



Centers for Disease Control and Prevention

# Sampling Procedure and Potential Sampling Sites

Protocol for collecting environmental samples for *Legionella* culture during a **cluster or outbreak** investigation or when **cases of disease** may be associated with a facility.

Sampling should only be performed after a thorough environmental assessment has been done and a sampling plan has been made. This protocol describes how to take standard biofilm swab, bulk water, and filter samples from commonly sampled sites. This protocol may be used in conjunction with the following tools:



**LEGIONELLA ENVIRONMENTAL ASSESSMENT FORM**



**SAMPLE DATA SHEET**



**LEGIONELLOSIS OUTBREAK INVESTIGATION VIDEOS:**

*Legionella Ecology and an Introduction to Environmental Health and Engineering*

*Conducting and Interpreting the Environmental Assessment*

*How to Make a Sampling Plan*

*How to Sample Potable Water*

*How to Sample Cooling Towers*

*How to Sample Spas and Fountains*

## MATERIALS NEEDED

- Sterile plastic 1 L bottles. (Glass bottles are not recommended due to risk of breakage during transport.)
- Sterile plastic 15 mL screw top tubes (with a tube rack) for biofilm swabs.
- Disposable Dacron/polypropylene-tipped swabs with wooden or plastic stems. Do NOT use cotton-tipped swabs as they inhibit *Legionella* growth.
- Labels.
- 0.1N solution of sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3$ ) (15.81 g/L in distilled water, filter sterilize, replace every 12 months).
- Pipettes and bulbs for adding 0.5 mL of 0.1N sodium thiosulfate solution into 1 L water samples.
- Sterile plastic 500 mL or 1 L bottle for testing chlorine level, pH, and temperature.
- pH test kit.
- Chlorine test kit sensitive enough to detect chlorine level below 2 ppm and up to 10 ppm (may need two kits). 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.
- Thermometer.
- Sample data sheet and pens.
- Large cooler, preferably with wheels. A 70 quart (66.2 L) horizontal cooler (a standard large picnic cooler) should fit twenty-five 1 L bottles, twenty-five 15 mL plastic tubes for biofilm swabs, thermometer, pH, and chlorine test kits. For sampling of a larger facility (60–100 samples total), a second cooler that holds nothing but bottles, swabs, and tubes may be needed. The cooler may be packed ahead of time and stored at ambient temperature for an unlimited time as long as the sodium thiosulfate solution is replaced every 12 months.

## OPTIONAL MATERIALS

- Packing tape and scissors for sealing the cooler for mailing or air travel.
- Biohazard waste bags are useful for collecting trash.



### SAFETY PRECAUTIONS

The facility should be notified in advance to turn off (but do not drain or disinfect) any aerosol-generating devices to minimize the risk to the sampling team. Persons at an increased risk of developing Legionnaires' disease if exposed to *Legionella* (e.g., immunocompromised individuals) should not accompany the sampling team.

#### **Optional personal protective equipment (PPE):**

- Gloves are useful for sampling whirlpool spa filters or other sites that may be heavily contaminated with organic material.
- Wearing a half-face air-purifying respirator equipped with an N95 filter may be appropriate in the following situations: a.) when sampling cooling towers if the fans cannot be turned off, or b.) in enclosed spaces with an aerosol-generating device that cannot be turned off. Respirators must be used in accordance with a comprehensive respiratory protection program, which includes fit testing, training, and medical clearance ahead of their use (see OSHA standard [29 CFR 1910.134](#)). For more information about N95 respirators, visit the National Institute for Occupational Safety and Health (NIOSH) [website](#).

## SAMPLING POTABLE WATER AT THE POINTS OF USE

Note: In most situations, it's appropriate to sample only the hot water. However, there are situations where taking some cold water samples is helpful. For example, in hot climates, the cold water may be warm enough for rapid *Legionella* amplification (>77°F). Desalination may also elevate cold water temperature. Cold water could also be warm due to lack of insulation between hot and cold water pipes.

**Collect one biofilm swab and one bulk water sample from each sampling site (i.e., each showerhead or faucet).**

1. For showers, ask facility maintenance personnel to remove the showerhead. For faucets, ask them to remove the aerator.

### **Take biofilm swabs:**

2. Turn on the water for a couple of seconds to moisten the pipe, and then turn it off. Insert a sterile Dacron- or polypropylene-tipped swab deep into the faucet/pipe. Try to get beyond the bend and swab around the inside surface firmly without breaking the swab stem. (If there is visible biofilm on the inside of the showerhead or faucet aerator when these are removed, they can also be swabbed.)
3. Place the swab into a 15 mL sterile plastic tube and add 3–5 mL of water from the same faucet to keep the swab tip moist during transport. Snap the wooden or plastic swab stem approximately 1 in. from the top of the tube. Add a drop of 0.1N sodium thiosulfate solution to neutralize residual disinfectants. Tighten the tube top to prevent leakage.
4. Label the tube with a unique identifier. Record the type and location of the sample on a Sample Data Sheet, and place the tube into a cooler.

### **Take bulk water samples:**

5. After the biofilm swab is collected, turn on the water and let it run for a few minutes until the water is warm but not hot. The goal is to obtain water currently in the distribution system along with any material shed from biofilm. Avoid heating water excessively (approximately 122°F or higher) since free-floating *Legionella* will die quickly at elevated temperatures. Collect 1 L of water from the faucet into a sterile 1 L bottle, leaving a 1 in. space at the top.
6. Add 0.5 mL of 0.1N sodium thiosulfate solution to the water sample to neutralize residual disinfectants. Tighten the bottle top to prevent leakage.
7. Label the bottle with a unique identifier. Record the type and location of the sample on the Sample Data Sheet, and place it into the cooler.

### **Measure water parameters:**

8. Run the hot water until it is as hot as it will get. Collect 100–300 mL of water 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 Data Sheet.

## SAMPLING POTABLE WATER AT THE HOT WATER HEATERS

1. Collect a bulk water sample only; it is rare that a biofilm sample can be obtained from a hot water heater since this would require completely draining the tank.
2. Ask facility maintenance personnel to open the drain valve of the hot water heater and collect 1 L of water into a sterile 1 L bottle, leaving a 1 in. space at the top.
3. Add 0.5 mL of 0.1N sodium thiosulfate solution to the water sample to neutralize residual disinfectants. Tighten the bottle top to prevent leakage.
4. Label the bottle with a unique identifier. Record the type and location of the sample on the Sample Data Sheet, and place it into the cooler.
5. Always measure and record the temperature, pH, and chlorine level of a bulk water sample collected from a hot water heater.

## SAMPLING WHIRLPOOL SPAS

1. Take biofilm swabs from inside several jets and at the water line.
2. Place each swab into a 15 mL sterile plastic tube (one swab per tube) and add 3–5 mL of water from the whirlpool spa tub to keep the swab tip moist during transport. Snap the wooden or plastic swab stem approximately 1 in. from the top of the tube. Add a drop of 0.1N sodium thiosulfate solution to neutralize residual disinfectants. Tighten the tube top to prevent leakage.
3. Label each tube with a unique identifier. Record the type and location of the sample on a Sample Data Sheet, and place the tube into a cooler.
4. If the whirlpool spa tub is not drained, collect a 1 L bulk water sample in a sterile 1 L bottle. If the pool is partially drained, a sterile 15 mL tube may be used to collect the remaining whirlpool water. If the spa has been completely drained, ask facility maintenance personnel for access to the compensation tank (for collection of overflow water) and take a bulk water sample from there.
5. Add 0.5 mL of 0.1N sodium thiosulfate solution to neutralize residual disinfectants to the 1 L water sample. Tighten the bottle top to prevent leakage.
6. Label the bottle with a unique identifier. Record the type and location of the sample on the Sample Data Sheet, and place it into the cooler.
7. Collect 100–300 mL of water from the whirlpool spa tub (or the compensation tank if drained) in a separate plastic sampling bottle. Measure temperature, pH, and free chlorine or bromine level of the sample. Record all measured data on the Sample Data Sheet.
8. It is very important to collect a filter sample from whirlpool spas. Request access to the filter (which is usually located in a separate maintenance room) from the facility maintenance personnel. Gloves should be worn due to heavy organic loads typically found in filters and the abrasive or caustic nature of some filter filling material. The methodology for filter sample collection depends on the filter type.
  - a. Sand filters: 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.
  - b. 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.
  - c. 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 in. of water.
  - d. Add 0.5 mL of 0.1N sodium thiosulfate solution to the sample to neutralize residual disinfectants. Tighten the bottle top to prevent leakage.
  - e. Label the bottle with a unique identifier. Record the type and location of the sample on the Sample Data Sheet, and place it into the cooler.

## LIST OF POTENTIAL SAMPLING SITES\*

Site	Approximate number of samples	Type of samples	Sample processing <sup>†</sup>
<b>Potable water</b>			
Incoming water main (where water enters the facility/campus/building from the municipality)	1	1L bulk water	Concentrate
Every well and water tower that supplies water to the facility/campus/building	1 per well or water tower	1L bulk water	Concentrate
Every holding tank or cistern	1 per holding tank/cistern	1L bulk water	Concentrate
Centralized water heater	1	1L bulk water (a biofilm swab if drained)	Direct
Expansion tank for hot water (absorbs excess water pressure caused by thermal expansion within the hot water heater)	1	1L bulk water	Concentrate
Hot and cold water returns	1 each for hot and cold	1L bulk water	Concentrate
For buildings with water softeners, special filters, and disinfection systems, sample water before and/or after these processes		1L bulk water	Concentrate
Shower	2 per shower <sup>‡</sup>	1 biofilm swab and 1L bulk water	Concentrate
Faucet	2 or 3 per faucet <sup>‡</sup>	1 biofilm swab inside the faucet, (1 biofilm swab of the inside of the aerator if visual inspection indicates that it's overgrown with biofilm), 1L bulk water	Concentrate
Whirlpool baths <sup>‡</sup> (i.e., Jacuzzis)	1	1 biofilm swab inside the jets	Concentrate
<b>Cooling towers<sup>‡</sup></b>			
Make-up water (water added to replace water loss because of evaporation, drift, or leakage)	1	1L bulk water	Direct
Collection basin (an area below the tower where cooled water is collected and directed to the sump)	2	1L bulk water and a biofilm swab at the water line	Direct
Sump (a depressed chamber contiguous to the basin, where water flows to facilitate pump suction; may also be used as collection point for silt and sludge)	2	1L bulk water and a biofilm swab at the water line	Direct
Storage tank or reservoir in the system	1	1L bulk water	Direct
Drift eliminators or other surfaces that remain moist	1	1 biofilm swab	Direct
Heat sources (e.g., chillers)	1	1L bulk water	Direct

Site	Approximate number of samples	Type of samples	Sample processing <sup>†</sup>
<b>Whirlpool spas</b>			
Water in the tub	1	1L bulk water	Direct (concentrate if chlorine is detected by odor or direct testing)
Biofilm at the water line	2	Biofilm swabs (the quantity depends on the size of the tub)	Direct
Water jets	2	Biofilm swabs of several jets	Direct
Filter	1 per filter	Combination of water and a filling (sand in sand filters, diatom powder in DE filters, or polyester filling in cartridge filters) to keep the filling moist during the transport	Direct
Compensation tank	1	1L bulk water	Direct (concentrate if chlorine is detected)
<b>Other sources</b>			
Decorative fountains	2	1L bulk water and a biofilm swab (number of swabs dependent on size and complexity of the fixture)	Direct
Sprinkler systems	>2	1L bulk water and one or several biofilm swab(s) of the sprinkler jets	Concentrate
Safety showers and eye wash stations	2	1L bulk water and a biofilm swab	Concentrate
Humidifiers	2	Bulk water (as close to 1L as possible) and at least one biofilm swab of moist surface	Concentrate
Nebulizers, hand-powered resuscitation bags, intermittent positive pressure breathing ventilators, and other respiratory care equipment that uses water for filling or cleaning	>2	1L bulk water used to clean the device and biofilm swabs of moist surfaces	Concentrate

\*This table is a list of commonly sampled sites; however, all sites need not be sampled during every outbreak. Important: Use CDC sampling procedures.

<sup>†</sup>This table provides general recommendations on whether a sample should be processed directly or concentrated based on the assumption that potable water samples typically contain less bacteria and fungi than non-potable water samples. For each sample, decisions about the processing strategy should be made depending on specific circumstances (e.g., a decorative fountain contains water with a strong odor of chlorine or a bulk water sample from a faucet that has no detectable chlorine level).

<sup>‡</sup>All showers and faucets in all case rooms should be sampled, along with showers and sink faucets in additional rooms. Choose rooms proximal and distal to risers or hot water heaters and on various floors based on the results of the environmental assessment. Ideally, sample at least a couple of outlets on every floor and/or wing.

<sup>¶</sup>Whirlpool baths are filled from the tap and drained after each use.

<sup>¶</sup>Not all cooling towers have all listed components. Engage an engineer or maintenance technician familiar with the facility to identify appropriate sampling sites for a particular cooling tower.