Legionella Questions and Answers
Public Health Ontario

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ORGANISM QUESTIONS

1. HOW DO LEGIONELLA BACTERIA ENTER THE POTABLE WATER SYSTEM?

Legionella bacteria are widespread within the natural water environment and in most soils and mud. The bacteria are fairly resistant to conventional chlorination and thus enter hot and cold water plumbing systems, cooling towers and whirlpool spas from rivers, lakes, ponds and reservoirs. Unless appropriate control measures are in place, given favourable conditions the bacteria may multiply, increasing the risk of exposure to aerosols containing Legionella.

2. WHAT FACTORS FAVOUR THE GROWTH OF LEGIONELLA

There are a number of factors that provide an environment for Legionella to multiply:

- Temperature appears to be one of the most important influences as to whether or not the bacteria will grow. Legionella can survive and multiply between 25-45°C with an optimal temperature between 32-42°C. This demonstrates why cooling towers, hot and cold water systems, spa pools and humidifiers provide ideal environments for Legionella to grow. Legionella can withstand temperatures of up to 50°C for several hours, but are destroyed within a few minutes at 60°C. Although it is uncommon to find proliferation below 20°C, Legionella can remain viable and dormant in cool water, multiplying when the temperature reaches a suitable level and when not prevented from multiplying by adequate disinfection. Arvand et al., in a study of cold water systems in health-care facilities, found that 94/265 (35%) and 43/156 (28%) of distal cold water samples that displayed a temperature of <20°C and <15°C were contaminated with Legionella. The authors concluded that the cold water supply of health-care facilities may be heavily contaminated with Legionella species.

- Another factor that is important for the survival and proliferation of Legionella is the formation of biofilms. A biofilm is a slimy lining the inside walls of water pipes, air conditioners, cooling towers, whirlpools, showerheads, taps and humidifiers. In addition to providing nutrients, the biofilm protects the Legionella bacteria from external stressors such as disinfectants; increases in temperature; and attempts at physical removal, especially in areas where surfaces are scaled (usually a function of water hardness) or corroded. The presence of scale and corrosion in a system will increase the available surface area and encourage the formation of biofilms. Preventing the growth of biofilms is an important control measure against proliferation of Legionella because once established, biofilms are difficult to remove.

- The growth and survival of Legionella are promoted by their ability to incorporate themselves and rapidly multiply within certain species of protozoa also found in biofilm. Once these protozoa die, a large number of Legionella bacteria will be released into the water.

- The materials used within a system may also be a factor favouring Legionella growth. For example, natural organic compounds such as rubber gaskets and hoses provide a good nutritional source for bacteria and other microorganisms to grow.
• The build-up of sediment can harbour *Legionella* bacteria and also provide a nutrient source for them.7

**EXPOSURE QUESTIONS**

3. **IS THERE ANY LITERATURE TO SUGGEST THAT NURSING HOME RESIDENTS WOULD BE AT RISK OF LEGIONNAIRES’ DISEASE IF THEY WERE TO USE TUB BATHING?**

Bathroom facilities such as bathtubs and faucets are sources of *Legionella*-containing bacteria and it is possible that aerosols produced during faucet use might be a cause of *Legionella* exposure.8 The risk of exposure will depend on the design of the faucet and the water flow rate.8 Caution is advised since it may not be possible to fill a tub without aerosolization occurring, because when the water pours in, it will splash when it hits the hard surface of the tub.

There is evidence showing that hot water taps containing *Legionella* aerosolize a low number of the organisms during routine use. Thus the use of hot water faucets is a plausible means of transmission of *Legionella* from potable water to resident.9

**ENVIRONMENTAL SAMPLING QUESTIONS**

4. **WHAT METHOD SHOULD BE USED FOR IDENTIFYING LEGIONELLA FROM ENVIRONMENTAL SAMPLES?**

Bacterial culture is the current standard for identifying *Legionella* in environmental samples. Standardized culture methods include ISO (International Organization for Standardization) 11731: “Detection and Enumeration of *Legionella*” and CDC (Centers for Disease Control and Prevention): “Procedures for the Recovery of *Legionella* from the Environment”. Laboratories chosen for processing of environmental samples should be certified for proficiency in the CDC Environmental *Legionella* Isolation Techniques Evaluation (ELITE) program (or an equivalent scheme accredited to ISO 17043:2010).

Culture is essential for identifying and typing *Legionella* strains during outbreaks.10 However, culture is time-consuming because of the slow growth rates of *Legionella*, and results may take 10 - 15 days. Standard culture techniques can also be complicated by difficulties in isolating *Legionella* in samples with high background levels of other microorganisms which can inhibit *Legionella* growth, or in situations where *Legionella* are protected within amoebae or protozoa. Additionally, the culture methods used for isolation of *Legionella* species were initially developed for the growth of *Legionella pneumophila* (as this is the species responsible for the majority of all outbreaks) and may not detect other disease-causing *Legionella* species present.4

Because of these limitations, polymerase chain reaction (PCR), is now widely used as an alternative method to detect and enumerate *Legionella*.7 PCR is a molecular test that amplifies deoxyribonucleic acid (DNA) from the microorganism under investigation. When using PCR to identify *Legionella*, the test is designed to detect DNA sequences specific for *Legionella* species. Although PCR is rapid and highly sensitive and specific, it cannot differentiate between viable and non-viable *Legionella* and there is no
consensus on when and how it should be used, or how to interpret the results. Therefore, although PCR may be useful for identifying potential sources of *Legionella* and monitoring the effectiveness of treatment programs, culture remains the “gold standard” for *Legionella* detection and enumeration.

**Public Health Ontario Laboratories (PHOL) does not perform PCR on environmental samples; however, it does utilize PCR technology when assessing respiratory specimens (see Question 14).**

### 5. HOW MANY SAMPLES SHOULD BE TAKEN WHEN MONITORING FOR *LEGIONELLA* IN ENVIRONMENTAL SPECIMENS?

The purpose of sampling and testing for the presence of *Legionella* bacteria is to establish the source of infection or to monitor the effectiveness of preventative measures. However, it is recognized that the number and type (water or swabs) of samples that are taken should be determined on an individual system basis following a thorough risk assessment of the water system and the identification of sites that pose the highest risk.

In their guidelines for the control of *Legionella* bacteria, both the European Working Group for *Legionella* Infections (EWGLI) and New Zealand’s Ministry of Health recommend that when performing environmental sampling, multiple sample sites should be chosen, and be representative of all areas where *Legionella* can live and grow. This includes areas where water temperatures will support growth, areas with stagnant water, areas where aerosols are created and areas most likely to have vulnerable populations. In terms of the number of samples to take, it has been suggested that if the facility (hospital) has less than 500 bed than 10 sites should be sampled. For greater than 500 beds, then the number of samples should be increased by two additional sites per 100 beds over 500.

**Public Health Ontario’s (PHO) Public Health Inspector’s Guide offers advice on how sampling for *Legionella* should be collected and includes a list of potential sampling sites.**

It is important to use the sterile containers provided by the laboratory as they contain sufficient sodium thiosulphate to neutralize any oxidizing biocides such as chlorine.

### 6. WHAT ARE THE BEST PRACTICES SURROUNDING ROUTINE ENVIRONMENTAL SAMPLING FOR *LEGIONELLA* IN HEALTH CARE FACILITIES, PARTICULARLY LONG-TERM CARE FACILITIES (LTCFS) AND RETIREMENT HOMES (RHS)?

Routine sampling of water for *Legionella* in health care facilities is a controversial issue with differing approaches advocated.
Disease surveillance
One approach touted by some organizations, including the CDC and the Florida Department of Health, advocates a disease surveillance strategy. This disease surveillance approach involves health care facilities maintaining a high degree of awareness for legionellosis among patients presenting with health care-associated pneumonia. Only when a *Legionella* outbreak is identified is environmental sampling initiated to identify the source of the *Legionella* and to assess the efficacy of remedial action.\(^{15,17}\) With the exception of facilities that have a transplant unit, the CDC does not recommend routine sampling for *Legionella*.

Transplant patients are at high risk for legionellosis because of their severely immunocompromised status; therefore, culturing of the potable water system should be considered a prevention strategy in these patient areas.\(^{18}\)

The main argument for this is as follows:

- Environmental sampling results are hard to interpret because of the ubiquitous nature of *Legionella* in hospital water systems.

- The concentration of *Legionella* in a water sample required to produce illness is unknown, i.e. there is no threshold level above which illness is known to occur.\(^4,19\)

- If *Legionella* is found during routine sampling, there may be pressure to unnecessarily try to eradicate the organism. It is important to keep in mind that *Legionella* may be present in water without causing disease. The risk of illness following exposure depends on many more factors than just the presence of *Legionella*, including strain virulence, host susceptibility, whether the *Legionella*-containing water could be aerosolized to a respirable size, and the survival of the organism in the aerosol.\(^3,17\)

- Negative routine testing results for *Legionella* may lead to complacency, and is not a substitute for primary prevention through good water system design, proper operation and maintenance practices, and effective water treatment.\(^{17}\)

Routine water monitoring approach
An alternative strategy of routine water monitoring approach has been proposed by Stout et al.\(^{18}\) This strategy advocates that routine environmental cultures for *Legionella* colonization are necessary to assess the risk of *Legionella* should be conducted even if no cases are detected. The main arguments for this approach are as follows:

- *Legionella* colonization will vary over time\(^{18}\)

- LD has not been reported in health care facilities where *Legionella* has not colonized the water system\(^{20}\)
- Positive environmental sampling results create a high degree of suspicion for legionellosis and active surveillance for cases can be initiated.
- Routine sampling allows evaluation of the effectiveness of preventative measures taken to control the growth of Legionella.

It is important to recognize that water testing by itself does nothing to reduce risk. It is only when steps are taken to identify water systems that present a significant risk to users and follow up action is taken to reduce that risk that we have an effective preventive program. To achieve that objective, several organizations use a hazard analysis and critical control point (HACCP) type approach to Legionella in water systems.

High risk areas
- There is overall consensus from public health authorities including WHO, CDC and HSE for routine environmental sampling in health care facilities that house people with increased susceptibility to Legionella such as transplant patients.
- Routine testing for Legionella is not conducted in isolation but as part of an overall risk management approach required to control Legionella growth and prevent legionellosis. WHO terms this risk management approach a “water safety plan (WSP).”
- The WSP is based on HACCP risk management principles. The key components of a WSP are provided in the World Health Organization’s guidance document, Legionella and the prevention of legionellosis. Monitoring for Legionella may be used to provide evidence that the WSP is effective and control measures are operating within specified target parameters.
- WHO notes that in high risk areas such as transplant units, water from outlets should be Legionella free (not detected). The CDC also notes that due to the lack of immune function in patients in these specialized parts of the hospital, eradication of Legionella from the potable water system should be the goal.

RHs and LTCFs
While there is no consensus on the need for routine environmental sampling in RHs and LTCFs, there is recognition that Legionella growth in the water system may result in the generation of infectious aerosols.

Furthermore,
- It is known that RHs and LTCFs tend to house older people with predisposing factors to Legionella infection and that sporadic cases of legionellosis may be under-recognized in this population.
Outbreaks of legionellosis have been reported among residents of LTCFs (24;25) and RHs although less frequently.3;26 Silk et al. identified the potential for outbreaks in this vulnerable population and suggested that retirement homes should consider sampling of the potable water in the context of a Legionella water safety plan (WSP).26 This recommendation was also echoed by Cristino et al. who noted the successful control of Legionella by four LTCFs who implemented environmental routine sampling as part of their overall WSP.21 Although these two authors recommend RHs and LTCFs develop a HACCP type WSP that where warranted may consider routine environmental sampling, they do not recommend routine water monitoring outside a WSP.

A prudent approach advocated by the UK Health and Safety Executive (HSE) in 2014 is to conduct a local risk assessment (RA) as an integral part of an overall water safety plan (WSP) to determine if Legionella monitoring is appropriate in the RH or LTCH.27

Public Health Ontario Laboratories do not provide testing of samples collected for routine environmental monitoring for Legionella, nor do they perform enumeration of colony forming units (CFU) when culturing for Legionella. If this testing is desired, another laboratory should be used for these services. The laboratory selected for processing of environmental samples should be certified for proficiency in the CDC Environmental Legionella Isolation Techniques Evaluation (ELITE) program (or an equivalent scheme accredited to ISO 17043:2010).

Based on the RA findings, a decision concerning environmental monitoring for Legionella can be made. According to the HSE, the circumstances when monitoring for Legionella may be appropriate include:

- where the potable hot water is stored or distribution at reduced temperatures
- water systems where the temperature or disinfectant concentrations are inadequate or not being consistently being achieved
- where there is a population with increased susceptibility to Legionella

**7. SPECIFICALLY, IN TERMS OF COOLING TOWERS, HOT AND COLD WATER SYSTEMS, AND WHIRLPOOLS, WHERE SHOULD SAMPLES BE TAKEN?**

**Cooling towers**
The New Zealand Ministry of Health suggests that samples should be taken from the sump water. If this is not practical, then the circulating water should be sampled as near to the heat source as possible.14;28

**Hot and cold water systems**
The Health and Safety Executive (HSE) proposed draft document “The control of Legionella bacteria in hot and cold water systems” states, wherever possible, samples should be taken as follows:27

- From separate hot and cold outlets which are not blended or thermostatically controlled
• In both hot and cold water systems
  o from areas where the control parameters are not met (i.e. where disinfectant levels are low or where temperatures are below 50°C for hot water systems or exceed 20°C for cold water systems)
  o from areas subject to low usage, stagnation, dead legs, blind ends, excessive heat loss

• In cold water systems, samples should be taken:
  o from the point of entry (or nearest outlet) if the water is from a private water supply or where the temperature of the incoming mains supply is above 20°C from the cold water storage tank or tanks
  o from the furthest and nearest outlet on each branch of the system

• In hot water systems, samples should be taken from:
  o the calorifier (hot water tank) hot water outlet
  o the base of the calorifier
  o the furthest and nearest outlet on each branch of a single pipe system
  o the furthest and nearest outlet on each loop of a circulating system

**Whirlpool spa**
Water samples should be collected from the pool, filter housing and balance tank. Filter material and biofilm from inside air jets, hoses, taps and pipes may also contain large numbers of *Legionella* and should be sampled by swabbing.\(^3\)

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**Because biofilm shields Legionella and enhances its multiplication, Ta et al. and the WHO recommend that swabs be taken in conjunction with water samples from sites where biofilms are likely to form; i.e., cooling tower sumps, potable water faucets, hoses, showerheads, and whirlpool spa filters.**\(^3,29\) **Research has shown that the number of Legionella organisms from biofilm swabs is greater than the number of Legionella organisms sampled from water.**\(^29\) **This can be explained by the fact that the swab technique results in direct sampling of the organisms present in the biofilm, which gives a greater yield than sampling of water.**\(^30\) **It has been recommended that swab samples be collected as part of any environmental Legionella sampling protocol.**\(^29\)
8. WHAT SITES SHOULD BE SAMPLED DURING A LEGIONELLA OUTBREAK?

Before samples are collected it is essential to involve PHOL at the onset, not only to make them aware of the outbreak and to allow them to prepare, but also to obtain advice on the number and types of samples required.

The PHO Public Health Inspector’s Guide to the Principles and Practices of Environmental Microbiology outlines when testing will be performed and provides basic guidance on the number of samples required, selection of sampling sites, sampling collection procedures and transportation and interpretation of the results. The guide is available at: http://www.publichealthontario.ca/en/eRepository/Public_Health_Inspectors_Guide_2013.pdf

According to the New Zealand Ministry of Health, during an outbreak, sample sites should be chosen to be representative of all the identified risk areas where *Legionella* can reside and grow. Selecting sample sites will require a detailed understanding of the layout of the water system. For large sites, taking numerous samples may be necessary. There may be no information available on the water system layout, or a risk assessment may not have previously been done. If this is so, a risk assessment must be done in order to support the outbreak investigation. Consideration should be given to the following:

- areas that contain water at temperatures likely to support the growth of *Legionella*
- cross-contamination between ‘dead’ (still or stagnant) and ‘live’ (flowing) water
- locations where water aerosols can be created such as showers and taps, decorative fountains, whirlpool spas, cooling towers and humidifiers

In the case of outbreak investigations involving patients, environmental sampling (water and swab) should involve water outlets (showers and taps) in the immediate environment of a suspected case.

The New Zealand Ministry of Health requires that duplicate samples be taken during an outbreak of Legionnaires’ disease because of variation in *Legionella* numbers in samples drawn from the same source at different locations around the same time. This is reasonably explained by the fact that *Legionella* bacteria are not continuously shed from biofilm. Release depends on mechanical and physical disruption, and sloughing of the biofilm.
9. FOR COOLING TOWERS, HOT AND COLD WATER SYSTEMS, AND WHIRLPOOLS, WHAT IS THE RECOMMENDED FREQUENCY OF SAMPLING, WHAT ARE THE ACTION (CRITERIA) LEVELS AND WHAT ACTIONS SHOULD BE TAKEN WHEN LEGIONELLA IS DETECTED?

PHOL does not perform enumeration of colony forming units (CFU) when culturing for Legionella. Samples are reported as being just positive or negative for Legionella.

Cooling Towers
The most recent document on the control of Legionella bacteria in cooling towers was published by the HSE. The HSE warns, “The absence of Legionella in sampling results does not indicate the absence of risk. Sporadic Legionella positive results are not uncommon and, provided the total viable counts (TVCs) and biocide control are good, are not cause for concern. However, repeated Legionella positives, or positives plus poor biocide control and/or poor TVC are (a potential concern), and should be investigated”.

Table 1 shows the sampling frequency, action levels and response to Legionella analysis results.
Table 1: Frequency, action levels and comments in response to *Legionella* analysis results

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Legionella Action level cfu/L</th>
<th>Comments and action required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monthly</td>
<td>Not detected or up to 100</td>
<td>Focus on maintaining control measures.</td>
</tr>
<tr>
<td></td>
<td>&gt;100 and up to 1000</td>
<td>Low-level <em>Legionella</em> count detected. This may be a sporadic result or could indicate a persistent problem. Reassess the control program. Ensure the water treatment system is operating correctly. Adjust the biocide dosage if the general aerobic count does not indicate good control (&lt; 10 cfu/L). Re-sample to verify the initial result and then again to check that remedial actions are effective.</td>
</tr>
<tr>
<td></td>
<td>&gt;1000 or persistent low-level results</td>
<td>Immediate action required. Re-sample, and as a precautionary measure, shock dose the water system with an appropriate biocide or increase the level of continuous dosage of biocide. Reassess the entire control program and take any corrective actions. Re-sample the system to verify the count and to determine the effectiveness of the corrective action, Re-sample again within 48 hours. If the high <em>Legionella</em> counts persist, review the risk assessment to identify further remedial actions.</td>
</tr>
</tbody>
</table>

Once the water system is colonized with *Legionella*, it may prove extremely difficult to reduce numbers to undetectable levels and periodic positive *Legionella* results may recur. Under such circumstances steps should be taken to make sure the risk assessment reflects this and control measures should be devised to ensure that, although likely to be present at low levels, *Legionella* cannot multiply to dangerous levels.
**Hot and cold water systems**

The HSE’s technical document “The control of *Legionella* bacteria in hot and cold water systems”, due to be published in spring 2014, gives guidance on *Legionella* monitoring. It does not recommend routine monitoring but suggests that monitoring for *Legionella* should be carried out where control levels of the treatment regime (e.g. temperatures, disinfectant concentrations) are reduced or not being achieved. The HSE also notes that it might be appropriate to consider monitoring *Legionella* in some high risk situations, such as certain health-care premises. Guidance on the sampling frequency, action levels, and action to be taken if *Legionella* is found in the water system, is shown in Table 2. For health-care premises with vulnerable patients, the action levels and recommended actions are shown in Table 3.

Table 2: Action levels following *Legionella* sampling in hot and cold water systems

<table>
<thead>
<tr>
<th>Circumstances when monitoring for <em>Legionella</em> would be appropriate</th>
<th>Legionella bacteria action level (cfu/L)</th>
<th>Recommended actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>For water systems treated with biocides, where water is stored or distribution temperatures are reduced to &lt;50°C to prevent scalding for example. Monthly testing is recommended and the frequency of testing reassessed when the results indicate the effectiveness of the regime.</td>
<td>&gt;100 but &lt;1000</td>
<td>Either:</td>
</tr>
<tr>
<td>Where there is doubt about the efficacy of the control regime, e.g. temperature or disinfectant concentrations are not being consistently achieved. Frequent testing, e.g. weekly, should be carried out until the system is brought back under control</td>
<td></td>
<td>a) If only one or two samples are positive, system should be re-sampled. If a similar count is found again, a review of the control measures and risk assessment should be carried out to identify any remedial actions necessary.</td>
</tr>
<tr>
<td>In some high-risk areas such as where there is a population with increased susceptibility (frequency not specified)</td>
<td></td>
<td>b) If multiple samples are positive, the system may be colonized with <em>Legionella</em>. Disinfection of the system should be considered but an immediate review of control measures and risk assessment should be carried out to identify any other remedial action required.</td>
</tr>
<tr>
<td>During a suspected outbreak</td>
<td>&gt;1000</td>
<td>The system should be re-sampled and an immediate review of the control measures and risk assessment carried out to identify any remedial actions, including possible disinfection of the system. Retesting should take place a few days after disinfection and at frequent intervals thereafter until a satisfactory level of control is achieved.</td>
</tr>
</tbody>
</table>
Table 3: Actions to be taken following *Legionella* sampling in hot and cold water systems in health-care premises with susceptible patients

<table>
<thead>
<tr>
<th><em>Legionella</em> Count (cfu/L)</th>
<th>Recommended actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 100 cfu/L</td>
<td>In health care, the primary concern is protecting susceptible patients, so any detection of <em>Legionella</em> should be investigated and, if necessary, the system re-sampled to aid interpretation of the results in line with the monitoring strategy and risk assessment.</td>
</tr>
<tr>
<td>&gt;100 cfu/L and up to 1000 cfu/L</td>
<td>If only one or two samples are positive, the system should be re-sampled. If a similar count is found again, a review of the control measures and risk assessment should be carried out to identify any remedial actions to be taken. If multiple samples are positive then the system may be colonized with <em>Legionella</em>, albeit at a low level. An immediate review of control measures and risk assessment should be carried out to identify any other remedial action required, which may include disinfection of the system.</td>
</tr>
<tr>
<td>&gt;1000 cfu/L</td>
<td>An immediate review of the control measures and risk assessment should be carried out to identify any remedial actions, including possible disinfection of the system. The system should be re-sampled. Retesting should take place a few days after disinfection and at frequent intervals thereafter until a satisfactory level of control is achieved.</td>
</tr>
</tbody>
</table>

**Whirlpool Spas**

Although there is limited risk of significant microbial contamination in a well-managed disinfected whirlpool spa, a variety of public health agencies and government agencies have recommended that in addition to monitoring physicochemical parameters and proper cleaning and maintenance, *Legionella* monitoring should be carried out at appropriate intervals. The reason for this is to ensure that optimum water treatment conditions are being maintained, and to validate the effectiveness of the maintenance measures. Table 4 gives examples of standards in the United Kingdom and by the WHO.

Table 4: Action level, frequency of testing and action required by various organizations for whirlpool spas

<table>
<thead>
<tr>
<th>Organization</th>
<th><em>Legionella</em> action level cfu/mL</th>
<th>Frequency of testing</th>
<th>Action required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health Protection Agency (UK)</td>
<td>&lt;100</td>
<td>Quarterly</td>
<td>Under control.</td>
</tr>
<tr>
<td></td>
<td>&gt;100 but &lt;1000</td>
<td></td>
<td>Disinfect, drain, clean. Review control and risk assessment, and carry out remedial actions identified. Refill and retest next day and 2-4 weeks later.</td>
</tr>
<tr>
<td>Organization</td>
<td>Legionella action level cfu/mL</td>
<td>Frequency of testing</td>
<td>Action required</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>--------------------------------</td>
<td>----------------------</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>&gt;1000</td>
<td></td>
<td>Immediate closure, disinfect, drain, clean. Review control and risk assessment, and carry out remedial actions identified. Refill and retest. Keep closed until results are negative and risk assessment is satisfactory.</td>
</tr>
<tr>
<td>World Health Organization ³</td>
<td>No detectable count should be the goal</td>
<td>Monthly</td>
<td>Failures should be investigated and the effectiveness of any remedial work should be monitored.</td>
</tr>
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10. WHAT IS THE RE-SAMPLING PROTOCOL FOR LEGIONELLA WHEN POST-TREATMENT SAMPLES COME BACK POSITIVE FOR LEGIONELLA AND WHEN ALL SAMPLES EVENTUALLY COME BACK NEGATIVE?

When a source has been identified and remediated following an outbreak, there is a clear need for testing for Legionella to ensure that the implemented control measures are effective. In a recent outbreak investigation the CDC recommended that testing for Legionella should be conducted every two weeks for three months, and then every three months to ensure that the remediation has been effective.23 The CDC recommends that any Legionella detected during this time frame must be re-remediated (using a modified remediation procedures) and the test cycle restarted.23 It must be recognized that once Legionella has colonized a water system eradication through disinfection is generally unachievable.34 One reason for this is that biofilm protects the Legionella bacteria from external stressors such as disinfectants; increases in temperature; and attempts at physical removal, especially in areas where surfaces are scaled (usually a function of water hardness) or corroded.3;6 Another explanation is that Legionella can incorporate themselves within protozoa also found in biofilm, some of which are very resistant to high temperatures and other disinfection procedures.3

It should be noted that post treatment, restarting a system such as a cooling tower can create conditions that are particularly favourable for detaching Legionella contaminated biofilm and airborne release35

11. HOW DOES ONE INTERPRET A POSITIVE ENVIRONMENTAL SAMPLE RESULT?

Legionella is widespread in the environment and a positive result does not necessarily imply that it is likely infection will occur. The factors that lead to outbreaks or cases of Legionnaires’ disease are not completely understood, but certain features are necessary for infection.1 These include:

- the presence of virulent bacteria in an aquatic environment
- amplification of the bacterium to an as yet unknown infectious dose
- transmission of the bacteria via aerosol to a susceptible human host

A number of guidelines and control strategies aimed at reducing the risk of Legionnaires’ disease are based on the concept that Legionnaires’ disease is a preventable illness; i.e., controlling or eliminating the bacteria in certain reservoirs prevents the disease. Some guidelines (Allegheny County for example) do not insist on having culture-negative water systems. They recognize that because of its widespread nature, persistence of Legionella in many instances will be inevitable, and may be of minimal significance from a public health standpoint. These guidelines recommend taking remedial action if 30% of environmental samples are positive for Legionella (see box below). On the other hand, the New Zealand Ministry of Health notes that “Legionella should not be detected in any wet cooling system at any time.” They note that whenever Legionella is detected the appropriate remediation is required to ensure the system is brought back under control.14
Several scientific guidelines propose taking remedial action based on the prevalence of *Legionella* in a hospital’s domestic water supply. It is assumed that if 30% of environmental samples are positive for *Legionella* then an elevated risk of health-care-acquired Legionnaires’ disease exists. This metric was first proposed in 1983, and is still cited in guidelines and research, examining the relationship between *Legionella* concentrations in water and health-care-acquired Legionnaires’ disease. However a recent study shows that the proposed 30% positivity metric has 59% sensitivity and 74% specificity (i.e., a 41% false-negative rate and a 26% false-positive rate). The authors concluded that the possible consequence of using a percent positivity metric with low sensitivity and specificity is that many hospitals might fail to mitigate when a true risk is present, or might unnecessarily allocate limited resources to deal with a negligible risk.

**CLINICAL ASPECTS QUESTIONS**

12. **HOW LONG SHOULD THE EXPOSURE HISTORY LOOK BACK?**

Uncertainty over the range of the incubation period exists. Initially, the incubation period for *Legionella* was thought to be 5 to 6 days, with a range of 2 to 10 days. However, evidence from some outbreaks shows that the range can be from 1 to 19 days. Therefore, the World Health Organization (WHO), Public Health England (PHE), and the Ministry of Health and Long Term Care (MOHLTC) all recommend assessing a 14 day exposure history in order to accommodate any uncertainty about the exact day of onset. This will cover the potential exposure source for 90% of cases.

13. **WHAT FACTORS MUST BE IN PLACE TO CREATE A RISK OF SOMEONE DEVELOPING LEGIONNAIRES’ DISEASE?**

The mere presence of *Legionella* bacteria in building water is not sufficient to cause Legionnaires’ disease. Legionnaires’ disease is principally acquired by inhaling minute airborne droplets of water (aerosols) containing the bacteria. It is not transmitted from person to person or from ingestion unless aspiration into the lungs occurs. Certain factors that increase the risk of someone acquiring Legionnaires’ disease include:

- conditions suitable for growth of the organisms, e.g. suitable water temperature (20°C–45°C) and deposits that are a source of nutrients for the organism, such as sludge, scale, rust, algae, other organic matter and biofilms
- a means of creating and spreading breathable droplets, e.g. the aerosol or mist generated by cooling towers, air conditioners, humidifiers, showers, or whirlpool spas
- exposure of vulnerable persons to colonized water that is inhaled or aspirated into the lungs. Particularly vulnerable persons at high risk for Legionnaires’ disease include the elderly, dialysis
patients, persons who smoke, newborn infants, persons with underlying medical conditions, or persons taking medications that weaken the immune system.¹²

14. SHOULD TESTING FOR LEGIONELLA CULTURES FROM SPUTUM BE ORDERED IN THE CASE OF COMMUNITY ACQUIRED PNEUMONIA?

It is very difficult to clinically distinguish patients with Legionnaires’ disease from other patients with community-acquired pneumonia (CAP) as clinical and radiological findings are similar. The key to diagnosis is performing appropriate clinical testing.¹

The CDC emphasizes the need for health-care providers to test and treat adults with potential CAP for Legionella.⁴⁰ Similarly, the Infectious Diseases Society of America/American Thoracic Society (IDSA/ATS) Consensus Guidelines on the Management of Community Acquired Pneumonia in Adults recommends that patients with severe CAP should have blood and sputum samples drawn for culture and urinary antigen tests for L. pneumophila.⁴¹ The report discusses the importance of identifying a Legionella case early in order to treat the patient and identify any possible outbreaks in the community. Spangnolo et al. notes that the isolation of the microorganism is of critical importance to public health investigations by allowing comparisons with environmental samples. They also note that sputum culturing is not very sensitive (10–80% sensitivity) and that less than one-half of patients with Legionnaires’ disease produce sputum.⁴²

At PHOL, molecular testing (PCR is performed) on respiratory specimens, which is quicker and more sensitive than culture. If PCR is positive for Legionella, the specimen is cultured in an attempt to isolate bacteria to help with speciation/serotyping of Legionella. For further information on laboratory testing see PHOL’s labstract, available at: http://www.publichealthontario.ca/en/eRepository/LAB_SD_084_Legionella_realtime_PCR_testing.pdf

15. IS THERE A URINE ANTIGEN TEST FOR LEGIONELLA?

The Legionella antigen can be found in the urine. Recently a new commercially available urine antigen test for Legionnaires’ disease has been introduced that can provide results within 15 minutes. Ulleryd et al. noted that given the low diagnostic yield, culturing sputum for confirmation of Legionnaires’ disease has limited importance when compared to the urine antigen test.⁴³ A very recent rapid review of the literature by Health Quality Ontario found a pooled specificity estimate of 99.1% and a specificity of 74%.⁴⁴ However, the author noted that the quality of the evidence was low in both specificity and sensitivity.

One disadvantage of the urine antigen test is that it is used primarily to investigate the presence of L. pneumophila serogroup 1 only, so suspected cases of Legionnaires’ can go undetected if the patient is infected with a different serogroup.⁴⁵-⁴⁶ A second disadvantage is that it does not allow for the comparison of clinical and environmental strains.
The urinary antigen detection should ideally be done in conjunction with sputum culture to isolate *Legionella*. This allows microbiological identification and subtyping in order to support the identification of the environmental source during an outbreak.47

**PREVENTION/CONTROL QUESTIONS**

16. **WHAT ARE THE PRACTICES AVAILABLE FOR CONTROL OF *LEGIONELLA* EXPOSURE?**

The risk from exposure should be avoided by measures that do not allow the growth of *Legionella* bacteria in the water system and that reduce exposure to water aerosols. Precautions should include (12):

- avoiding water temperatures between 20°C and 50°C which favour the growth of *Legionella* bacteria
- avoiding water stagnation and low flow – stagnation can encourage the growth of biofilm which can protect *Legionella* and provide conditions that encourages its proliferation
- avoiding the use of materials such as rubber washers and hoses that harbour *Legionella*, or provide nutrients for microbial growth
- controlling the release of water spray
- maintaining the cleanliness of the system to avoid the accumulation of sediments which can harbour *Legionella* and provide a nutritional source
- using water treatment techniques to control the *Legionella* population
- ensuring that the system operates correctly and is well maintained

17. **OUTSIDE OF CHLORINATION AND THERMAL HEATING, ARE THERE ANY ALTERNATIVE TECHNIQUES OF WATER TREATMENT AVAILABLE TO INSTITUTIONS FOR CONTROLLING BIOFILMS/*LEGIONELLA*/?**

There are a number of alternative techniques of water treatment available that can be used as stand-alone techniques or in combination with traditional chlorination.

*Copper-Silver ionization*

The application of copper-silver ionization results in cell lysing. A recent review suggests that copper-silver ionization is currently the most well-studied and effective control method for *Legionella* in hospital water systems.48 This review found that it is the only method where multiple field evaluations have been published in the peer-reviewed literature including those for hospitals, long-term care facilities, office buildings and apartment buildings. They note that this disinfection method is also easily installed and maintained. However, the review noted that some hospitals are reporting copper-silver ion resistant *L. pneumophila* a few years after installation of the system.49 This system requires that adequate
concentrations of copper-silver ions are maintained and that levels be frequently monitored. Poor water quality can affect the effectiveness of this type water treatment method. Dead legs require frequent flushing to ensure an appropriate ion residual. Depending on the water quality, the electrodes may have to be changed regularly, which can be expensive.

**Ultraviolet irradiation**
An ultraviolet (UV) irradiation system is an attractive option for disinfecting water at the point of entry because no chemicals are added to the water. However, its effectiveness against *Legionella* within biofilms is limited. Because it produces no residual effect and *Legionella* remains in biofilm and stagnant areas, it is commonly used in conjunction with a biocide. It is important to consider the quality of the water system, as hardness and iron can lead to scaling or discoloration of the UV lamp, making the system less efficient.

**Ozone**
Ozone can be used to kill *L. pneumophila*. Ozone instantaneously inactivates *Legionella*, however, ozone does not remain in water for long enough to provide a residual effect against potential contamination in the water distribution system. These systems are not intended to be dispersive and are usually designed to have their effect at, or very close to, the point of application. A second form of disinfection may be required in the distribution system for residual protection.

**Monochloramine**
Experimental studies show that monochloramine is effective against *Legionella* and against biofilm-associated *Legionella* in model plumbing systems, however, long-term studies still remain to be reported. Monochloramine provides a stable residual that penetrates biofilms and has a wider working pH range than copper-silver ionization and chlorine. Lin et al. conclude that monochloramine disinfection appears to be a promising approach for decreasing *Legionella* colonization.

**Chlorine dioxide**
Chlorine dioxide systems have been used by numerous establishments for *Legionella* disinfection. Its penetration into biofilms is superior to chlorine and its biocidal action is maintained over a wider range of pH than chlorine and copper-silver ionization. The limits of chlorine dioxide disinfection include the following:

- The major challenge for chlorine dioxide is maintenance of an effective residual concentration (0.3–0.5 mg/L) throughout the water distribution system. Because of this it tends to be used in under certain conditions where contamination prevents the use of chlorine.
- A prolonged duration is necessary to demonstrate significant reductions in the *Legionella* positivity rate.
- Reactions with organic material and corrosion scale in piping can cause rapid conversion of chlorine dioxide to its by-products: chlorite and chlorate. These by-products, although not suspected carcinogens, may pose health risks for consumers.
- Corrosion of galvanized pipes can cause loss of chlorine dioxide; this may affect efficacy.
Overall, chlorine dioxide is regarded as a promising disinfection for controlling *Legionella*\(^4^9\).

**Point-of-use filters**
There are commercially available bacterial filters that can be fitted to water outlets to prevent *Legionella* and other microbes from being released. Point-of-use filters (0.2-mm pore size) have been used for prevention of nosocomial infections due to *Legionella* and *Pseudomonas aeruginosa*. It was found that point-of-use filters completely eliminated *L. pneumophila* and *Mycobacterium* from hot water samples through eight days of use and yielded a 99% reduction in total heterotrophic plate count (HPC) bacteria through seven days of use.\(^4^8;\(^5^2\) The authors concluded that the microbiologic quality of tap water was significantly improved with point-of-use filters.\(^5^2\) A disadvantage of using filters is that they require regular replacement.

18. **HOW EFFECTIVE IS HYDROGEN PEROXIDE IN INACTIVATING LEGIONELLA IN DISTRIBUTION PIPES? AND WHAT IS THE IDEAL DOSE?**

There is very little literature on the effectiveness of hydrogen peroxide for inactivating *Legionella* in distribution pipes. In a document prepared by the WHO on *Legionella* and a review of the literature on hospital control methods for *Legionella*, hydrogen peroxide was not mentioned as a control method.\(^3;\(^4^8\). A study has found that stabilized hydrogen peroxide with silver did effectively control *Legionella* in a hospital’s closed loop hot water system.\(^5^3\) Treatment is carried out using a stable concentration solution of hydrogen peroxide and silver, exploiting the bacterial action of each. Also, some research does show that hydrogen peroxide may be effective against biofilm formation.\(^5^4\) The use of this technique requires further research and field trials to determine its effectiveness on *Legionella*.\(^7\)

19. **TO WHAT TEMPERATURE SHOULD THE HEATER SOURCE BE SET PRIOR TO WATER DISTRIBUTION TO THE REST OF THE HOSPITAL?**

Temperatures between 20° and 50°C tend to promote the growth of *Legionella*. Therefore, the best way to prevent colonization of *Legionella* in a water distribution system is the maintenance of temperatures outside the 20–50°C temperature range.\(^3\) Ideally, the cold water temperature should be below 20°C after running for up to two minutes, and the hot water temperature should be maintained above 50°C within one minute of running the water.\(^3;\(^5^5\) However, it should be noted that regulations designed to reduce the risk of scalding may require that hot water temperatures be kept below 50°C.

20. **WHAT IS THE REQUIRED CHLORINE RESIDUAL IN THE WATER SYSTEM?**

Where a water system is relatively free from established biofilm, maintaining a free chlorine residual of 0.5 - 1.0 mg/L will help reduce the development of biofilm in the pipework and aid the control of *Legionella*.\(^2^7\)

The WHO has set a health-based guideline maximum value of 5.0 mg/L for total chlorine as a residual disinfectant in drinking water.\(^3\) In practice, domestic water in buildings usually does not exceed 1.0mg/L as this would render the water unpalatable and might lead to an unacceptable level of corrosion. Levels higher than this do not usually provide additional disinfection capability.\(^2^7\)
21. HOW OFTEN SHOULD THE BUILDING’S WATER SYSTEM BE TESTED FOR CHLORINE LEVELS?

There is no standard on how often the chlorine residual level should be tested. The frequency of testing can be based on a risk assessment which takes into account such factors as the pH level, temperature, concentration of organic matter, history and pattern of the residual levels, the type and level of contamination, the population that could be exposed and the number and types of microorganisms in the water.

For most water systems, the HSE recommends the following routine inspection and maintenance to ensure control: 27

- Weekly - check the system operation and chlorine stocks in the reservoir.
- Monthly – measure the concentration of free chlorine at the sentinel taps - the concentration should be 0.5 - 1.0 mg/L; and adjust the chlorine product dosage to establish the required residual at the sentinel sample points.
- Annually – test the chlorine product concentration at a representative selection of outlets throughout the distribution system - the target concentration should be at least 0.5mg/L free chlorine.

22. WATER MAY REMAIN STAGNANT IN PIPES UNTIL THE POINT OF USE IS ACTIVATED/TURNED ON. WHAT ARE THE RECOMMENDATIONS AROUND THIS?

The risk from Legionella growing in parts of the water system where there is an absence of water circulation can be minimized by using these outlets regularly. When outlets are not in regular use, the HSE recommends weekly flushing for several minutes. 27 They note that once implemented, flushing has to be maintained or critical increase in Legionella can occur at the outlet. A risk assessment may indicate the need for more frequent flushing where there is a more susceptible population present, e.g. where immunocompromised individuals are present. It is important that flushing be carried out with minimum creation of aerosols since inhalation is the route of exposure.

PUBLIC HEALTH MANAGEMENT QUESTIONS

23. WHY IS IT DIFFICULT TO FIND A COMMON ENVIRONMENTAL SOURCE OF LEGIONNAIRES’ DISEASE IF TYING CAN BE CONDUCTED THROUGH CULTURE TESTING?

While sequence-based typing of cultures can be used to conclude if the Legionella in the clinical samples match that of the environmental samples, it can be difficult to establish an epidemiological link for a variety of reasons.

- Levels of Legionella in water systems can fluctuate. 35 Because of this fluctuation, the time difference between an outbreak becoming evident (which can be weeks or months in the case of pseudo-outbreaks) and samples being taken may make it difficult to interpret the results and establish an epidemiological link between the causative agent and environmental source.
- *Legionella* can reside within amoebae or protozoa therefore standard culture methods can produce false negative results.\(^5^6\)

- There is difficulty in isolating *Legionella* if the samples contain high background levels of other microorganisms.\(^4\)

- Standard culture methods will not detect active but non-culturable *Legionella* (ABNC) (live bacteria unable to multiply on culture media). There is mounting evidence that ABNC *Legionella* spp. are present at higher densities than colony forming units, and some fraction of these ABNC cells may still be infectious to humans.\(^5^7\)

- Some *Legionella* species capable of causing disease do not grow well using the traditional culture method for the isolation of *Legionella*.\(^4\)

Furthermore, with large outbreaks involving environmental sources such as cooling towers, it is possible to identify the strain responsible for clinical cases in multiple environmental sources. In such situations it is only through a good epidemiological investigation that the most likely source can be identified but not confirmed.\(^43^5^8\)

24. **IN THE CASES THAT HAVE OCCURRED IN ONTARIO IN 2013, WERE THEY OF THE SAME SPECIES OR SEROGROUPS?**

Most of the cases are due to the same species and serogroup, *L. pneumophila*, serogroup 1. It is important to note that most cases (>80%) are only confirmed via urine antigen test, which only detects *L. pneumophila*, serogroup 1. Therefore, unless appropriate samples are taken for PCL/culture, other *L. pneumophila* serogroups and other *Legionella* species cannot be identified. However, there are studies that indicate that most reported *Legionella* identified through culture-based methods are also *L. pneumophila*, serogroup 1.

In Ontario during 2013, 3.2% (269/8,530) of clinical specimens tested at Public Health Ontario Laboratories (PHOL) were positive for *Legionella* species. *L. pneumophila* was identified in 92% (247/269) of positive specimens with serogroup 1 representing 87% (233/269) of the positive specimens. Only 8% (22/269) of positive specimens were *L. non-pneumophila* species. However, these results should be interpreted with caution as urine testing is the predominant testing method for *Legionella* at PHOL and this method only detects *L. pneumophila* serogroup 1.

25. **ARE THERE ANY RECOMMENDATIONS FOR INVESTIGATION TRAVEL-RELATED CASES? E.G., SAMPLING THE WATER IN HOTELS?**

The European Working Group for *Legionella* Infections (EWGLI) has produced Technical Guidelines for the Investigation, Control and Prevention of Travel Associated Legionnaires’ Disease. Part 2 of the guideline specifically deals with methods for investigating and controlling Legionnaires’ disease in hotels, including environmental sampling of the hotel’s water system.\(^7\)
26. WHEN SHOULD ONE START AN ENVIRONMENTAL INVESTIGATION - AFTER ONLY ONE CASE OR SEVERAL?

The MOHLTC’s Infectious Disease Protocol states that (every reported) case should be investigated to determine the source of infection.\textsuperscript{39,59} This investigation should include information on the date of symptom onset, travel history, history of exposure to high risk sources, risk factors, exposure dates, occupation and place of residence or attendance at a facility or institution. The document states that an exposure investigation should be done to determine if the case was institution-acquired and whether a common source of exposure is present. The protocol goes on to say that environmental sampling should be reserved only for investigations involving institutions and disease clusters or an outbreak where a potential common exposure has been identified.

The Public Health Agency of Canada notes that because \textit{Legionella} is not spread from person to person, any institutionally acquired case indicates a probable environmental source and should prompt further investigation.\textsuperscript{60}

New Zealand’s Ministry of Health states that investigations of single case reports are important because they may uncover other cases and may point to common exposures, such as air or water in commercial or institutional settings during the incubation period. They recommend that if there is a single hospital-acquired case, that the institution’s water system should be sampled because of potential risk to other patients.\textsuperscript{14} Public Health England has guidelines that recommend that when a case of Legionnaires’ disease has been diagnosed, it should be investigated in a systematic way. They note that examination of the potential environmental sources of infection for these single cases is likely to highlight problems that might otherwise remain undetected and could contribute to the occurrence of further cases of Legionnaires’ disease.\textsuperscript{61}

27. IF A CASE OF LEGIONNAIRES’ DISEASE IS FOUND, AND IT TAKES UP TO 14 DAYS FOR A POSITIVE CULTURE FROM AN ENVIRONMENTAL SOURCE, WHAT SHOULD BE DONE IN THE MEANTIME?

There are few specific recommendations on what to do while waiting for environmental sampling results after a positive case of Legionnaires’ disease has been identified. The actions that are taken will likely depend on the results of the epidemiological investigation that has been conducted in relation to the identified case(s). This investigation may determine whether the case(s) is likely associated with the facility in question, and if the facility is the only potential source of exposure or one of many. Actions taken may also depend on whether an outbreak (two or more cases) has been detected in association with the facility or if there is a single case. Often environmental sampling is reserved for health-care investigations or an outbreak where a potential common exposure has been identified (see Question 26). In a recent investigation, Ulleryd et al. noted that an outbreak investigation is a race against time.\textsuperscript{43} The outbreak team has to gather as many facts as possible in a short time in order to identify the most probable source(s) of the outbreak. The closing or elimination of the probable source must often be expedited without waiting for all results of sampling in order to reduce further cases.\textsuperscript{43}
28. ARE THERE PLANS TO CREATE A FORMAL DEFINITION FOR NOSOCOMIAL LEGIONNAIRES’ DISEASE FOR EPIDEMIOLOGICAL INVESTIGATIONS?

The WHO’s definition: A nosocomial outbreak is defined as two or more confirmed cases of Legionnaires’ disease in the same hospital or residential institution within a six-month period. Location of the outbreak is defined in terms of geographical proximity of the cases and requires a certain level of judgement. Depending on length of stay in hospital before onset and environmental investigation results, cases are definitely, probably or possibly nosocomial.

**Definite nosocomial** — Legionnaires’ disease in a person who was in hospital for ten days before the onset of symptoms.

Probable nosocomial — Legionnaires’ disease in a person who was in hospital for one to nine of the ten days before the onset of symptoms, and either became ill in a hospital associated with one or more previous cases of Legionnaires’ disease, or yielded an isolate that was indistinguishable (by monoclonal antibody subgrouping or by molecular typing methods) from isolates obtained from the hospital water system at about the same time.

**Possible nosocomial** — Legionnaires’ disease in a person who was in hospital for one to nine of the ten days before the onset of symptoms in a hospital not previously known to be associated with any case of Legionnaires’ disease, and where no microbiological link has been established between the infection and the hospital (or the residential institution).
References


