NJHealth
New Jersey Department of Health
Communicable Disease Service

Environmental *Legionella* Sampling and Testing Guidance

During an outbreak investigation, environmental sampling for *Legionella* culture testing is needed to identify sources of transmission and the extent of colonization. Sampling should only be performed after conducting a comprehensive environmental assessment of the building water systems to identify potentially hazardous conditions and developing an environmental sampling plan. It is important that the sampling event occur as soon as possible after the environmental assessment and should not be delayed pending implementation of other recommendations.

1. Developing a Sampling Plan

It is the environmental consultant's responsibility to develop the environmental sampling plan while adhering to the New Jersey Department of Health's (NJDOH) guidance. Sampling plans are based on the inventory of the building water systems, the findings of the environmental assessment, and the available epidemiological data. To ensure comprehensive representation of the entire building water system, plumbing riser diagrams should be utilized to determine proximal, mid, and distal locations from the heating source. Figure 1 presents an illustrative example of a plumbing riser diagram.

Table 1 outlines recommended sampling locations that should be considered based on the findings of the environmental assessment and the available epidemiological data. At minimum, NJDOH recommends including all centralized building water system points (e.g., incoming cold water, water heaters, expansion tanks, conditioner systems, hot water return lines), as well at aerosol generating devices (e.g., cooling towers, decorative fountains, hot tubs), and a representative sampling from approximately 10% of rooms/areas (e.g., resident/guest rooms, dining, laundry, restrooms, etc.). It is essential to include locations where the case-patient(s) may have been exposed, as well as areas identified during the environmental assessment that may have hazardous conditions that could promote *Legionella* growth.

2. Collecting Environmental Samples

Note: Initial sampling as part of an environmental assessment during an outbreak investigation is typically conducted by facility staff and/or a third-party consultant on behalf of the facility. However, if the Local Health Department would prefer to have the initial samples sent to the Legionella Reference Center (supported by APHL/CDC) for testing and has additional resources to support sample collection and shipping, please contact NJDOH (preventLD@doh.nj.gov) for further guidance and approval.

a. Bring enough sample bottles per the environmental sampling plan. A 1-liter (1000 mL) sample volume is recommended for investigative sampling, such as during an investigation into a case of Legionnaires' disease. The laboratory should be told the reason for testing.
    i. Note: 250 mL is the minimum recommended sample volume for **routine** environmental sampling of potable water for *Legionella* in the absence of cases. **This is not an acceptable sample volume for investigative sampling**.
b. Ensure that any 0.2-micron point-of-use filters are removed prior to sample collection. Re-install filters immediately after the sample is collected.
c. Each sample should be a first draw, hot water sample, unless otherwise specified.
d. If the sample bottles are not pre-treated with sodium thiosulfate, then 0.5 mL of 0.1N sodium thiosulfate must be added to each 1000 mL bottle to neutralize residual disinfectants like chlorine.
e. Biofilm swab collection is recommended for outlets in visible poor condition.
f. Label the bottle with a unique location identifier. Record the type and location of the sample on the Sample Data Sheet and chain of custody.
    i. For example, "guest room 140 bathroom sink hot"
g. Samples should be transported to the laboratory in insulated containers as soon as possible after the time of collection, preferably within 24 hours. If the time must exceed 48 hours, consult with the laboratory for instructions.

3. Measuring Water Quality Parameters

a. For each sampling location, measure pre-flush and post-flush water quality parameters (WQPs), including temperature, disinfectant residual, and pH.
    i. To collect post-flush WQPs, run the water until the temperature stabilizes (may be hot or cold water depending on the sample being collected). Collect 100-300 mL of water in a separate bottle designated only for measuring WQPs (the same bottle can be used for measuring WQPs at each sampling location). Record all measured data on the sample data sheet (see link below).
    ii. Record how long it takes for each sampling location to reach its maximum water temperature. Further investigate locations where it takes more than a minute for the water to reach its maximum temperature.
    iii. Please refer to NJDOH's Environmental Sampling Data Sheet as an example of information that should be collected.

4. Processing and Analyzing Environmental Samples

a. Samples must be analyzed at a CDC ELITE member laboratory that is accredited by a regional, national, or international accrediting body and can retain isolates of *Legionella*-positive samples.
b. The laboratory must process the entire volume for the type of sample collected and the test conducted. For instance, collecting and processing a full liter (1000 mL) for culture is recommended for potable water.
c. Each sample must be analyzed using traditional *Legionella* culture methods, including enumeration and species/serogroup identification.
d. The limit of detection (LOD) for *Legionella* culture testing for potable water must be less than or equal to 0.1 colony forming units per milliliter (CFU/mL) and for non-potable LOD must be less than or equal to 5 CFU/mL.

Health and Safety Considerations

Notify the facility in advance to turn off aerosol-generating devices (without draining or disinfecting) to reduce risk to the sampling team. Individuals at high risk for Legionnaires' disease should not accompany the team.

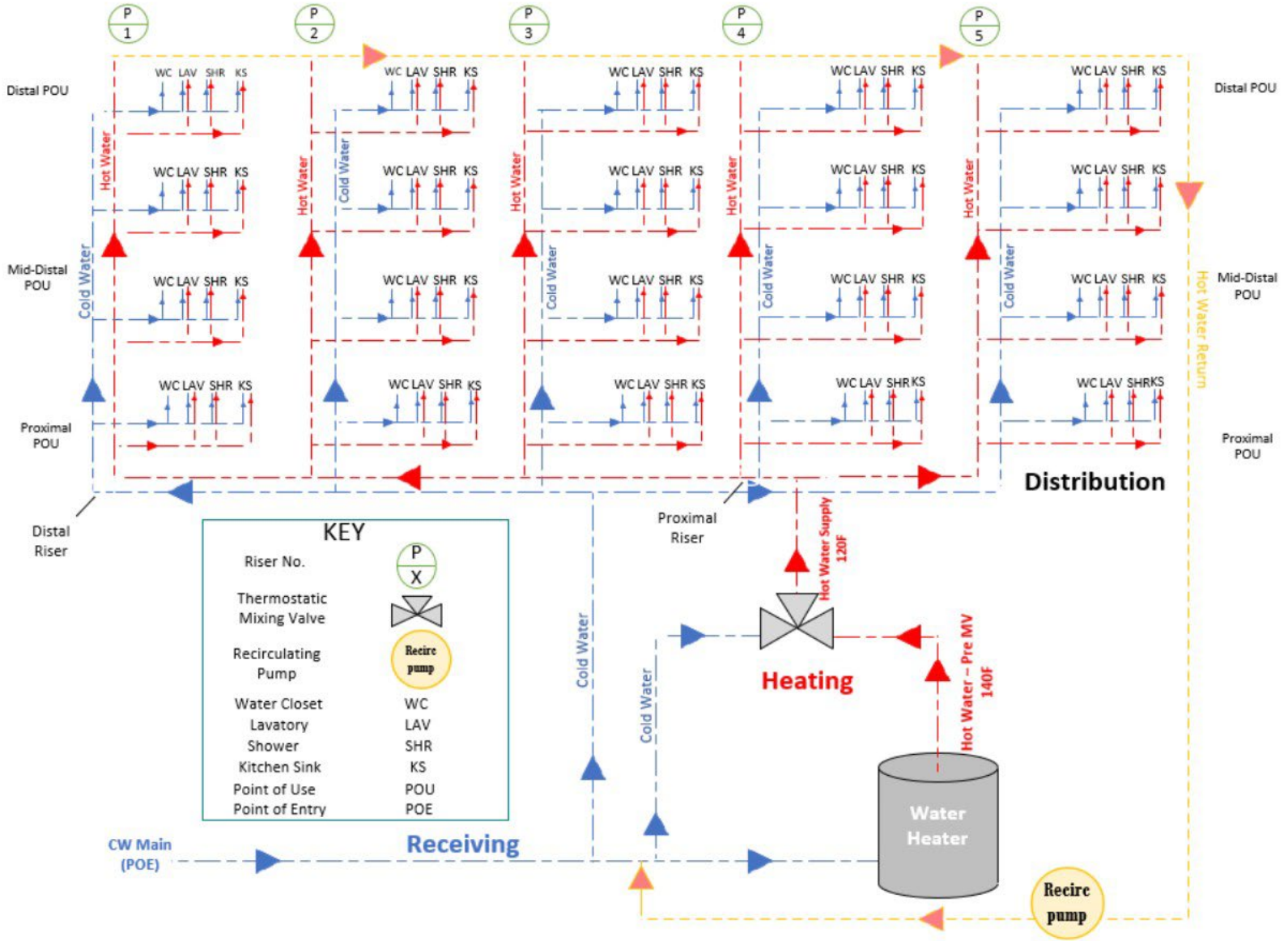
Optional Personal Protective Equipment (PPE):

- **Gloves:** Useful for handling heavily contaminated sites like whirlpool spa filters.
- **Respirators:** A half-face respirator with an N95 filter is recommended for:
    - Sampling cooling towers with active fans, or
    - Enclosed spaces with active aerosol-generating devices.

Ensure respirators are used per OSHA standards (29 CFR 1910.134), including fit testing, training, and medical clearance. For more on N95 respirators, visit the NIOSH website.

Other Hazards: Common hazards include slips, falls, cuts from corroded equipment, and electrical shocks. Wear non-slip shoes, goggles, nitrile gloves, and a hard hat may be appropriate when entering a cooling tower. Avoid loose clothing and be cautious of cleaning chemicals and stinging insects (e.g., wasps, bees) that may be in or near the cooling tower.

Figure 1. Example of Plumbing Riser Diagram



Utilizing Riser Diagrams to Develop a Sampling Plan

Riser diagrams visually show how cold water enters the building and is distributed throughout the building to point of use outlets. For buildings with a centralized hot water system, some of the cold water goes to a centralized water heater to be heated. The hot water is then distributed throughout the building. Buildings with multiple floors typically have vertical pipes (risers) that serve a “stack” of rooms. The hot water is recirculated throughout the system by a pump and returned to the water heater to be re-heated.

It is important to identify sample locations from risers that are representative of the building water system (including risers that are proximal, mid, and distal from the centralized water heater). Additionally, select locations on each individual vertical riser to ensure various levels/floors of the building are represented (e.g., rooms that are proximal, mid, and distal on a riser line).

I cTable 1. Potential Sampling Locations

Sample Location	Sample Type	Sampling Description
Incoming cold water	1L bulk water	A flushed water sample collected at a location closest to the water meter. Flushing duration is dependent on the length of the service line and water usage patterns.
All hot water and cold water storage tanks	1L bulk water	A sample from the drain valve at or near the bottom of each tank
All centralized water heaters	1L bulk water	A sample from the drain valve at or near the bottom of water heater
All expansion and pressure tanks for potable water	1L bulk water	Collect a first draw sample if tap or nearby sampling port is available
All hot water return lines	1L bulk water	Collect a first draw sample closest to recirculatory pump prior to the return water blending with cold, hot, or both cold and hot water
Water softeners, special filters, and disinfection systems	1L bulk water	Collect a sample before and/or after these processes
All fixtures in case rooms	1L bulk water	Hot water – first draw
All fixtures in case rooms	1L bulk water	Cold water – first draw
All other areas the case-patient may have been exposed to (e.g., salon sink, common shower room)	1L bulk water	Hot water – first draw
Representative number of point-of-use (POU) fixtures from the hot water system (including proximal, mid, and distal locations)	1L bulk water	Hot water – first draw
Cooling tower system	1L bulk water	Collect 1 sample from the collection basin (an area below the tower where cooled water is collected) and 1 sample from the lowest point in the open condenser water circuit.
Hot tubs	1L bulk water	Collect 1-2 samples from the tub and 1 sample per filter
	Biofilm swabs	Number of swabs dependent on size and complexity of water system
Decorative fountains	1L bulk water	Collect 1 sample
	Biofilm swabs	Number of swabs dependent on size and complexity of water system
Note: Additional bulk water or biofilm swab samples may be recommended based on the condition, size, and complexity of water systems and devices.		