



Monterey County Health Department
Consolidated Environmental Laboratory
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COLLECTION WATER SAMPLES FOR *LEGIONELLA PNEUMOPHILA*

PURPOSE

The genus *Legionella* are Gram-negative bacteria including *Legionella pneumophila*, which can cause legionellosis (pneumonia-type illness known as Legionnaires' disease or milder, flu-like illness called Pontiac fever).

Legionella are naturally occurring in freshwater environments, including lakes and streams. Water contaminated with *Legionella* can become a health concern when the bacteria grows and spreads in human-made water systems including: showers and faucets, cooling towers (air-conditioning units for large buildings), hot tubs that aren't drained after each use, decorative fountains and water features, hot water tanks and heaters, and large plumbing systems. High concentrations of *Legionella* may be present in biofilm (i.e. slime) on surfaces constantly exposed to water, such as pipes and aerators. Legionnaires' disease may develop in individuals who breathe in aerosols (small water droplets) containing *L. pneumophila*.

Our laboratory offers 3 different methods for detecting *Legionella spp. (species)* and *L. pneumophila* in environmental samples: Legiolert (IDEXX), conventional culture (BYCE agar) and PCR (*Legionella spp. & L. pneumophila*). Legiolert is a simple and reliable method used to detect and quantify *L. pneumophila* in water samples. This test is based on enzyme substrate detection technology and can produce results within 7 days with a detection level of ≥ 1 organism / 10ml for potable water and ≥ 1 organism / 0.1ml for non-potable water.

The conventional culture method, still considered the gold standard for detecting and quantifying *Legionella spp. and L. pneumophila* is useful for validating Legiolert test results and isolating *Legionella* to perform additional testing, including speciation and strain typing for *Legionella* investigations. The culture and PCR methods are used for testing biofilm samples and may be used for testing water samples in conjunction with Legiolert.

Please contact the laboratory for information for guidance on method selection, sample collection and testing costs.

SAMPLE COLLECTION

Water samples taken for *L. pneumophila* testing must be collected and handled carefully to ensure that the sample taken truly represents the bacteriological quality of water in the system. See procedures below.

For information on sample collection during outbreak investigations or when cases of disease are associated with a facility, refer to the *CDC Sampling Procedure and Potential Sampling Sites* in the REFERENCE section.

1. Sterile plastic bottles (2 x 500 ml or 1 x 1000 ml) provided by your laboratory must be used.
2. Do not touch or otherwise contaminate the inside of the sample bottle, the inside of the cap, or the threads inside of the container. The bottle contains a chemical to neutralize chlorine; do not rinse the container.
3. Be sure to wear gloves. Use safety goggles and a respirator when collecting samples that could generate aerosols.
4. Select a sample location where *Legionella* contamination is suspected. Common examples of potable water sample sites include drinking fountains, kitchen and bathroom faucets, showers, and ice machines. Non-potable water sites include cooling towers, air conditioners, whirlpools, pools, decorative fountains, and untreated waters.
5. Record water chlorine residual on chain of custody.
6. CDC recommends collecting both hot and cold water if available since *Legionella* bacteria prefer warmer temperatures. To collect water from faucets, open it just enough to produce a flow which can be collected without splashing. Collect pre- and post-flush water. After collecting the pre-flush (warm) water, let the water run until the temperature stabilizes and collect a post-flush (cooler) sample. Carefully fill the container, leaving an open air space; if 100 ml IDEXX bottles are used for sample collection, fill the bottle up to the 100ml mark, or fill to the neck of the 1L bottle. Immediately replace the cap (tightly), and label the sample with description of sample point, date and time of collection, and name of sample collector.
7. Refrigerate the sample and/or place the sample in a cooler with blue ice until it can be delivered to the laboratory. If ice is used, avoid placing the sample directly on ice. It is recommended that samples are held at below 10 °C if received at the laboratory more than 2 hours after collection.
8. Complete the laboratory form including mailing address, name of sample collector, description of sample point, date and time of collection, and test ordered.
9. Biofilm samples may be collected using a sterile dacron or polypropylene swab; do not use cotton-tipped swabs as they will inhibit *Legionella* growth. Wear gloves to avoid contaminating the swab. After collecting the biofilm, place the swab into a sterile tube containing 3 - 5 ml sterile water to keep the swab from drying during transport to the laboratory; snap the wooden or plastic swab stem approximately 1 in. from the top of the tube. Add a drop of 0.1N sodium thiosulfate.

SAMPLE TRANSPORT

SAMPLES MUST BE SUBMITTED DIRECTLY TO THE LABORATORY WITHIN 24 HOURS OF COLLECTION. Samples that cannot be dropped off at the laboratory can be submitted on the same day of collection to one of the following Health Department offices:

Monterey Co. Environmental Health
1200 Aguajito Rd.
Monterey, CA
831-647-7654

Monterey Co. Environmental Health
200 Broadway St. Suite 70
King City, CA
831-386-6899

Monterey - Drop off by 9:00 a.m.

King City – Drop off by 9:00 a.m.

Please notify the laboratory when samples are dropped off at the Health Department office.

REMEMBER: SAMPLES SHOULD BE COOLED AFTER COLLECTION UNTIL RECEIVED AT THE LABORATORY (e.g. iced cooler). Health Department offices can provide cold storage/transport from point of receipt.

TESTING FEES

Please contact the laboratory regarding testing fees. If you do not have an account with our laboratory, please pay the laboratory before testing. Clients who have an account will be billed at the end of the month for which results are reported.

REFERENCES

1. CDC Sampling Procedure and Potential Sampling Sites.
<https://www.cdc.gov/legionella/downloads/cdc-sampling-procedure.pdf>
2. U.S. Dept. of Labor, OSHA, Section II:E. Water Sampling Guidelines.
<https://www.osha.gov/dts/osta/otm/legionnaires/sampling.html>
3. IDEXX Legiolert Testing Procedure and Information.
<https://www.idexx.com/en/water/water-products-services/legiolert/>