Testing Plan by identifying the specific protocols to be used in *Legionella* isolation and characterization, which laboratorians would be responsible for performing each test, and the expected timeframe for results. Additional information related to laboratory workflow, workforce, specimen and sample collection, shipping, and storage were recorded in the In-house Testing Plan Considerations section of the complete LDLRP (not shown).

PHL X In-house Testing Overview

In-house Process Step	In-house Protocol Name(s)	Staff Member Responsible (multiple names may be listed)	Results Reported (genus, spp, serogroup, etc.)	Turn-around Time
Legionella Isolation				
Clinical Specimens	*Specimens culture*	*Ellen, Sarah*	*genus*	*3-14 days*
	Cysteine auxotrophy screen			*1-2 days*
Environmental Samples	*Sample culture*	*Ellen, Claressa, Jeff*	*genus*	*3-7 days*
	Cysteine auxotrophy screen			*1-2 days*
Isolate Characterization				
Species and *L. pneumphilia* Serogroup Determination	*DNA extraction*	*Natalia, Brian*	*genus, Lp, Lp1*	*1-2 days*
	Isolate qPCR assay			

Referral Laboratory Plan

To complete the referral laboratory section of the plan, PHL X contacted the state PHL to confirm they would be able to perform isolate characterization for non-*L. pneumophila* serogroup 1 isolates and molecular typing in the event of a Legionnaires' disease cluster or outbreak response. Additional information, including contact information, types of samples, shipping protocols, and financial arrangements, were agreed upon and recorded within the Referral Laboratory Plan section of the complete LDLRP (not shown).

PHL X Referral Laboratory Overview

Referral Laboratory Process Step	**Materials to Be Shipped?** (specimens/ samples/ isolates)	**Tests Requested**	**Cost** (per specimen, sample, or isolate)	**Results** (genus, spp, sg, molecular typing)	**Turn-around Time**
Isolate Characterization	*Legionella isolates (BCYE slants)*	*Direct Fluorescent Antibody (DFA)*	*State-funded*	*Non-pneumophila spp and L. pneumophila serogroup 2-14 identification*	*1 day to ship, 2 days for results*
Isolate Molecular Typing	*Legionella isolates (BCYE slants)*	*Whole genome sequencing*	*State-funded*	*Genomic comparisons*	*1 day to ship, 5-8 days for results*

Example Legionnaires' Disease Laboratory Response Scenario

After noting a significant increase in reports of Legionnaires' disease cases confirmed by UAT, epidemiologists from the local health department launched an initial investigation. Patient interviews revealed two common links among the majority of cases: Healthcare Facility Q (HCF Q) and Hotel Z, where HCF Q patients often stay.

An epidemiologist from the Legionnaires' disease Response Team referred to the LDLRP and notified PHL X of the scope of the outbreak so far and that a full investigation was about to begin. The epidemiology and laboratory Response Team members discussed the clinical specimens and environmental samples expected to be sent to the laboratory for *Legionella* testing. While clinical laboratories arranged the collection and transport of respiratory specimens, members of the Legionnaires' disease Response Team coordinated the collection of water samples, and the PHL X laboratorians referred to the LDLRP to prepare for the response.

On the first day of the laboratory response (see Example Response Workflow and Example Response Calendar), PHL X received, processed, and plated one clinical specimen (specimen A) for culture. PHL X has a standard operating procedure of monitoring culture growth from clinical specimens for 14 days. On day 5, a second clinical specimen (specimen B) arrived and was processed and plated for culture. Eight days after culturing specimen A, suspect *Legionella* colonies were detected and screened for cysteine auxotrophy by streaking on both BCYE and blood agar. A few days later, selected colonies from clinical specimen B were screened for cysteine auxotrophy.

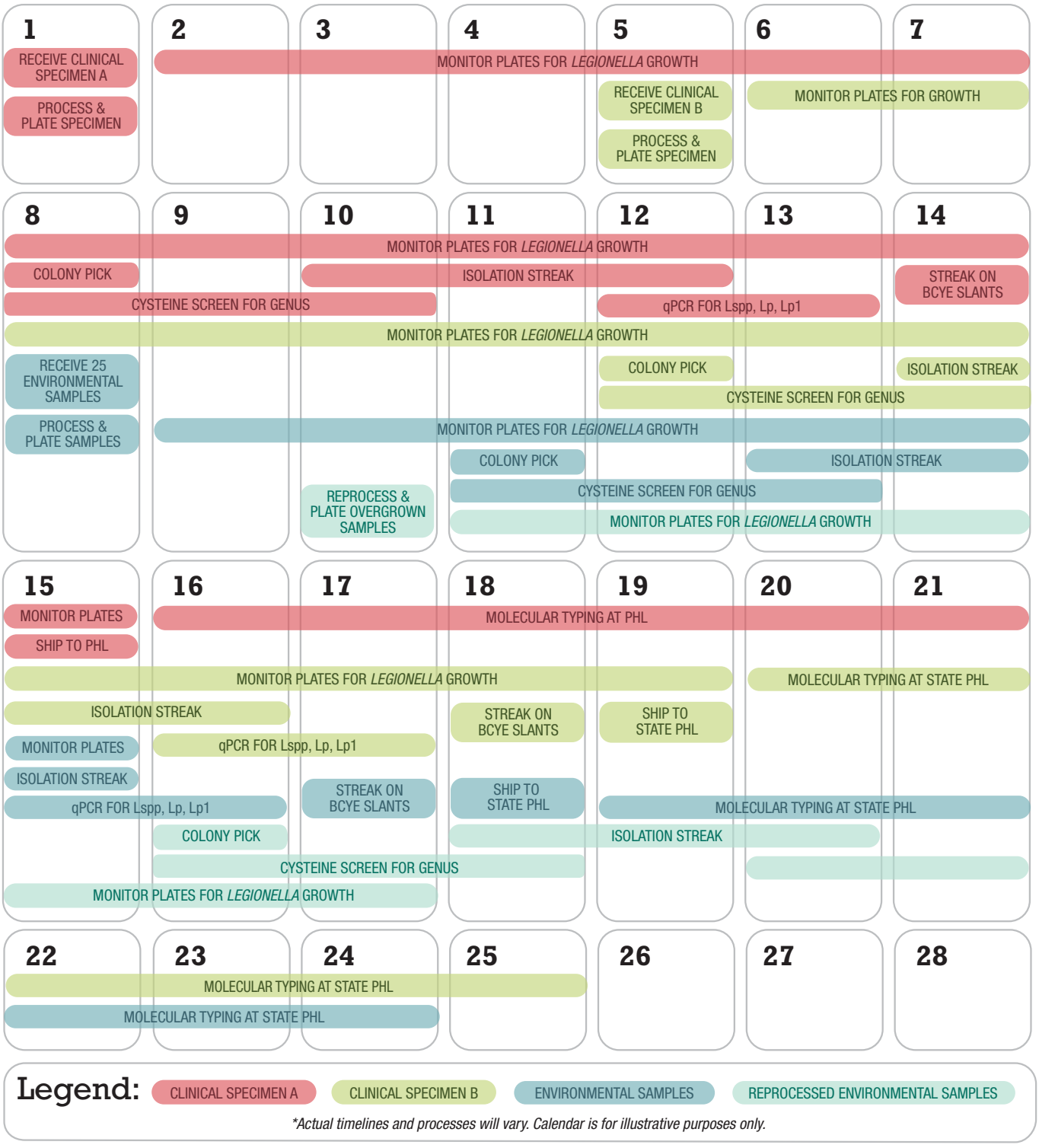
The same day that clinical specimen B arrived, PHL X received 25 one-liter potable water samples collected in total from HCF Q and Hotel Z. Each sample was filter-concentrated and plated on selective and non-selective BCYE agar (125 plates total). PHL X has a standard operating procedure of monitoring growth from environmental samples for 7 days. Two days after initial plating, plates from some of the environmental samples were overgrown with heterotrophs and fungi. These samples were reprocessed with additional acid treatment. The next day, suspect *Legionella* colonies selected from the environmental sample plates that did not overgrow were screened for cysteine auxotrophy on both BCYE and blood agar. After several days, suspect *Legionella* colonies were also identified from the reprocessed plates and screened for cysteine auxotrophy.

All confirmed selected colonies were streaked for isolation once cysteine auxotrophy was confirmed. Then gDNA was extracted from each isolate and qPCR performed for the detection of *Legionella* genus, *L. pneumophila*, and *L. pneumophila* serogroup 1 (Lp1) genetic markers. All of the isolates cultured from clinical specimens A and B were identified by qPCR as Lp1. Upon identification, BCYE agar slants were prepared for each clinical specimen isolate and shipped to the state PHL for whole genome analysis. Assay results from the environmental sample isolates indicated that, like the clinical samples, both HCF Q and Hotel Z samples also contained Lp1. In addition, non-Lp1 isolates were identified in Hotel Z samples and, later, the reprocessed samples. Since only Lp1 was isolated from the clinical specimens, just the Lp1 environmental sample isolates from both facilities were prepared on agar slants and shipped to the state PHL.

As soon as *Legionella* was identified in samples from both HCF Q and Hotel Z, PHL X notified the epidemiology and environmental health members of the Legionnaires' disease Response Team. The state PHL performed whole genome analysis and comparison among the clinical specimen and water sample isolates. Results of these analyses indicated that the genomes from the clinical isolates were a close genetic match to each other and to the Hotel Z isolates. The state reported these results to the local PHL who in turn reported these results to the Response Team.

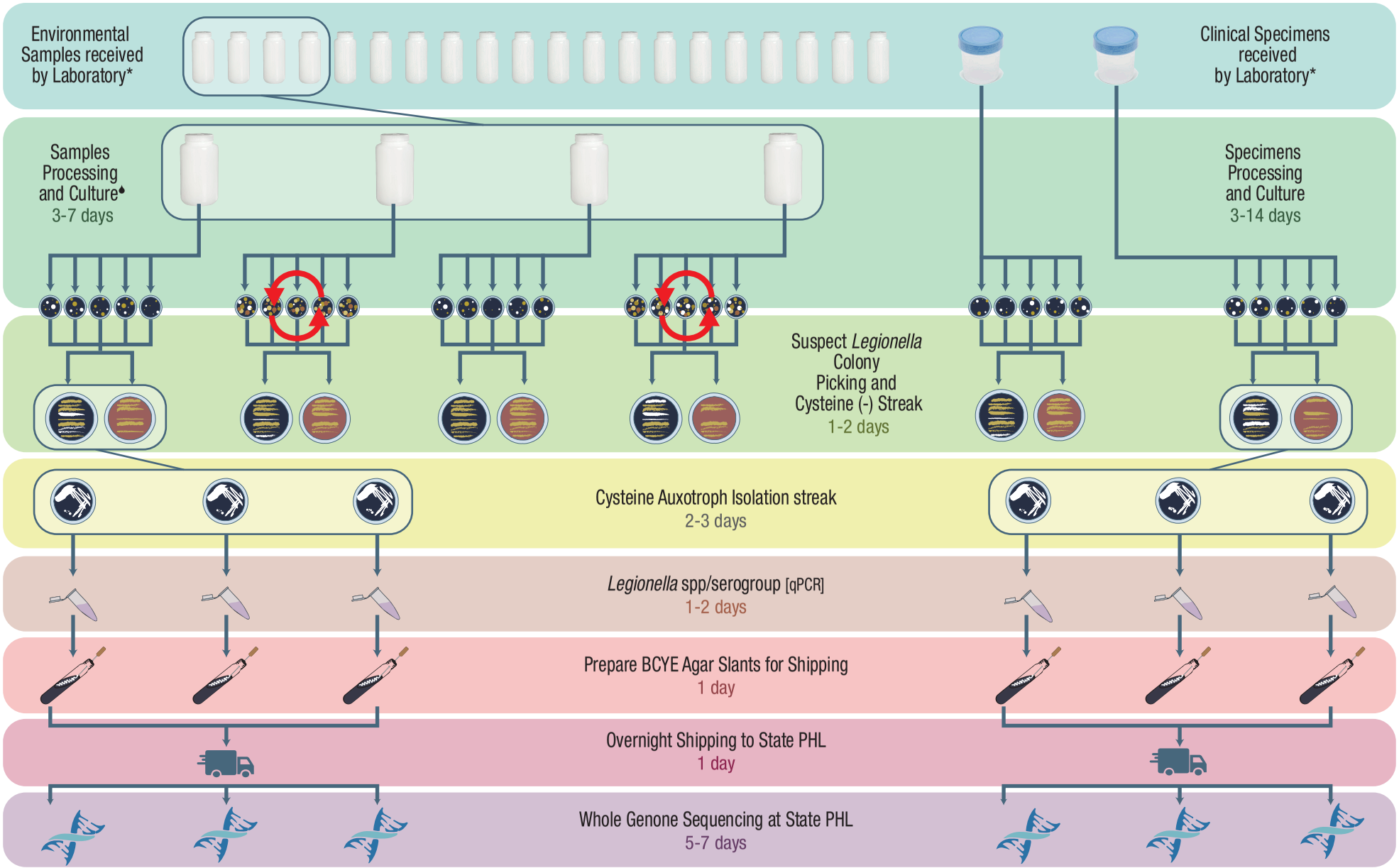
Upon completion of Legionnaires' disease response activities, PHL X placed *Legionella* isolates and remaining clinical specimens in long-term storage. Remaining environmental samples were discarded. Documents and reports related to the response were compiled and archived.

Example Response Timeline*



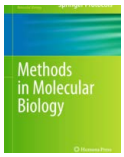
*Actual timelines and processes will vary. Calendar is for illustrative purposes only.

Example Response Workflow

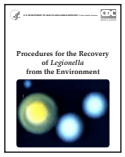


Resources

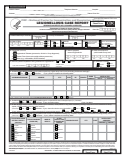
Environmental *Legionella* Isolation Techniques Evaluation (ELITE) Member Laboratories
https://wwwn.cdc.gov/elite/Public/MemberList.aspx



Book chapter
N.A. Kozak, C.E. Lucas, J.M. Winchell. Identification of *Legionella* in the environment: *Legionella* Methods and Protocols. *Methods in Molecular Biology.* 954 (2013), pp 3-25.



Processing Environmental Samples
https://www.cdc.gov/investigate-legionella/php/publications/lab-procedures-manual.html



Legionnaires' Disease Epidemiological Investigation Tools
https://www.cdc.gov/investigate-legionella/php/public-health-strategy/investigations.html

***Legionella* Environmental Investigation Tools**
https://www.cdc.gov/investigate-legionella/php/public-health-strategy/environmental-assessments.html

CDC Toolkit
Developing a Water Management Program to Reduce *Legionella* Growth and Spread in Buildings
https://www.cdc.gov/control-legionella/php/toolkit/wmp-toolkit.html

CDC Contact Information

- Additional *Legionella* laboratory queries may be directed to legionellalab@cdc.gov
- Legionnaires' disease epidemiologic investigation consultation requests should be directed to travellegionella@cdc.gov

Appendix (fillable Word and Excel files)

- Appendix A: LDLRP Customizable Tables
- Appendices B, C, and D:
  - Environmental Sample Culture
  - Clinical Specimen Culture Worksheet
  - Colony Testing Worksheet

Appendix A: Customizable Tables

Response Plan Checklist

The checklist, Table A (below), reflects the steps that are required in a complete investigation, though additional steps may be performed.

- Check "In-house" for any steps that your lab currently performs.
- Check "Referral" for any steps that you would need to refer to another laboratory.

Specific *Legionella* tests performed by your laboratory are recorded in Table D.

Table A: Checklist

Laboratory Response Step	In-house	Referral	Notes
Legionella Isolation			
Clinical Specimens	☐	☐	
Environmental Samples	☐	☐	
Isolate Characterization (e.g., qPCR/MALDI-TOF/Antibody-based)			
Species	☐	☐	
L. pneumophila Serogroup	☐	☐	
Molecular Typing (e.g., PFGE/SBT/WGS)			
Legionella Isolate Comparison	☐	☐	

Response Team Plan

Identify and record contact information for primary responders within your public health department who would be involved in a Legionnaires' disease investigation in Table B (below).This team should include representatives from your laboratory and the state or local public health department's epidemiology, environmental health, and communications divisions with whom you will coordinate activities in the event of a response. Response Team information should be reviewed and updated annually or with any staff changes to ensure accuracy.

Table B: Response Team Contact Information

		Name	Email Address	Office Phone Number	Mobile Phone Number
Laboratory	Primary Contact				
	Secondary Contact				
Epidemiology	Primary Contact				
	Secondary Contact				
Environmental Health	Primary Contact				
	Secondary Contact				
Communications	Primary Contact				
	Secondary Contact				

Response Team Plan

Complete the plan in Table C (below) in coordination with Response Team representatives. This plan allows the team to establish a framework for key activities that will be coordinated across health department epidemiology, laboratory, environmental health, and communications staff in the event of a response. In addition, the completed LDLRP can be shared with the Response Team to inform expectations of laboratory requirements, processes, and turn-around times. The end of this section provides signature lines for documentation of Response Plan agreement.

Table C: Response Team Plan

Response Communications	
Who will contact the laboratory in the event of an investigation?	
Which laboratorian should be contacted first?	
Which laboratorian will report results?	
Who should receive laboratory reports?	
How will laboratory results be reported?	
Environmental Samples Handling	
What types of environmental samples (e.g., potable water, swabs, cooling tower water) might be collected in an investigation?	
Who will collect environmental samples? *Note: Include the lead person responsible, a back-up, and all of their contact information.*	
What protocols will be followed for environmental sample collection? *See the resources section of this toolkit for links to instructional videos and detailed information regarding environmental sample collection for* Legionella *investigations.*	

Who will provide environmental sample collection materials (e.g., sterile watertight containers, swabs)? *See the resources section of this toolkit for links to instructional videos and detailed information regarding environmental sample collection for* Legionella *investigations.*	
What are acceptable methods of environmental sample delivery and storage? *See sample requirements in Table F.*	
Clinical Specimens Handling	
What sources (e.g., hospital laboratories) might send clinical specimens (e.g., sputum, lung tissue, bronchoalveolar lavage fluid)?	
Do sources know to collect respiratory specimens during a Legionnaires' disease response? **If not,** how will sources be made aware?	
Do you have specimen collection requirements and shipping instructions that can be shared with labs/providers that are shipping you samples? *See specimen requirements in Table F and example shipping requirements in the resources section of this toolkit.*	

Response Team Signatures

Use Table D (below) to document the LDLRP agreement among your health department's Legionnaires' disease Response Team members.

Table D: LDLRP Team Signatures

Title	Name	Signature	Date
Laboratory Primary Contact			
Epidemiology Primary Contact			
Environmental Health Primary Contact			
Communications Primary Contact			

In-house Testing Plan

The In-house Testing Plan outlines your laboratory's Legionnaires' disease response workflow. Establishing the processes and tests to be performed, who will be performing them, and when to expect results will increase your laboratory's efficiency and manage expectations for obtaining results during a response.

Use Table E (below) to designate test protocols that will be performed in-house, who is trained and responsible for performing these tests, what results will be generated, and the turn-around time for results. Non-culture methods of *Legionella* detection directly from specimens or samples have also been included below. However, this step is not required for a complete *Legionella* investigation. The In-house Testing Plan should be reviewed and updated annually or with any staff changes to ensure accuracy.

Table E: In-house Testing Responsibilities

In-house Process Step	**In-house Protocol Name(s)**	**Staff Member Responsible** (multiple names may be listed)	**Results Reported** (genus, spp, serogroup, etc.)	**Turn-around Time**
Legionella Detection (e.g., qPCR/DFA/UAT)				
Clinical Specimens				
Environmental Samples				
Legionella Isolation				
Clinical Specimens				
Environmental Samples				
Isolate Characterization (e.g., qPCR/MALDI-TOF/DFA/latex agglutination)				
Species				
L. pneumophila Serogroup				
Molecular Typing (e.g., PFGE/SBT/WGS)				
Legionella Isolate Comparison				

Specimen and Sample Requirements and Storage Policies

Table F (below) provides a space to document the minimum requirements or acceptance criteria for:

- Clinical specimens or environmental samples that may be processed during a response
- Short- and long-term storage conditions
- Locations for each material
- How long each material will be retained by your laboratory

Examples are listed.

Note: Distribute information recorded in the table below related to acceptance criteria, pre-shipment storage instructions, and shipping requirements to entities that may send clinical specimens or environmental samples to your laboratory for processing. Having a pre-made document helps ensure the integrity of specimens and samples received and reduce shipping delays. An example shipping document is available in the resources section of this toolkit.

Table F: Specimen and Sample Requirements and Storage Policies

Accepted Specimens/ Samples	**Specimen/ Sample Minimum Requirements** (volume, other specifications, etc.)	**Storage Before Shipment** (temperature, duration, etc.)	**Shipping Conditions** (temperature, cold packs, dry ice, etc.)	**Length of Retention After Testing**
Clinical Specimens				
Urine				
Sputum				
Tissue				
Bronchoalveolar Lavage				
Other				
Environmental Samples				
Potable Water				
Cooling Tower Water				
Swabs				
Filters				
Others				
Legionella Isolates				

In-house Testing Plan

Table G (below) contains additional questions to consider regarding in-house testing. Space is provided for responses or notes related to each question.

Table G: In-house Testing Plan

Documentation	
Which documents will be used to record the chain of custody of clinical specimens or environmental samples?	
Which documents will be used to record results from each in-house laboratory process or test that will be performed on clinical specimens or environmental samples?	
Which documents will be used for reporting results from clinical specimens or environmental samples?	
How will you use your document management system to help prepare for information requests related to a potential legal investigation?	
Clinical Specimens	
Most legionellosis cases are identified by UAT, but respiratory specimens are important for linking clinical isolates to environmental sources. How will clinicians be asked to obtain respiratory samples?	
Do you have a document with shipping instructions for labs/providers?	
Have you successfully completed proficiency testing or an alternative assessment for each of your tests?	
Environmental Samples	
Do you plan to screen environmental samples for the presence of *Legionella* DNA with qPCR? **If yes,** does this qPCR include an inhibition control? *This control is required due to the composition of environmental water samples.* **If yes,** does your laboratory retain qPCR-negative environmental samples to attempt isolation if needed? **If yes,** have your tests been validated within your laboratory? *If qPCR is used with environmental samples, CDC recommends that it be implemented primarily to screen and prioritize processing of large numbers of samples for culture.*	
Have you successfully completed proficiency testing or an alternative assessment for each of your tests? *Information related to the Environmental* Legionella *Isolation Techniques Evaluation (ELITE) alternative assessment program can be found here: http://www.cdc.gov/legionella/elite.html*	

Workforce Capacity	
What is the maximum test volume that may be processed by your laboratory using currently trained and competent staff?	
List additional staff that can be trained if a large scale outbreak occurs.	
List any laboratory activities or testing that may be affected by redirecting staff.	
Are staff available to work weekend or holiday hours in the event of a high-priority response? *If staff are not available to work extended hours during an investigation, the Response Team should be informed at the onset and expectations about the timeline for results adjusted accordingly.*	
Culture	
Will your laboratory make or order BCYE agar plates? *BCYE agar is a specialized media specific for* Legionella *growth. This media has an extensive ingredient list and can be challenging to make correctly. BCYE agar is available commercially, but can be expensive and is often back-ordered in the summer months, the most common time of year for Legionnaires' disease outbreaks. See the resources section of this toolkit for links to detailed protocols.*	
How many BCYE plates does your laboratory normally keep in supply? *While actual plate requirements will vary by investigation and laboratory protocols, a mid-size investigation with 25 environmental samples and 2 clinical specimens, for example, could require 250 or more BCYE agar plates total. See the Example Laboratory Response Scenario for more details.*	
What types of selective BCYE agar will you use? *CDC recommends the use of selective BCYE agar plates containing the following types of selection:* 1. *Polymyxin, vancomycin, and cyclohexamide* 2. *Polymyxin, vancomycin, cyclohexamide, and glycine* ***Note:** Other antibiotics may be substituted. See the resources section of this toolkit for links to detailed protocols.*	
***Legionella* Identification**	
How many colonies do you pick per plate from original specimen/sample plates to screen for cysteine auxotrophy? *This informs the number of downstream isolates to be processed and BCYE agar plates used. Weigh increased workload vs. sensitivity of detection.*	

Storage	
Do you have overflow storage space at required temperatures to accommodate environmental samples and related culture plates that can be used during a response? **If yes,** what is the maximum capacity?	
Referral Laboratory	
How many environmental samples can your lab handle before surge assistance is required?	
What other factors will determine if you need support from a referral laboratory? *(i.e., type of isolates recovered, need for molecular typing)*	
Administration	
What are the funding mechanisms for a Legionnaires' disease response? *A Legionnaires' disease response often requires increased use of lab supplies, test reagents, and personnel overtime.*	
Post-response Activities	
Will clinical specimens be retained? **If yes,** for how long?	
Will environmental samples be retained? **If yes,** for how long?	
Will any *Legionella* isolates from clinical specimens or environmental samples be stored? **If yes,** for how long?	
If indicated, will there be remediation sampling and in-house laboratory testing?	

Related Documents

List any laboratory documents (policies, processes, technical procedures, or protocols) related to the In-house Testing Plan in Table H (below).

Table H: Related Documents

Document Title	Document Number/Location

In-house Testing Plan Notes

Record any additional information or notes related to your In-house Testing Plan in the space below.

Referral Laboratory Contact Information

List the contact information for the referral laboratories you have established in Table I (below).

Table I: Referral Laboratory Contact Information

	Primary Referral Laboratory	Secondary Referral Laboratory
Referral Laboratory Name		
Tests Requested		
Primary Point of Contact		
Name		
Phone Number		
Email		
Secondary Point of Contact		
Name		
Phone Number		
Email		
Who is responsible for contacting the referral laboratory?		
At what point in response should the referral laboratory be contacted?		
Additional Notes		

Referral Laboratory Plan Overview

Document expectations of the referral laboratory in advance of a response in Table J (below).Record each type of specimen or sample, requested tests, anticipated cost, resulting information, and expected turn-around time. In the case of more than one referral laboratory, adding a "Referral Laboratory Name" column may be helpful.

Table J: Referral Laboratory Plan

Referral Laboratory Process Step	**Materials to Be Shipped** (specimen/ sample/ isolate)	**Tests Requested**	**Cost** (per specimen, sample, or isolate)	**Results Reported** (genus, spp, serogroup, molecular typing)	**Turn-Around Time**
Legionella Isolation					
Clinical Specimens					
Environmental Samples					
Isolate Characterization (e.g., qPCR/MALDI-TOF/Antibody-based)					
Species					
L. pneumophila Serogroup					
Molecular Typing (e.g., PFGE/SBT/WGS)					
Legionella Isolate Comparison					

Referral Laboratory Storage and Shipping Requirements

Complete Table K (below) by contacting the referral laboratory to determine specific requirements for storing and shipping specimens or samples. This information is essential for ensuring materials being shipped will meet the referral laboratory's acceptance criteria and that the integrity of the materials remain uncompromised. Example specimen and sample types are listed.

Table K: Referral Laboratory Storage and Shipping Requirements

Referred Specimens/Samples	**Referral Lab Minimum Requirements** (volume, other specifications)	**Referral Lab Requirements Before Shipment** (temperature, duration)	**Referral Lab Shipping Specifications** (temperature, packaging, delivery service, acceptable dates)
Clinical Specimens			
Sputum			
Respiratory Tissue			
Bronchoalveolar Lavage (BAL)			
Other			
Environmental Samples			
Potable Water			
Cooling Tower Water			
Swabs			
Filters			
Other			
Legionella Isolates			

Referral Laboratory Plan Notes

Record any additional information or notes related to your Referral Laboratory Plan in the space below.

Legionella Environmental Sample Culture Worksheet

Document #xx-xxx

ABC
Laboratorian Initials

Investigation:	PHLX-2020-1
Date Sample Collected:	11/12/20
Date Sample Processed:	11/13/20

Microbial Growth Key

NG:	No growth	**MG:**	Moderate growth
LG:	Light growth	**HG:**	Heavy growth

Sample # ZYX-01 | First Observation | Date: 11/16/20 | Last Observation | Date: 11/20/20 | ***Reprocess Sample?

Sample Type: Potable (hot, 1 liter)

Sample Processing:
Direct _____
Acid _____
Concentrate ___X___

	Growth	Colony pick #s		Growth	Colony pick #s
BCYE	HG	N/A	BCYE	HG	overgrown
*BCYE-S	MG	1,2	BCYE-S	HG	N/A
*BCYE-S	MG	N/A	BCYE-S	HG	N/A
**BCYE-SG	LG	3	BCYE-SG	MG	6,7
**BCYE-SG	LG	4, 5	BCYE-SG	MG	8

no

Sample # | First Observation | Date: | Last Observation | Date: | Reprocess Sample?

Sample Type:

Sample Processing:
Direct _____
Acid _____
Concentrate _____

	Growth	Colony pick #s		Growth	Colony pick #s
BCYE			BCYE		
BCYE-S			BCYE-S		
BCYE-S			BCYE-S		
BCYE-SG			BCYE-SG		
BCYE-SG			BCYE-SG		

Sample # | First Observation | Date: | Last Plate Read | Date: | Reprocess Sample?

Sample Type:

Sample Processing:
Direct _____
Acid _____
Concentrate _____

	Growth	Colony pick #s		Growth	Colony pick #s
BCYE			BCYE		
BCYE-S			BCYE-S		
BCYE-S			BCYE-S		
BCYE-SG			BCYE-SG		
BCYE-SG			BCYE-SG		

Sample # | First Observation | Date: | Last Observation | Date: | Reprocess Sample?

Sample Type:

Sample Processing:
Direct _____
Acid _____
Concentrate _____

	Growth	Colony pick #s		Growth	Colony pick #s
BCYE			BCYE		
BCYE-S			BCYE-S		
BCYE-S			BCYE-S		
BCYE-SG			BCYE-SG		
BCYE-SG			BCYE-SG		

*BCYE-S: BCYE + selection

**BCYE-SG: BCYE-S + glycine

*This text is for example purposes only.

***If plates are highly contaminated and samples require reprocessing, record information here

Legionella Environmental Sample Culture Worksheet

Worksheet Instructions:

1. Record investigation, date, sample, and laboratorian information.
2. Refer to the Microbial Growth Key to record the first reading of culture plates.
    a. First plate reads are typically performed 3-4 days post-inoculation.
3. Record a number for each suspect *Legionella* colony picked for testing.
4. Record if any samples require reprocessing due to overgrown cultures.
5. Record final plate growth and colony picks.
    a. Final plate reads are typically performed 7 days post-inoculation.

Legionella Clinical Specimen Culture Worksheet

Document #xx-xxx

ABC

Laboratorian Initials

Specimen #: XYZ-000*	Investigation: PHLX-2020-1
Specimen Type: Sputum	
Date Specimen Collected: 11/11/20	
Date Specimen Processed and Plated: 11/12/20	

Microbial Growth Key

NG:	No growth	**MG:**	Moderate growth
LG:	Light growth	**HG:**	Heavy growth

Day Post-inoculation (PI)	Direct Inoculation						1:10 Specimen Dilution						1:100 Specimen Dilution					
	BCYE		**BCYE-S**		**BCYE-SG**		**BCYE**		**BCYE-S**		**BCYE-SG**		**BCYE**		**BCYE-S**		**BCYE-SG**	
	Growth	Colony #s	Growth	Colony #s	Growth	Colony #s	Growth	Colony #s	Growth	Colony #s	Growth	Colony #s	Growth	Colony #s	Growth	Colony #s	Growth	Colony #s
3	MG	1,2	LG	3	LG	4	LG	N/A	LG	4	NG	N/A	NG	N/A	NG	N/A	NG	N/A
4																		
5																		
6																		
7																		
8																		
9																		
10																		
11																		
12																		
13																		
14																		

*BCYE-S: BCYE + selection **BCYE-SG: BCYE-S + glycine

*This text is for example purposes only.

Worksheet Instructions:

1. Record investigation, specimen, date, and laboratorian information.
2. Beginning 3 days post-inoculation, refer to the Growth Key to record observed microbial growth on each culture plate.
3. Record a number for each suspect *Legionella* colony picked for testing.
4. Record plate growth and colony picks daily until 14 days post-inoculation.

Legionella Colony Testing Worksheet

Document #xx-xxx

Investigation:	PHLX-2020-1

Results Key

L spp:	*Legionella* species	Lp1:	*L. pneumophila* serogroup 1
Lp:	*L. pneumophila*	Lp2-14:	*L. pneumophila* serogroup 2-14

Colony ID #	**Agar/ BCYE** (L spp)	Result Date	Initials	**PCR**	Result Date	Initials	**Latex Agglutination**	Result Date	Initials	**DFA**	Result Date	Initials	**Final Result** (L spp, Lp, Lp1-14)	Result Date	Initials
XYZ-000-1	L spp	11/17	ABC	Lp	11/20	DEF	N/A	N/A	N/A	Lp6	11/21	ABC	Lp6	11/21	ABC

*This text is for example purposes only.

Worksheet instructions: Record colony numbers, results, dates, and laboratorian initials in the spaces provided.