Legionella Bacteria in the Built Environment

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Since the first documented outbreak of Legionnaire disease in Philadelphia in 1976, new outbreaks have been reported worldwide almost yearly. It is well understood that *Legionella* bacteria, the causative agent of Legionnaire disease, have a worldwide distribution in fresh water and in moist soils. Another form of disease caused by *Legionella* bacteria is Pontiac fever. They are collectively known as legionellosis.

Legionnaire disease is transmitted by aerosols containing living, viable *Legionella* bacteria. Susceptible individuals, including those who have a weakened or suppressed immune system, are particularly vulnerable to the infection, which includes fever and pneumonia symptoms. The susceptible population includes the elderly, AIDS patients, diabetics, organ and bone marrow transplant patients, and smokers. Smokers over the age of 50 are particularly susceptible. Incubation period is usually 2 to 10 days, mostly 5-6 days. The infections can be lethal if patients are not given proper and timely treatment. Several antibiotics are effective in treating Legionnaire disease. There has been no person-to-person transmission reported.

Legionella bacteria grow in fresh water. Hot and cold water systems, cooling towers of the HVAC system and other building-associated cooling water are potential sources for harboring *Legionella* bacteria. The bacteria reproduce and multiply favorably at a warm water temperature of approximately 30-35ºC. This is why it is not uncommon to find outbreaks of Legionnaire disease in the summer season or during extended warm weather. Inhalation of water droplets or mists containing *Legionella* bacteria from contaminated water sources is the primary route of the infection. There have been documented cases showing that *Legionella* bacteria in water droplets can travel and survive over a distance of 250 ft. *Legionella*-containing water droplets that are down drafted from rooftop cooling towers are believed to be a common route of transmission. Some believe HVAC systems are another route of transmission. Another important route is hot water showers. Other sources include whirlpools, vegetable stand sprinklers, spas, pools or industrial waters. The potential is there when there are sources generating water mist or droplets.

There are currently no governmental or professional sampling and analytical protocols for regulatory purposes. For those who are interested in a building evaluation and investigation for *Legionella* bacteria, ASTM and ASHRAE have published documents on the subject. There are currently no governmental or professional numeric standards (such as PEL or TLV) for allowable *Legionella* concentrations in air or water, although attempts have been made by individual consultants or laboratories to establish such standards. Since there are no standardized analytical methods, it is difficult to define a numeric standard.

Although this group of bacteria is ubiquitous and can be lethal, its importance and significance in hospitals, nursing homes, offices and commercial buildings, or even residences has been overlooked by environmental professionals. This technical information sheet discusses and offers guidance to environmental professionals on the sampling and testing for such an important group of bacteria in buildings.

1. Why and when to sample and test for Legionella bacteria

There are at least two reasons for taking samples for *Legionella* testing. The first one is a proactive sampling. Because of the common prevalence of *Legionella* in building water systems, early detection will facilitate better control and management of the bacteria. This will minimize the possibility for the bacteria to settle and establish colonies in the systems, and increase successful control rates. In proactive sampling and testing, ask the laboratory to detect, identify and quantify *Legionella* levels. No serotyping is necessary. This will reduce costs for testing. For cooling water systems, proactive sampling may be performed 2-4 times a year, depending on the geographic location (Northeast US v Texas), frequency of operation of the water systems (a few months a year or all year round), or history (previous *Legionella* infestation or not) of the water system. Those located in the NE can perform their sampling around the warm months (June to October). Two to three samplings are usually sufficient

if the water sources have no history of *Legionella* infestation. Southern locations and those with a *Legionella* infestation history require increasing the frequency of samplings. For building water supply systems, quarterly sampling is recommended. For water systems with a history of *Legionella* infestation, sampling monthly or once every two months is recommended.

The second reason for *Legionella* testing is in response to a report of Legionnaire disease, whether one case or an outbreak. An outbreak is defined as two or more confirmed cases. During the investigation and testing of an outbreak, request that the laboratory identify detected *Legionella* bacteria to species as well as serotype. With this information, an investigator can determine whether *Legionella* bacteria identified in environmental samples are related to clinical specimens or not. *Legionella pneumophila* serotype 1 is the most common strain associated with Legionnaire disease. The concentration of *Legionella* species and their serotypes is also important. Sampling in response to an outbreak investigation is usually done as soon as it is discovered, ideally before any biocide treatments are added. Follow-up samplings are necessary but depend on the progress and need of the investigation. After *Legionella* bacteria are controlled or eliminated from the water systems, a sampling schedule should be prepared. The schedule should include 4-6 samplings (once every two or three months) in the first year. If all samples test negative for *Legionella* bacteria in the first year, follow the proactive sampling for the second year and each year thereafter.

Buildings which known to be occupied by immune-deficient people should have scheduled sampling, whether proactive or responding to outbreaks. Hospitals, health-care facilities, nursing homes, or specialty treatment facilities (such as alcohol and smoking cessation) should have a routine monitoring program. Buildings with a history of *Legionella* bacteria in their water system should have a routine monitoring and treatment program to keep *Legionella* bacteria under control or eliminate them all together.

2. How to Select an Environmental Microbiology Laboratory for Testing of Legionella bacteria

After sample collections, samples must be iced and shipped to a selected environmental microbiology laboratory for testing. Because there are no formal training courses for isolating *Legionella* bacteria from environmental water sources available, it is critically important to select a laboratory that has a qualified staff with demonstrated training and experience in *Legionella* testing and a laboratory quality assurance and quality control program. Dr. Chin S Yang of our company was formally trained with the Centers for Disease Control and Prevention (CDC). A laboratory staff including a microbiologist with advanced degrees, such as Ph.D. or masters, is essential. American Industrial Hygiene Association's (AIHA) Environmental Microbiology Laboratory Accreditation Program (EMLAP) does not include or cover *Legionella* bacteria testing. Most laboratories accredited under the program do not do *Legionella* testing or even know how to do the testing. However, a few laboratories have tried to use the cover for business gains.

3. Selecting sampling methods

For both proactive and outbreak samplings, water and swab samples are recommended. Although air sampling for *Legionella* testing is possible, it is not usually recommended because the chance of detection is very low. Thus, negative results from air samples do not guarantee there are no *Legionella* bacteria in the air.

Water samples are collected from water sources, such as hot water tanks, faucets and shower heads, cooling towers, and other water sources in a building. Large water sources may need more than one sample, depending on the size and configuration of the system. The person performing sampling should make an informed decision. If there are questions on sampling, call us at 856-767-8300 or send an e-mail to Chin.Yang@PrestigeEM.com.

For potable (drinking) water sources, each sample should contain 500-ml or more. For non-potable water, each sample should have 250-ml. Sample bottles must be sterile and contain sodium thiosulfate, which is used to neutralize residual chlorine in water. The bottles are available from our laboratory. Call our laboratory to order. Make sure you inform the lab whether your samples are potable or non-potable, because such information is necessary to determine the best way to process your samples.

In addition to collecting water samples, swab samples are often taken as supplements. Swab samples taken from inside the aerators or shower heads are useful to determine whether *Legionella* bacteria grow there or not. Swab sample kits are available from our laboratory.

All samples should be water-tight and secured in a box or ice chest with ice packs to keep the samples cool. Please **do not** use ice cubes (too wet) or dry ice (too cold). Deliver your samples to our laboratory ASAP.

4. Selecting analyte

It is obvious that *Legionella* bacteria are the analyte of choice. There are two analytical methods for the detection or isolation of *Legionella* bacteria. The first one is the culture method. The second method is the polymerase chain reaction (PCR) method. The culture method is considered the "Gold standard" and has a couple of variations. The commonly used method in the US is the Centers for Disease Control and Prevention method (or CDC method), which was developed during the original investigation of the outbreak in Philadelphia in 1976. The second variant method is the ISO method, which is essentially a modified CDC method. The primary difference is the use of acid treatment to reduce interference by non-*Legionella* bacteria in the CDC method, while both acid and heat treatments were used in the ISO method. Acid and heat treatments are common techniques used in microbiology to reduce interference by junk bacteria.

Treated samples are inoculated onto three selective media, incubated at 35-37ºC for 10-14 days, and examined under microscopes. Suspect *Legionella* bacteria are isolated, subjected to confirmation tests, and identified with the direct fluorescent assay method (DFA). Identified *Legionella* colonies are counted and quantified to CFU/ml or CFU/1,000 ml. DFA is a method that can also be applied directly to the water samples. However, it is not recommended for that application because false positive results are common. The DFA reagents are used to identify presumptive *Legionella* cultures to species, and then to serotype if necessary.

The PCR method was developed relatively recently. This method detects sections of DNA unique to *Legionella* bacteria or to *L. pneumophila*. The method can also quantify the amount of unique DNA in the sample. The primary advantage of this method is its quick turnaround time of 1-2 days. It is also a very sensitive and accurate technique. It can be a very important testing tool during an outbreak situation when information is urgently needed. However, DNA does equal living bacterial cells and does not by itself cause infection. The current PCR test can only detect a few (no more than 3) species of *Legionella* and cannot differentiate *Legionella* bacteria into serotypes.

5. Sensitivity of the tests

The culture method is the gold standard in *Legionella* testing. It provides both qualitative and quantitative sensitivities. However, we have experienced comparative testing situations when several laboratories were asked to analyze samples from the same water source. The inferior laboratories reported negative results, when other laboratories detected the presence of *Legionella* by both culture and PCR methods. The inferior laboratories tended to be those known in asbestos testing, in water testing or in environmental chemistry testing.

Qualitative sensitivities indicate the accuracy and reliability of *Legionella* identification and serotyping. As indicated previously, there are very few trained or qualified microbiologists who are capable of identifying *Legionella* bacteria and their serotypes. Mis-identifications of *Legionella* bacteria are not uncommon. Even experienced microbiologists who have doctoral degree in microbiology will frequently mis-identify non-*Legionella* bacteria as *Legionella* bacteria, or vice versa.

Quantitative sensitivities indicate the accuracy, precision and reproducibility of *Legionella* concentrations. Because the testing typically involves the use of three different selective media, the number of colonies may develop differently on the three media. Laboratory staff must determine which one is a better representation of *Legionella* bacteria in the sample. Inexperienced laboratory staff is likely to under or over estimate *Legionella*

counts in the sample. Finally, quantitative results depend heavily on sample processing in the lab, lab training and experience, and lab QA/QC programs. AIHA's EMLAP does not cover *Legionella* testing. Users should follow the recommendations discussed in ***2. How to Select an Environmental Microbiology Laboratory for Testing of Legionella bacteria?***

6. Field sampling QA/QC

Collection and handling of samples in the field can have a major impact on the samples and their results. It is critically important for sample collectors to observe the following field QA/QC procedures. Aseptic techniques should always be observed during sampling by wearing gloves and cleaning with 70% rubbing alcohol. Under certain situations, sample collectors may want to wear respiratory protection equipment, such as a respirator with a 95% or higher cartridge. The samples should be tightly secured, packed into a cooler, and then shipped with appropriate precautions, such as on ice packs and with plenty of cushion to protect samples from damage, by over night delivery. A complete documentation of samples on a chain of custody is very important.

7. Reporting and laboratory support

A *Legionella* report typically includes both qualitative (ID's) and quantitative results (concentrations). Users of the reports should fully understand how to use the results and what the results mean. Qualitative results may include presumptive identification, species, serotypes, or any combination. Presumptive identification means the isolates are not fully identified through the entire confirmation and identification steps because of unavailability of reagents. Species identification indicates that confirmation and final identification is to the species. Serotypes indicate that identified species are further identified to the subspecies levels in serotypes. There may be one or more than one serotypes in each *Legionella* species. For example, *Legionella pneumophila* may have as many a 15 serotypes.

Quantitative results include concentrations of *Legionella* bacteria in CFU/ml, CFU/100ml, or CFU/L. Because these microbes can grow and can die, their concentrations may change over time. Users should understand the relativity of such data. There are no numeric standards or guidelines for Legionella bacteria in water. Laboratory personnel should be available to assist environmental consultants on the interpretation of the results. There are questions including why, how, and what *Legionella* bacteria like to grow in building water systems. Good laboratories can also provide useful information on the control and elimination of *Legionella* bacteria from the water systems.

8. Result interpretation

As in most microbiological testing, qualitative results are more important than quantitative results. Identification to species and serotype from culture methods indicate positive results for the presence of living *Legionella* bacteria. The results, particularly serotypes, are useful in establishing the relationship between environmental and clinical strains in an outbreak. Quantitative results are also important. However, numbers are relative in microbiology. *Legionella* bacteria in the water source can change due to reproduction or death. Samples taken from the same source but on different dates may yield different results, particularly quantitative ones. Quantitative results reflect *Legionella* concentrations on the date of sampling. The concentrations may be different on the date laboratory results become available. If biocide treatments are used, differences in both qualitative and quantitative results can be obvious and significant.

Results from the PCR method also include qualitative and quantitative ones. However, PCR results yield limited detection capability of probably no more than three species. More importantly, serotyping is not available at this time and DNA does not cause infection.

9. Conclusions

There are very few environmental or medical microbiology laboratories capable of analyzing environmental samples for *Legionella* bacteria. Users should do their homework and use due diligence by talking to and visiting with the laboratory.

Current analysis is based on the CDC (2005 version) or ISO culture method. Because CDC was and still is the pioneer in the investigation, detection and identification of *Legionella* bacteria, we believe in using the CDC method. The PCR method is a useful tool and can be used under certain circumstances. However, users should understand the pros and cons of using PCR method and the results.

There may be additional methods used by individual laboratories. Users should be aware of their reliability and usefulness. There are no more than five environmental laboratories in the US that are capable of analyzing environmental samples for *Legionella* bacteria with confidence. Users should do their homework.

Collecting Samples for the Analysis of *Legionella* Bacteria in Building Water Systems

During preparation of sample collection, please make sure taking your safety into consideration. You should prepare to wear a fit-tested HEPA respirator during sampling when *Legionella*-containing aerosols may be generated. Cooling tower fan should be turned off when collecting samples. Protective gloves should be woven during sampling.

Cooling towers or recycled industrial water reservoirs: To collect samples from these water sources, use sterile 250-ml wide mouth plastic bottles. Take water from each reservoir as well as from drains and make-up water supply. Record the water temperature. If biocides are used, record trade names as well as chemical names of the biocides.

Hot and cold (pot) water systems: To collect samples from these water sources, obtain sterile 500ml or 1,000ml wide mouth plastic bottles. Consider taking 1st and 2nd draw (pre-flush and post-flush) samples. 1st draw sample is collected after the water system has been idled for at least overnight. 2nd draw sample is taken after the 1st draw sample and the outlet has been drained for at least one minute or until hot water is felt for a hot water system. Take samples from faucets, shower heads, water mains, hot water tanks, and all outlets of the water systems.

Other water sources: To collected water samples from sources other than the two discussed above, take 250ml samples if the water is considered non-potable or 500-1000ml if it is potable. Non-potable water may include condensate drain pans, fire suppression reservoirs, decorative fountains, whirl-pools and Jacuzzi, etc. Other potable water sources may include well water and drinking fountains.

Collection bottles: 250-ml, 500-ml and 1000-ml wide mouth plastic bottles are available from our laboratory. They contain sodium thiosulfate for neutralization of halogens (such as Cl or Br) and are sterilized. Call our lab as soon as your collection is scheduled.

Documentation: Label your samples with a unique number for each sample and record the numbers on a Chain-of-Custody sheet. Complete all chain-of-custody information for sample submittal. Record water temperature if a thermometer is available. Make sure to disinfect the thermometer with 70% alcohol between samples. Record trade names and chemical names of any biocides used in the cooling water system. Make notes of any changes and treatments to the water systems prior to sampling.

Packing and shipping samples: Tighten and secure screw cap of each bottle to avoid leaks. Place and pack samples in coolers or insulated boxes for shipping. Put ice packs (never use dry ice or ice cubes in a plastic bag) in the coolers or insulated boxes, particularly in the summer. Place additional packing materials, such as paper, styrofoam peanuts, or plastic air packs, to cushion and protect samples. Ship samples by overnight express curriers or deliver them in person same day or next day. Call our lab to forewarn us incoming samples.

Additional considerations in *Legionella* sampling are:

1. Routine maintenance sampling
2. Potable v. non-potable water sources
3. Disease outbreaks v. proactive & preventive sampling.

It is highly recommended that building managers conduct routine maintenance sampling for *Legionella* analysis to monitor their water systems. This is particularly important for health care facilities, such as nursing homes and hospitals. It is well understood that *Legionella* bacteria are common in natural and man-made water system even if a building has had no report of Legionellosis or Legionnaire's disease. Routine maintenance sampling offers the opportunity to detect *Legionella* bacteria at an early stage of contamination and colonization. Once *Legionella* bacteria are detected, the system can be quickly and easily treated and rid of the bacteria. Typically, routine maintenance sampling should be done at least twice a year depending on the use, maintenance and history of the systems. If a water system has previously been tested for *Legionella* bacteria and the results have always been negative, sampling every six months should be able to provide sufficient warning. Cooling towers that are operated only during cooling season are sampled in mid- (June or July) and late (August or September) cooling season. If cooling towers are operated year round, additional samplings are necessary.

Potable or drinkable water sources, particularly hot water lines, are susceptible to colonization by *Legionella* bacteria. However, their concentrations are usually, but not always, low and lower than non-potable water sources, such as cooling towers. To facilitate better detection, potable water samples are usually concentrated by filtration. A 500 to 1,000ml water sample is recommended. Some suggest that 1st and 2nd draw (pre-flush and post-flush) samples should be collected from water outlets, such as shower heads or faucets. To collect 1st draw sample, one should prepare to take the sample after the water system has been idled overnight. After the first draw sample, take the second draw sample after allowing the water outlet to run for at least a one-full minute. For hot water system, collect the second draw samples until the water is hot. Swab samples may also be collected from inside of the faucet or shower head. Slime inside the faucet and shower head may harbor significant number of *Legionella* bacteria. For non-potable water, such as HVAC condensate water, reservoir water, or fire suppression water, decorative fountain water, whirl pool and Jacuzzi water, etc, collect 250ml water in a sterile container.

In a disease outbreak (defined as two or more confirmed cases of Legionnaire disease) investigation, a systematic inspection and sampling must be carried out. Identify all water sources and outlets in the building. Collect samples from every water sources and every outlets according to the discussions above. Make sure all personal safety and precautions are observed because the chance of contracting aerosolized *Legionella* bacteria is high.

In hospitals or other health care facilities where elderly or immune-suppressed populations are common, proactive & preventive sampling for *Legionella* testing is highly recommended. The State of New York Department of Health and other governmental health agencies have issued directives recommending proactive monitoring for *Legionella* bacteria in water systems in health care facilities as a part of nosocomial infection control protocols. Commercial gyms or health and wellness centers where whirl-pool and Jacuzzi are equipped should consider proactive monitoring for *Legionella* bacteria. Hotels should also have their water systems proactively monitored.

Additional scenarios may occur. Call us at 856-767-8300 to discuss.