

SAMPLING AND CONTROLLING *LEGIONELLA* BACTERIA IN DOMESTIC WATER SYSTEMS

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Introduction

Following the 1976 Legionnaires' disease outbreak in Philadelphia, tremendous strides have been made in our understanding of and ability to control the causative agent, *Legionella* bacteria. The original outbreak was traced to the cooling tower on the rooftop of the Bellevue Hotel. It was later found that *Legionella* bacteria could also colonize and amplify in domestic water systems, such as the hot water system. This particular issue discusses *Legionella* bacteria in the domestic water system: its assessment, sampling, testing, and control of the bacteria.

Legionella Bacteria And Their Ecology

After the outbreak of the disease in 1976 in Philadelphia, significant efforts were made at the Centers for Disease Control and Prevention (CDC) to identify the etiologic agent. A previously unknown bacterium was isolated and demonstrated in 1977 to be the etiologic agent responsible for causing the disease. It was named *Legionella* (to honor the victims of the Legionnaires' convention) *pneumophila* (pneumonia symptoms and occurred in Philadelphia). More than forty species in the genus *Legionella* have since been identified and named. At least twenty of the species have been linked to human diseases. The first species, *L. pneumophila*, is still the most common and the primary species associated with the disease. In addition, *L. pneumophila* is found to include at least 14 serotypes. *Legionella pneumophila* serotype 1 is not only the most common type (followed by serotypes 4 and 6) detected in environmental samples but also the most common one associated with the disease. It is estimated that *L. pneumophila* accounts for 80 to 85% of the disease as well as the strains recovered from water sources associated with buildings. Its serotype 1 is the primary subtype encountered in the disease and the environmental sources.

Legionella bacteria can survive in a temperature range of 20-50°C (or 68 to 122°F) and grow in a temperature range of 25-42°C (or 77 to 108°F), although there are indications that they can survive in temperatures lower than 20°C. Temperatures approaching 55°C or 131°F start to kill the organism. With this information, it is easy to understand why they like to grow in cooling tower water or in domestic hot water systems, particularly those set at a tepid water temperature range. It is also clear that high temperatures, over 55°C or 131°F, can be used to control *Legionella* bacteria.

Legionella bacteria are considered to have a worldwide distribution, although outbreaks of Legionnaires' disease are a more common occurrence in the northeast United States, England, Australia, the Netherlands, and a few other countries. Many regions of the US have had reports of Legionnaires' disease. Because of the wide distribution, close attentions should be paid to monitoring the presence and amplification of *Legionella* bacteria and to their control in building water systems.

The ecology of this group of bacteria is important in their spread. They are water-borne and can be aerosolized. It was found that *L. pneumophila* may survive as long as 139 days at room temperature in distilled water and for over a year in tap water. They grow, not just survive, in tap water in association with amoebae. The organisms can survive in aerosols and have been found as far as 200 m away from the aerosol source. Proteinaceous material and extracts of blue-green algae were found to stabilize the organism in aerosols. They have been found to spread through HVAC systems when the bacteria growing in cooling tower are aerosolized and get into the air-intake system. Recent research suggests that the bacteria establish a symbiotic relationship with a variety of protozoa and amoebae. They grow and multiply inside these organisms. That association makes treatment to rid the bacteria much more difficult. In addition to cooling tower water, *Legionella* bacteria have been isolated from potable water supplies, lakes, soils, etc.

Legionella bacteria are fastidious microbes, and can be isolated with selective media, which are incubated at 35-37°C. Treatment of samples briefly with acid or with elevated temperature has been found to increase recovery of the bacterium. *Legionella pneumophila* has at least fourteen serotypes at this time. Serotyping is useful in tracing the source of infection. Monoclonal antibody typing, direct immunofluorescence staining, and the slide agglutination test (SAT) are the primary methods to speciate and sub-speciate *Legionella* bacteria. Unfortunately, many of the reagents required to perform these tests are not available commercially.

Legionella Bacteria And The Diseases

Legionella bacteria cause two forms of disease: Legionnaires' disease and Pontiac fever. They are collectively called legionellosis. Both diseases are transmitted through airway exposure, and there has been no report of human-to-human transmission. *Legionella* bacteria in airborne water droplets or mists from contaminated water sources are the primary source of human exposure. The incubation period for the disease is 2-10 days, with an average of 5-6 days, from initial exposure. Legionnaires' disease is caused by several species of *Legionella* bacteria. The disease is a form of pneumonia and includes symptoms of pneumonia. Legionnaire's disease affects mostly smokers, transplant patients, the elderly and immuno-deficient people. Usually less than 5% of exposed people develop the disease. On occasion, mortality is likely if patients are not appropriately treated, and the fatality rate may reach 15%.

Legionnaires' disease is very treatable with antibiotics (e.g. erythromycin, azithromycin, levofloxacin, etc.) if it is diagnosed quickly. The diagnosis is based on pneumonia symptoms and confirmed with chest x-ray and various laboratory diagnostic tests for evidence of recent exposure to *Legionella* bacteria. Common laboratory tests include isolation and confirmation of *Legionella* bacteria from a clinical specimen; a four-fold increase of antibody titer against *Legionella* bacteria; detection of *Legionella* bacteria in a clinical sample using the direct fluorescent antibody assay (DFA); and the detection of *Legionella* specific antigen in urine (available only for limited *Legionella* species).

Pontiac fever is considered a milder form of the disease caused by *Legionella* bacteria. As indicated by the name, fever and flu-like illness are the primary symptoms of Pontiac fever. This disease is not considered lethal. However, over 95% of the exposed population may develop symptoms after a short incubation period. A full recovery follows a couple of days later.

Regulations And Guidelines

There are currently no federal or State regulations in the United States. However, recommendations to monitor *Legionella* bacteria in water systems have been recommended by State agencies, professional organizations and trade groups. The DOL-OSHA maintains an informative website on *Legionella* bacteria and legionellosis. The web address is <http://www.osha.gov>. In addition to OSHA, the U. S. Environmental Protection Agency (USEPA) and the Centers for Disease Control and Prevention (CDC) have been actively involved in research, water quality and public health issues concerning *Legionella* bacteria and legionellosis. Their websites are www.epa.gov and www.cdc.gov, respectively.

There are no nationally recognized standards or guidelines in the United States for prevention or control of *Legionella*. The federal government included *Legionella* in the OSHA Proposed Rule on Indoor Air Quality and Environmental Tobacco Smoke. The regulation, however, is not likely to be enacted soon. The State of Wisconsin published a set of guidance notes, commonly known as Wisconsin protocol. However, the document covers only cooling towers and emphasizes responses to an outbreak. Furthermore, the document is more than 15 years old and some consider it out of date. The CDC included legionellosis in a set of guidelines for the prevention of nosocomial pneumonia in hospitals and health care facilities.

The American Society of Testing and Materials (ASTM) has produced a Standard Guide (D5952-96) for investigation of legionellosis outbreaks, sample collection, and testing. The document covers clinical diagnosis as well as environmental sampling and is primarily designed to assist in the investigation of outbreaks. The Standard does contain information for routine monitoring.

Recently, the American Society of Heating, Refrigeration and Air-conditioning Engineers, Inc. (ASHRAE) published a new set of guidelines (ASHRAE Guideline 12-2000) on the prevention and control of Legionellosis in building water systems. The document presents the first set of guidelines available in the United States that cover all of the water systems in a building.

Legionella Bacteria In Domestic Water Systems

Legionella bacteria are known to survive and colonize domestic water systems, particularly the hot water system. The colonization of *Legionella* bacteria in the domestic water system poses a significant problem because it is the water source for the occupant's use. In addition, *Legionella* bacteria are difficult to control in the domestic water system because they may hide and survive in the biofilm on the surface of the pipes.

The origin of *Legionella* bacteria in the domestic water system is believed to be the drinking water supply because they are very common in surface water. In general, the concentrations of *Legionella* bacteria in building water supplies are very low. They may not be detectable in routine sampling and testing of the water supply. However, a small amount of *Legionella* bacteria may colonize the water system if they find their way into the system. Older water systems are more likely to have *Legionella* colonization because of thicker biofilm buildup and the increased probability that *Legionella* bacteria may reach the system.

Because of the warm temperature requirements for *Legionella* amplification, hot water systems (as well as the cooling towers) are particularly susceptible. In addition, dead legs and corners of the pipes and the hot water heater, and temperature stratification in the heater can allow *Legionella* bacteria to survive and even amplify. Biofilm on the interior surfaces of the water system is relatively heat inert and can protect the bacteria from exceedingly high temperatures.

Sampling, Testing and Analytical Methodology

Although CDC does not recommend routine sampling and testing for *Legionella* bacteria in building water systems other than hospitals and healthcare facilities, several state and local governmental agencies have recommended guidelines for monitoring and testing water systems for *Legionella* bacteria. It is also a good idea that building owners and operators establish a monitoring and testing program for *Legionella* bacteria in their building water systems, particularly if the buildings are in geographic areas where high incidences of Legionnaires' disease have been reported.

In sampling and testing *Legionella* bacteria in domestic water systems, we recommend that the floor plan or blue print of the domestic water system be located. If the blue print or floor plan is not available, the water systems are identified and a schematic drawing of the systems is marked onto a floor plan. For routine monitoring and sampling, random selection, but inclusive of all water lines and outlets, of sample sites is suggested. Faucets, showerheads, hot water tanks (water heater) and the supply main are common outlets for sampling. In situations where remediation of *Legionella* bacteria in the system is performed, post-remediation quality assurance testing is recommended. For specific sampling, specific outlets at selected locations plus random sampling from the same water system are recommended. This will help to determine whether the contamination is localized or has spread.

Water samples are usually collected for the testing. For domestic potable water systems, a volume of 500 to 1000 ml is collected in a sterile, 1000-ml wide mouth, plastic collection bottle with preservatives (sodium thiosulfate). In addition, swab samples are strongly recommended for collecting biofilm on the interior surfaces in the faucets and showerheads. The swab samples can be directly analyzed or re-suspended and mixed with the water sample. The standard testing method is the culture method (*Procedures for the Recovery of Legionella from the Environment*, 1994.) developed by the Centers for Disease Control and Prevention (CDC). The normal turnaround time is ten days to two weeks. If samples appear negative, the plates are held for observation for at least ten days. If samples are positive for *Legionella* bacteria, results may be available in 7-10 days. The USEPA has developed and patented a Quantitative Polymerase Chain Reaction (QPCR) method, which is based on the exponential duplication of DNA *in vitro*. We have successfully used the method in conjunction with the CDC culture method in several outbreaks in the summer 2003. The PCR method can provide results the same day or the next day. However, the QPCR method is available only for three *Legionella* species at this time, including the most common species *L. pneumophila*.

To indicate the effectiveness of the control measures taken and to provide an early warning of potential problems, all high-risk systems, such as cooling towers and the domestic water systems, should be tested for *Legionella* bacteria on a routine basis (quarterly for normal systems or monthly for towers in healthcare facilities). Common tests performed for water quality of cooling towers, such as pH and heterotrophic plate counts (also known as standard plate counts), have no direct correlation with cooling water containing *Legionella* bacteria. It is also important to point out that testing should be used to complement the maintenance practices being used or to indicate where such practices may be inadequate.

Analyses of samples should always be performed by culture method specifically for *Legionella* bacteria, in accordance with the CDC method. The International Standard Organization (ISO) also published a similar testing protocol. A reputable microbiology laboratory should be used to ensure consistency and quality assurance. There are no more than five qualified, competent commercial laboratories available in the USA at this time. Although the American Industrial Hygiene Association Environmental Microbiology Proficiency Analytical Testing (AIHA EMPAT) program includes *Legionella* bacteria on their list of

potential test organisms, the bacteria have never been officially included in the proficiency testing as of December 2003. The EMPAT program became a part of the Environmental Microbiology Accreditation program in 1999.

While other test methods are available, such as quantitative polymerase chain reaction (QPCR) and direct fluorescent antibody (DFA) assay, DFA is prone to false negatives, false positives, and is often imprecise in the results generated when used directly in environmental water samples. QPCR is also prone to false negatives or false positives unless the analytical laboratories are knowledgeable and proficient in QPCR testing. Because PCR detects DNA and DNA does not cause the infection, PCR testing should always be coupled with the culture method.

Result Interpretation

Interpretation of results is very straightforward. Viable and culturable *Legionella* bacteria of any species and serotype should not be present in building water systems above the limit of detection (1 colony per test or <1 CFU/mL). Several studies have shown that this goal is attainable in a cost-effective maintenance program. An escalating course of action is required depending on the concentration of *Legionella* bacteria detected. If *Legionella* is not detected, the existing testing program and maintenance should be continued. If low levels of bacteria (< 10 CFU/mL) are detected, control measures should be implemented. Please keep in mind that, for convenience, the level is defined based on professional experience. The system should be retested within one to two weeks. If higher levels of bacteria are detected or if bacteria are present in a retest, the system should be re-treated.

Prevention, Maintenance And Control

Because *Legionella* bacteria are ubiquitous at low levels in surface water, it is impossible to prevent them from coming to the building and contaminating the water systems. Therefore, routine and systematic monitoring can serve as an alarm system to determine whether there is contamination and if remediation is necessary or not.

In the event the domestic water systems are contaminated, several remediation and control methods may be used. Three methods are discussed here. They are: super-chlorination, super-heating, and drying and flushing.

The super-chlorination method is to introduce free chlorine (Cl) gas into the water system and allow the increased Cl levels to circulate the entire water system for a few hours. All outlets are opened and flushed so proper disinfection can be achieved. Monitoring of free Cl from selected outlets is done to ensure that there is an effective free Cl level in the water. A free Cl concentration of greater than 5ppm is recommended for super-chlorination. In addition, a chlorine gas injector may be installed to constantly introduce Cl gas into the water system to maintain a free Cl level at 1-2 ppm to control any re-contamination. Chlorine gas, although it is highly effective at concentrations over 5-10 ppm, is corrosive to the plumbing system at the 2 ppm level or higher. In addition, Cl gas is not stable at high temperatures and may produce chlorinated organics, which are potentially carcinogenic.

The super-heating method is to raise the hot water temperature to at least 140°F or 60°C, or preferably to 160°F or 70°C or higher. The hot water is to circulate and flush the entire water system and the outlets for a period of time. There is no standard duration for allowing the super-hot water to flush the system. Flushing for 5 to 30 minutes at 160°F (70°C) or for at least 30 minutes at 140°F (60°C) has been suggested. However, this does not take into consideration the age of the plumbing system and the thickness of accumulated biofilm, which is not a good heat conductor. Our recommendation is to treat and flush the system for one hour for every ten years of age. For example, for a plumbing system between the ages of 11-20 years two hours of treatment and flushing are recommended. In addition to ensuring sufficient duration of flushing, the hot water temperature must be properly maintained. Most hot water heaters or boilers cannot make up the loss of super-hot water during the treatment. We recommend that the flow of hot water be reduced to trickling after the out-flowing hot water reaches the desired temperature (check with a thermometer). The reduction to trickling conserves hot water but maintains the desirable hot water temperature.

Another method, which is new and experimental, is to disconnect the water system, drain all water in the system, and blow hot, dry air through the pipes. After the entire water system is dry, reconnect it to the water source and flushed the system. Because water-borne bacteria are susceptible to desiccation, the hot, dry air likely kills *Legionella* bacteria. The drying process

also facilitates removal of biofilm and scale deposits. Dried biofilm on the interior surface can be easily flushed out and removed from the pipe system.

The three discussed methods eliminate *Legionella* bacteria from the system if they are conducted properly. However, re-contamination is likely. A maintenance method using a copper-silver ionization (Cu-Ag) system can effectively control re-contamination and re-colonization of the system. The Cu-Ag system releases Cu and Ag ions into water as disinfectants. The system has been found effective in maintaining the water system free of re-contamination by *Legionella* bacteria in most cases. However, we do not recommend the system for disinfecting a contaminated system since it may take several months to reduce the *Legionella* bacterial population down to the non-detectable level. In addition, monitoring of Cu and Ag ion levels in the hot water is recommended to ensure they meet the EPA drinking water standards, although hot water is normally not recommended for drinking. The Cu and Ag electrodes should also be removed for cleaning and/or replacement on a regular basis. This system is a good insurance policy against re-contamination in conjunction with one of the three methods discussed above.

There are other methods potentially available for treating water systems contaminated with *Legionella* bacteria. Ultraviolet radiation and ozonation treatments are two methods frequently mentioned. Our evaluations suggest that their disadvantages outweigh the benefits.

Conclusion

At present, there are no Federal regulations applicable to *Legionella* bacteria in domestic water systems or to the prevention and control of legionellosis (except for hospitals and healthcare facilities). At least two guidelines (ASTM and ASHRAE) on dealing with *Legionella* bacteria in building water systems have been published over the last few years. They are useful reference materials. However, each building should have its own *Legionella* control and risk management plan.

The only way to prove that *Legionella* is not present in a water system is to test specifically for the bacteria. A properly-designed testing program will assist in determining if a water treatment and maintenance program is effective for the system. The testing cost is miniscule when compared to the negative publicity and liability associated with and the cost of responding to an outbreak.

Sampling Protocol for Environmental Water for *Legionella* Analysis:

1. Personal safety and precautions should be observed during sampling. Avoid breathing aerosols that may be contaminated with *Legionella* bacteria. Avoid generating aerosols or water mists during sampling of the water system. Turn off the cooling tower fan during sampling, or wear a respirator equipped with a HEPA cartridge.
2. Prepare or obtain sterile 250 ml or 1 liter screw-capped plastic bottles for sampling. Obtain an extra bottle as a field blank. Sodium thiosulfate is routinely added to the bottle as a preservative and halogen (chlorine)-neutralizing agent. A 1 liter water sample is generally recommended for potable water sampling for *Legionella* analysis and 250 ml for non-potable water sampling.
3. For drinking or potable water, such as water fountains, faucets, and showerheads, collect two samples if possible. Collect the "pre-flush or first draw" sample by draining the first 250 (or 1000) ml of water from the faucets or flush drains into a bottle. Allow the water to run for approximately 60 seconds (or longer) and collect the second draw of 250 (or 1000) ml of water. Leave a one-inch space on top of the water sample. The second sample is also called "post-flush or second draw" sample.
4. When sampling faucet aerators and showerheads, swabs of faucet aerators and showerheads should be taken. The swabs should be kept moistened after sampling. The Culturette® kit comes with some wetting agent in the tube for that purpose. The swabs can also be submerged in 5-10 ml of water taken from the same water source (shower) in screw-capped plastic bottles.
5. For non-drinking or non-potable samples from such sources as cooling towers, chillers, condensate pans, surface water in reservoirs, sprinklers, etc., collect 250 ml water from the bottom or side of the vessel or reservoir. Leave a one-inch space on top of the sample. Record any biocide used in water treatment when collecting non-drinking water.

6. Label sample number on the bottle and record on the sample data sheet. Use a distinctive number for each sample. Complete all sample information on a sample data sheet for your own record. Send a copy with the samples to the laboratory.
7. Tightly cap the bottles. Make sure that water does not leak out during shipping and transporting. Taping of bottle around the cap and neck with electric vinyl tape is recommended. Place taped bottles in a clean plastic bag.

Place the samples in insulated boxes to protect specimens from extreme temperature fluctuations. Stuff the box with foam chips to cushion, and seal the box securely for shipping. Send samples by overnight express carrier. Call and inform the laboratory. Schedule sampling between Monday and Thursday so that samples can be delivered to the laboratory no later than Friday. Take holidays into consideration.

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