

SAMPLE COLLECTION GUIDELINES

Note: Sample integrity must be maintained during the entire sampling process, from the initial sample collection to the completed final report. Chain of Custody forms are provided by Environmental Safety Technologies, Inc. (EST) for all clients to use in order to document sample collection, identification, location, conditions, and sample traceability.

Legionella Analysis

When sampling, avoid generating aerosols that may be contaminated with the bacteria. Observe safety precautions when sampling. Wear a disposable N-95 mask and disposable gloves compatible with chemicals that may be present in treated water.

- **Test Codes:** **L001 Legionella Culture and DFA – water/bulk liquid and swabs**
L002 Legionella DFA only
- **Sampling Device for Water Sample:** Use a clean, dry sample bottle (do NOT use a recycled/used beverage bottle). EST recommends using a high density polyethylene bottle containing one 30 mg sodium thiosulfate tablet.

(*Legionella* analysis from samples taken from chlorinated samples (potable water, swimming pools, jacuzzi, etc.) may result in a false negative test due to the residual halogen biocides. Therefore, it is recommended by the CDC (US) that water samples contain sodium thiosulfate at a concentration of 0.5 ml of 0.1 N solution per liter of sample water to neutralize the disinfectant. This concentration may be achieved by collecting 500 ml of samples in a bottle containing one 30 mg sodium thiosulfate tablet, a neutralizing agent, to inactivate the chlorine.)

- **Sampling Device for Swab Sample:** EST recommends using a Copan™ Swab 114C – swab, Aimes with charcoal, rayon tip (black top). * **Note: DFA only analysis (L002) is not available for swab samples.**
- **Sample Transport:** Samples should be shipped on the day of collection Monday through Thursday for overnight delivery to the laboratory before noon the following day. Call to arrange for rush weekend processing/analysis; extra charges will apply.

(Guidelines established by the U. S. Department of Health and Human Services Centers for Disease Control and Prevention (CDC) in the document, “Procedures for the Recovery of *Legionella* from the Environment.” The directions for specimen collection states: “Samples that will not reach the laboratory within 72 hours should be refrigerated before shipping. Samples should be shipped so that they are protected from extreme heat or cold.” The validity of microbial counts beyond recommended shipping times becomes questionable since the environmental conditions during shipping could either artificially increase or decrease total viable bacteria and viable *Legionella* counts in a sample. Therefore, in order to obtain the most accurate analysis possible, samples should be shipped within 72 hours, preferably overnight. Results obtained from samples arriving later will be processed with customer approval, but may not accurately reflect the bacteria in the site at the time of sampling. Samples delayed beyond 72 hours will have a delayed transit comment on the sample report).

- **Sampling Method for Cooling Towers, Evaporative Condensers, and Air Handling Units, and Decorative Fountains**

Water Sample – **A minimum of 100 milliliters of water is required.** The Limit of Sensitivity for this assay is 10 colony forming units per milliliter of sample (CFU/ml). Depending on the accessibility of the tower, collect samples from the tower basin at the furthest most point from the source of the make-up water. Take caution to prevent losing the tablet from the bottle during sampling. Shake each sample for thirty seconds to help dissolve the tablet. Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well).

- If possible, samples should be free of sediment. Please do not fill the containers to maximum capacity, leave approximately one inch of air space in the neck of the container. If there is not enough water available at the sample location, a swab sample may be collected from any biofilm and/or moisture present on a drain, condensate pan, or coils.
- **IMPORTANT:** Samples should NOT be collected immediately following the addition of disinfectants/biocides to the cooling tower. It is strongly recommended that samples be collected no sooner than 48 hours after the addition of any disinfectants/biocides to the system.
- Sample Decorative Fountains while operational.

Swab Sample - If there is not enough water available at a sample location, a swab sample may be collected from any biofilm and/or moisture present on the drain, condensate pans, or coils.

- **Sampling Method for Potable Water Systems (Hot Water Tanks, Faucets and Showerheads, City Water, and Whirlpool Spas, etc.)**

Water Samples - **A minimum of 500 milliliters of water is required.** The Limit of Sensitivity for this assay is less than one CFU/ml of sample.

- **Hot Water Tanks** – Water samples should be collected from the bottom of the tank immediately after opening the drain valve (if necessary, let water run for approximately 10 seconds to purge the drain line). Please do not fill the containers to maximum capacity, leave approximately one inch of air space in the neck of the container. The water temperature of this sample should be recorded on the Chain of Custody (it is unlikely to be the same as at the top of the tank). Take caution to prevent losing the tablet from the bottle during sampling. Shake each sample for thirty seconds to help dissolve the tablet. Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well).
- **Faucets and Showerheads** - Water samples should be collected at various sites throughout the facility, depending on the type of exposure that may be encountered and the distance from the hot water heaters. Because

Legionella bacteria may multiply inside a faucet, the concentrations will be highest immediately after turning on the water. Thus, the best sample will be the first liter of water out of the faucet, preferably a faucet that has not been used for several hours. Please do not fill the containers to maximum capacity, leave approximately one inch of air space in the neck of the container. After obtaining the sample, allow the hot water to run until it has reached its maximum temperature (approximately one to two minutes). Record this temperature on the Chain of Custody. Take caution to prevent losing the tablet from the bottle during sampling. Shake each sample for thirty seconds to help dissolve the tablet. Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well).

Swab samples - Samples should be collected with a Aimes swabs with charcoal prior to collection of bulk water samples collected at the same site. The faucet and/or showerhead should be extensively swabbed inside as far as possible. If the faucet has an aerator, it should be removed and swabbed on the inside, particularly the rubber gasket. Insert the swab sample into the transport tube and securely push together to close.

- **Sampling Method for City Water** - This sample is intended to monitor the *Legionella* bacteria entering the facility from the city water supply. As such, this sample should be taken from a point as close as possible to where the water enters the facility. Since this sample is intended to analyze the incoming water, the faucet or drain valve should be opened and flushed for 30 seconds before collection of sample. Please do not fill the containers to maximum capacity, leave approximately one inch of air space in the neck of the container. Take caution to prevent losing the tablet from the bottle during sampling. Shake each sample for thirty seconds to help dissolve the tablet. Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well).
- **Sampling Method for Whirlpool, Jacuzzi, Swimming Pool** - The number of bacteria in pools, particularly whirlpool spas, often varies greatly during the day and from day to day. Thus, any one sample is only a spot check of the water at these sites. Ideally, these samples should be taken when the disinfection conditions are at their weakest (i.e. generally toward the end of the day or after a period of heavy usage), although other times are also acceptable. The sample bottles should be filled by collecting from the surface of the water. It is also a good idea to collect these samples while pool pumps, blowers, etc. are operating, although it is not absolutely necessary. Take caution to prevent losing the tablet from the bottle during sampling. Shake each sample for thirty seconds to help dissolve the tablet. Securely tighten each sample bottle top to prevent leaking during shipping (electrical or Teflon tape around the bottle top works well).