**Physical Survey and Water Sampling Protocol\***

Arrange with the OnSiteLegionellaTesting for supply and shipment of sterile sampling containers, and for analysis of water samples.

During the initial walk-through, estimate the size of the building and the number of water services at the facility to determine the number of samples and the size of the purchase order. When investigating the water services within a building, it will be helpful to obtain or prepare a simple schematic diagram of the water services. Note the following features:

* The location of the incoming supply and/or private source.
* The location of storage tanks, water treatment systems, and pumps.
* The location of water heaters and boilers.
* The type of fittings used in the system (e.g., taps, showers, valves) and the material from which the pipework is made.
* The location of all cooling towers, evaporative condensers, and fluid coolers at the facility. The location and type of all systems served by the cooling tower water including sump tanks, condensers, and indirect evaporative cooling coils in air handling units.
* The location of any evaporative cooling systems or humidifiers.
* The location of ornamental fountains, whirlpools, eye washes, safety showers, or other water sources within or near the facility.

Trace the route of the service from the point of entry of the water supply. Note the condition of pipes, jointing methods used, insulation, sources of heat, and the kind of insulation in water storage tanks. Also note carefully any disconnected fittings, "dead legs," and cross-connections with other services. Once you have identified these features, take water samples from:

* The incoming water supply.
* Each storage tank and water heater.
* A representative number of faucets for each of the hot and cold water systems in the facility.
* All cooling towers, evaporative condensers, humidifiers, spas, showers, etc.
* The water entering or leaving any other type of fitting or piece of equipment under particular suspicion.

It is important not to overlook any potential water sources in the building. Water from ice machines, hand spray bottles, decorative fountains, and for plastic injection molding equipment have been implicated in past outbreaks or have been found to be significantly contaminated. The ability to maintain an open mind is essential in conducting an investigation because of the variety of potential sources of contamination at a facility.

**Water Sampling Procedure**

Wear appropriate respiratory protection in the form of a half-face piece respirator equipped with a HEPA filter or a similar type of filter media capable of effectively collecting particles in the one micron size range during the examination of water systems if a significant potential exists for exposure to high concentrations of contaminated aerosols.

Collect samples in polypropylene (nalgene) containers (250 mL-1 L) that have been autoclaved at 121°C for 15 minutes. The microbiological laboratory that will analyze the samples should be able to provide the bottles. A local hospital or state health department should be able to autoclave the bottles. It is important not to flush the system to be sampled before collecting samples. Collect at least a 250 mL sample. Measure the temperature of the sampled water. It is preferable to accomplished this by measuring the water stream flowing from the water source and not by placing the thermometer in the sample container. To avoid cross-contamination of the samples, sanitize the thermometer with isopropyl alcohol before measuring the temperature of each sample. When measuring temperature from faucets, showers, water fountains, etc., record the initial water temperature, and then allow the fixture to discharge until the temperature stabilizes. Record the initial and final temperatures, and the time needed to stabilize.

**Domestic Water Heaters**

* Take a sample of water from the bottom drain.
* Collect a sample of water from the outlet pipe if the plumbing provides for access.

**Faucets and Showers**

* Collect a "before-flush" (initial flow) sample of water.
* Collect an "after-flush" sample of water when the maximum temperature has been reached.

The initial (before-flush) sample is intended to indicate the level of contamination at the sample point or fitting, and the final sample should reveal the quality of the water being supplied to the fitting. Collect sterile-swab samples from faucets or shower heads by removing the fitting and vigorously swabbing the interior. Swab samples may be positive for *Legionella* even when water samples from the source are negative. Sterile test tubes containing sterilized swabs are available for convenient sampling and shipping.

**Cooling Towers**

* Take a sample from the incoming supply to the tower.
* Take samples from any storage tanks or reservoirs in the system (i.e., chilled-water return tanks or header tanks).
* Take a sample from the basin of the cooling tower at a location distant from the incoming make-up water, and from the water returning from the circulation system at the point of entry to the tower.
* Take a sample of any standing water in the condensate trays or from the cooling coils.

**Humidifiers, Swamp Coolers, and Spas**

* Take a sample from the water reservoirs. Sample the incoming water supply if it is accessible.
* For cooling towers, humidifiers, swamp coolers, and building water services, collect samples of sludge, slimes, or sediments, particularly where accumulations occur.
* Take swabs of shower heads, pipes, and faucets and rehydrate from water taken from the sampling site. Swab areas of scale buildup (i.e., remove shower heads, faucet screens, and aerators). Use prepackaged sterile swabs and small glass or polypropylene bottles (autoclaved) for this purpose.

**Sample Transportation if not using OnSiteLegionellaTesting method**

Prepare samples for shipment carefully, as follows:

* Wrap vinyl tape clockwise around the neck of each bottle to hold its screw cap firmly in place and seal the interface between the cap and the bottle.
* Wrap absorbent paper around bottles, and place the bottles in a sealable (zip-lock) plastic bag.
* Place the sealed plastic bag in an insulated container (styrofoam chest or box).

Samples should not be refrigerated or shipped at reduced temperature. They should be protected from temperature extremes such as sunlight or other external heat or cold sources. Ship to laboratory using overnight mail. If shipping on a Friday, make arrangements for weekend receipt. The samples should be stored at room temperature (20° ± 5°C) and processed within 2 days.