



SAMPLING AND ANALYSIS OF *LEGIONELLA PNEUMOPHILA*

Introduction

Legionella are ubiquitous natural inhabitants of fresh water. They may be found in natural water bodies like rivers, lakes and springs and also in man-made water reservoirs. In homes and commercial buildings, *Legionella* species are often found in damp and warm places where biofilm forms, such as water storage and heating systems, sinks, faucets, shower heads, humidifiers, air conditioning systems, water fountains, and water cooling towers. Improperly maintained swimming pools, whirlpool spas, hot spas have also been reported as sites for *Legionella*.

Legionellosis was first known when 34 people attending the American Legion bicentennial conference in Philadelphia, USA in 1976 suddenly died of severe respiratory flu-like disease. The bacterium responsible for the outbreak was identified and named *Legionella pneumophila*. Contaminated water source containing the bacteria were believed to be the source of the disease. Since then numerous similar outbreaks have been reported in various regions of the world. Governments and health offices send out hazard alerts and educate people about regular maintenance and testing of water systems. This article covers the basic biology of *Legionella* and laboratory detection of the bacteria.

Health Risk

Many people may be in regular contact with the bacteria without being sick. Not all species of *Legionella* are harmful and a person in general good health is not necessarily diseased by the bacteria. High risk groups are heavy smokers, immuno-compromised individuals, elderly people and others with underlying disease conditions. Transmission of these bacteria is not found to be contagious. Amongst the health risk groups, more likely modes of transmission are aerosol inhalation, aspiration and drinking of contaminated water.

Legionella contamination of building water systems can lead to illness and in some instances the death of building occupants; especially those having lung problems and other immuno-compromised conditions. Conditions favorable to amplification include hot water system with a temperature range of 77-108°F, stagnation, scale and sediment, bio-film, and the presence of Amoebae. *Legionella* growth may also be influenced by certain materials. Natural rubber, wood, and some plastics may support amplification while materials such as copper inhibit growth. Amplification, dissemination, can be controlled by good engineering design and/or maintenance practices.

Outbreaks and deaths due to *L. pneumophila* have been reported as recently as June of 2005. In the United States, Legionellosis has been reported from all 50 states and an average of 8,000 to 18,000 cases of the disease is reported each year. Higher numbers are believed to either go unnoticed or not reported. Mortality rate of this disease is 5-30%.

Legionellosis caused primarily by *Legionella pneumophila*, generates symptoms ranging from flu-like symptoms with fever, dry cough and headache to severe pneumonia and diarrhea. Different serotypes of *L. pneumophila* have varying virulence and the severity of the disease depends both on the bacteria as well as the general health and age group of the patient. A less severe disease, Pontiac fever, is also caused by *Legionella*. Effective treatment is available by the use of appropriate antibiotics.

Environmental Management and Preventive Measures

General awareness amongst the public of the disease, causing agent, and mode of transmission is very important in the deterrence of any major outbreaks. Proper maintenance of air conditioners, humidifiers, plumbing lines etc. by regular cleaning and supervision is the best preventive measure. The bacteria are, however, not completely eliminated by the chlorination used to purify domestic water supplies. Raising the heating water temperature to above 68°C at homes of immuno-compromised or high risk individuals has also shown to decrease incidents of bacterial contamination and infection. Decontamination of the water at the source is the best way to avoid any outbreaks. The use of biocides and water flashing technique is also strongly recommended by various organizations.

Scheduled monitoring of potable water for *Legionella* might be warranted in places where persons are susceptible to Legionnaires' disease, such as hospitals and other health care facilities, or where people with diverse health conditions aggregate, such as hotels, office buildings, universities, health and fitness centers, stores, industries with water cooling facilities, etc. Other places that have or are near cooling towers, hot water reservoirs etc, may also at risk for high concentration of *Legionella* bacteria.. An effective risk assessment and monitoring program would reduce the risk of acquisition and transmission of *Legionella* caused diseases.

JCAHO [<http://www.jcaho.org>] has recently published **2005 Infection Control Standards (Pre-publication Edition)** for ambulatory care, behavioral health care, home care, hospital, laboratory, and long term care. Health care organizations seeking accreditation are required to “perform an ongoing assessment to identify its risks for the acquisition and transmission of infectious agents” and to “effectively conduct surveillance, collects data, and interprets the data”. While some health care organizations may have dedicated in-house infection control personnel, external help from industrial hygienists may be needed for the special expertise in sampling methodologies and for quality control purposes.

Potential Sources of *Legionella*

- Air Washers
- Architectural Fountains and Waterfalls
- Cooling Towers
- Direct Evaporative Air Coolers
- Emergency Water Systems (Fire Protection)
- Evaporative Condensers
- Fluid Coolers (Closed Circuit Cooling Towers)
- Humidifiers
- Indirect Evaporative Air Coolers
- Metal Working Systems

- Mistert and Atomizers (grocery store display cases)
- Municipal Water Supplies
- Pools, whirlpools and spas
- Potable Water Systems
- Any other source of aerosolized water

Potential Sampling Sites

Potable Water

- Incoming water main
- Water softener
- Holding tank, cistern
- Water heater tank (at the inflows and outflows)
- Potable water outlet, especially near patient rooms
- Faucet or tap
- Shower

Cooling Tower, Evaporative Condensers

- Make-up water
- Basin and/or Sump
- Heat sources (e.g. Chillers)

Humidifiers (e.g. Nebulizers)

- Bubblers for oxygen
- Water used for respiratory therapy equipment

Other Sources

- Decorative fountains
- Irrigation equipment
- Fire protection equipment (if recently used)
- Whirlpools
- Spas

Sample Collection

When sampling for *Legionella*, water samples and swabs from point of use devices or system surfaces should be collected. System surface swabs can evaluate the biofilm which frequently contains *Legionella*. When evaluating faucet aerators and shower heads, the surface swabs should be collected first. Water samples are collected after removing the shower heads and aerators. Swabs can be streaked directly onto buffered charcoal yeast extract agar plates if plates are available at the collection site. If the swabs and water samples must be transported to a laboratory for processing, immersing individual swabs in sample water minimizes drying in transport. Place swabs and water samples in insulated containers to protect specimens from temperature extremes.

Procedure for collecting and processing environmental specimens for *Legionella* spp.:

1. Collect water sample (100 mL - 1L) using the sterile bottles provided by the laboratory and secure the cap after collecting.

2. Collect swab sample of internal surfaces of faucets, aerators, and shower heads with a sterile swab provided by the laboratory. Fill the swab tube up to ½ full (about 5 ml) with the sample water from the same device from which the swab sample was taken and insert the swab into the tube and secure the top.
3. If your goal is to sample the faucets and pipes, then take water samples right after you turn on the water. If your goal is to sample source of the water, then let water run a few minutes before sampling.
4. Sampling in the presence of biocide: If the water sampled contains or is thought to contain an oxidizing biocide, then add an excess of an appropriate inactivating agent to the container before or at the time of sampling. NOTE: Chlorine and other oxidizing biocides are inactivated by the addition of potassium thiosulphate or sodium thiosulphate to the container. (Vials/bottles containing the potassium thiosulphate are provided by the laboratory, free of charge if returned for analysis, otherwise \$2.00 per 100 ml vial and \$3.00 per 1L bottle).
5. Deliver the samples to the laboratory as soon as possible or transport the samples in insulated containers to the laboratory via standard overnight shipping.
6. As a rule microbiological analysis should be commenced as soon as possible after arrival in the laboratory, particularly samples known to contain biocides, ideally within 24 hours and should not exceed 2 days. Store the samples at $(5 \pm 3)^{\circ}\text{C}$ before analysis.
7. In the laboratory, the samples can be analyzed by culture method or by QPCR, as specified by the client.

***Legionella* Detection and Enumeration Using Culture Methods**

Standard Methods 9260J is used for analyzing water samples with high bacterial count, and the ISO 11731 protocol is used for water with low bacterial counts.

ISO-11731– Part 2: Direct membrane filtration method for waters with low bacterial counts (ISO-11731-2:2004 E)

Scope: This protocol is a monitoring method for the isolation and enumeration of *Legionella* organisms in water intended for human use (e.g., hot and cold water, washing water), for human consumption and for treated bathing waters (e.g., swimming pools). It is especially suitable for waters expected to contain low numbers of *Legionella*. As the growth of *Legionella* may be inhibited by overgrowth of other bacterial colonies on the membrane, the method is only suitable for waters containing low bacterial counts. If necessary, we will also adopt additional procedures to increase the recovery of *Legionella*.

Additional Test: Latex agglutination test for *Legionella pneumophila* serogroup 1, 2 through 14, and non-pneumophilia group, sensitivity is 98.8%, specificity is 100%.

Turn around time: 10 days

Laboratory fee for culture analysis: \$85 per sample

Advantages and Limitations of Culture Methods: Culture method can provide information on viability of *Legionella*. However it requires precaution to maintain the viability during sampling and handling as well as shipping, and the turn around time is at minimum 10 days.

***Legionella* Detection and Enumeration Using QPCR**

QPCR method uses specific primer to detect and quantify the presence of target *Legionella pneumophila* genes. It is sensitive and reliable, and the results can be obtained within 1- 2 days.

Advantages and Limitations of the QPCR Method: The fast turn around time is advantageous when surveying a building suspected of *Legionella* contamination. If the results are positive, immediate decontamination action can be taken to reduce the potential exposure. No special precaution needed to maintain viability of the bacteria during sampling and handling. However, QPCR method does not provide viability and serogroup information. To verify the effectiveness of anti-bacterial treatment, it is best that the culture method be used.

Laboratory fee for QPCR analysis: \$85 per sample (Minimum 3 samples).

About Aemtek:

Aemtek is an environmental microbiology laboratory located in Fremont, CA. We provide accurate, fast, reliable, and legally defensible data in the detection and analysis of fungi and bacteria. We are committed to excellence in quality, service, and technology.

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Appendix CDC's Guideline for *Legionella* Sampling

Water Sampling Strategies and Culture Techniques for Detecting Legionellae

Legionella spp. are ubiquitous and can be isolated from 20%--40% of freshwater environments, including man-made water systems (1,2). In health-care facilities, where legionellae in potable water rarely result in disease among non immuno-compromised patients, courses of remedial action are unclear.

Scheduled microbiologic monitoring for legionellae remains controversial because the presence of legionellae is not necessarily evidence of a potential for causing disease (3). CDC recommends aggressive disinfection measures for cleaning and maintaining devices known to transmit legionellae, but does not recommend regular scheduled microbiologic assays for the bacteria (4). However, scheduled monitoring of potable water within a hospital might be considered in certain settings where persons are highly susceptible to illness and mortality from *Legionella* infection (e.g., hematopoietic stem cell transplantation units and solid organ transplant units) (5). Also, after an outbreak of legionellosis, health officials agree monitoring is necessary to identify the source and to evaluate the efficacy of biocides or other prevention measures.

Examination of water samples is the most efficient microbiologic method for identifying sources of legionellae and is an integral part of an epidemiologic investigation into health-care--associated Legionnaires' disease. Because of the diversity of plumbing and HVAC systems in health-care facilities, the number and types of sites to be tested must be determined before collection of water samples. One environmental sampling protocol that addresses sampling site selection in hospitals might serve as a prototype for sampling in other institutions (6). Any water source that might be aerosolized should be considered a potential source for transmission of legionellae. The bacteria are rarely found in municipal water supplies and tend to colonize plumbing systems and point-of-use devices. To colonize, legionellae usually require a temperature range of 77°F--108°F (25°C--42.2°C) (7) and are most commonly located in hot water systems. Legionellae do not survive drying. Therefore, air-conditioning equipment condensate, which frequently evaporates, is not a likely source (8).

Water samples and swabs from point-of-use devices or system surfaces should be collected when sampling for legionellae (9). Swabs of system surfaces allow sampling of biofilms, which frequently contain legionellae. When culturing faucet aerators and shower heads, swabs of surface areas should be collected first; water samples are collected after aerators or shower heads are removed from their pipes. Swabs can be streaked directly onto buffered charcoal yeast extract agar plates if the plates are available at the collection site. If the swabs and water samples must be transported back to a laboratory for processing, immersing individual swabs in sample water minimizes drying during transit. Place swabs and water samples in insulated coolers to protect specimens from temperature extremes.

References

1. Arnow PM, Weil D, Para MF. Prevalence and significance of *Legionella pneumophila* contamination of residential hot- tap water systems. J Infect Dis 1985;152:145--51.
2. Shelton BG, Morris GK, Gorman GW. Reducing risks associated with *Legionella* bacteria in building water systems. In: Legionella: current status and emerging perspectives. Barbaree JM, Breiman RF, Dufour AP, eds. Washington DC: American Society for Microbiology Press, 1993:279--81.
3. Joly JR. Monitoring for the presence of *Legionella*: where, when, and how? In: Legionella: current status and emerging perspectives. Barbaree JM, Breiman RF, Dufour AP, eds. Washington DC: American Society for Microbiology Press, 1993:211--6.
4. Hoge CW, Breiman RF. Advances in the epidemiology and control of *Legionella* infections. Epidemiol Rev 1991;13:329--40.
5. [CDC. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. MMWR 2000;49\(No. RR-10\).](#)
6. Barbaree JM, Gorman GW, Martin WT, Fields BS, Morrill WE. Protocol for sampling environmental sites for Legionellae. Appl Environ Microbiol 1987;53:1454--8.
7. Brenner DJ, Feeley JC, Weaver RE. Family VII. Legionellaceae. In: Bergey's manual of systemic bacteriology. Vol. 1. Krieg NR, Holt JG, eds. Baltimore, MD: Williams and Wilkins, 1984:279--88.
8. Katz SM, Hammel JM. The effect of drying, heat, and pH on the survival of *Legionella pneumophila*. Ann Clin Lab Sci 1987;17:150--6.
9. CDC. Procedures for the recovery of *Legionella* from the environment. Atlanta, GA: US Department of Health and Human Services, Public Health Service, CDC, 1992:1--13.