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.