

Legionella: questions and answers, 2nd edition

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Public Health Ontario

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Organism

1. How do Legionella bacteria enter the water system of a building?

Legionella bacteria are widespread within the natural water environment (rivers, lakes, ponds and reservoirs) and can be found in soils and mud.¹ *Legionella* can live and reproduce within certain protozoa (intracellular parasite), such as amoeba, and is commonly found within biofilm growing inside a building's plumbing (see box below). This ability to live within biofilm and as an intracellular parasite, grants the bacteria protection from traditional water disinfection treatments such as chlorination. *Legionella* can move from municipal water systems into a building's hot and cold water pipes, where, given suitable conditions, it can form biofilm from which the bacteria may be released from taps, showerheads and other points of discharge.^{1,2}

Biofilm: The slimy matrix produced by microorganisms (including *Legionella*) that forms inside pipes and other continually moist surfaces. *Legionella* can live and multiply within amoeba that inhabit biofilm. Both living within the amoeba and the biofilm matrix helps to protect the *Legionella* bacteria from chemical and thermal disinfection. Once established, biofilm is extremely hard to eliminate since no disinfection method is truly effective against it.³ Water pressure and vibration can dislodge pieces of *Legionella*-contaminated biofilm which can go on to colonize other parts of the water distribution system.⁴

Other means by which *Legionella* can enter a building water system include external construction, water main breaks and water pressure surges. These events can create vibration or changes in water pressure leading to contaminated biofilm shearing off or legionella naturally present in the environment to enter the water distribution system.⁵⁻⁷

2. What conditions favour the growth of Legionella?

There are a number of important factors that provide an environment for *Legionella* to multiply; water temperature, the presence of biofilm, water stagnation and the type of plumbing material and components present.

Water temperature

- The water temperature required for optimal *Legionella* growth is between 25°C and 45°C. This characteristic illustrates why cooling towers, hot and cold water systems, spa pools and humidifiers provide ideal environments for *Legionella* to grow.

It should be noted that *L. pneumophila* has been found in water systems at temperatures up to 60°C⁸ and it has been shown that hot water distribution systems set below 60°C are more likely to be contaminated with *L. pneumophila*.⁹

- Traditionally, thermal control is the primary means of controlling (but not eradicating) *Legionella* in water systems is to have hot water stored at 60°C and distributed so that it reaches the outlets at a minimum of 50°C to 55°C within a minute.¹⁰ *L. pneumophila* has the ability to recolonize water systems despite routine thermal disinfection.^{3,9}
- 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.¹¹
- In a study of cold water systems in health care facilities, Arvand et al. found that 94/265 (35%) and 43/156 (28%) of distal cold water samples displaying a temperature of less than 20°C and less than 15°C respectively contained *Legionella*.¹¹ The authors concluded that the cold water supply of health-care facilities may be heavily contaminated with *Legionella* species.¹¹

Biofilm

- Biofilms are the slimes that form on surfaces in contact with water, such as the inside walls of water pipes, air conditioners, cooling towers, whirlpools, showerheads, taps and humidifiers.
- Once integrated into biofilm, the *Legionella* bacteria obtain some protection from external stressors such as the action of disinfectants and increases in water temperature. They can thus persist and even thrive within biofilm.^{12,13} In addition to offering protection, biofilm provides an important nutritional source for *Legionella*.
- The presence of scale, corrosion and sediment in a water distribution system not only encourages the formation of biofilms but also provides the *Legionella* bacteria with nutrients.^{12,13}
- Preventing the growth of biofilms is an important control measure against proliferation of *Legionella* because once established, biofilms are extremely difficult to eradicate.¹⁴

Water stagnation or low flow

- During periods of water stagnation or low flow, hot water temperature decreases and cold water temperature increases. Also, a significant decay in residual disinfectant levels can occur. Both of these factors are favorable to the development of biofilm and can produce an environment in which *Legionella* thrives.¹² Facilities that have undergone extensive renovations over time often contain redundant pipework/dead legs in which water can stagnate. Infrequently used fixtures can also allow water to stagnate.

Other factors

- Inadequate disinfection: Reduction in disinfectant levels in a building's water systems can lead to the proliferation of *Legionella*. Factors such as temperature and water flow can reduce the amount of available disinfectant in the water, permitting *Legionella* to grow. Supplemental disinfectants can be used to boost disinfectant residual throughout the water system and help control *Legionella* growth.¹³
- Plumbing material: The type and characteristics of plumbing materials can favour biofilm colonization in potable water systems. For example, research has shown plastic and rubber materials found in plumbing components, such flexible hoses or thermostatic mixing valves, are susceptible to such colonization.^{13,15,16}

Exposure

3. Is there any evidence 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, e.g., filling the bath tub, might be a cause of *Legionella* exposure.^{17,18} The risk of exposure depends on the design of the faucet and the water flow rate.¹⁷

One outbreak investigation found that risk of Legionnaires' disease was lower for seniors in an apartment complex who reported tub bathing instead of showering. The authors noted that the finding that tub bathing is protective is not unexpected given that exposure to water aerosols is less than would otherwise be experienced while showering.¹⁹

Environmental sampling

4. What method should be used for identifying *Legionella* from environmental samples during an outbreak?

Culture-based testing for *Legionella* is generally recognized as the “gold standard” for *Legionella* detection and enumeration.^{20,21} Cultures can identify all known *Legionella* species and subspecies. It allows for identification of the environmental source of an outbreak through the matching of genetic sequences between clinical specimens and environmental samples, allowing control measures to be implemented before further cases can occur. Culture is time-consuming (results may take 10 to 14 days) and 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 in a viable but non-culturable state (VBNC) (see box below).²¹ These difficulties can lead to an underestimation of the presence of *Legionella* in water systems, which can have serious public health consequences.²²

Legionella spp. can enter into a VBNC state in order to withstand harsh environmental conditions (presence of disinfection, low nutrients, high temperatures and low oxygen levels). While within the VBNC state, *Legionella* are generally dormant and cannot be cultured, even though they continue to retain their viability and virulence.²³ However, given suitable conditions they can return to a culturable state.²⁴

Because of the limitations associated with culturing, polymerase chain reaction (PCR) is gaining use as an alternative method to detect *Legionella*. PCR is a rapid test designed to detect genetic sequences specific for all *Legionella* species and is highly sensitive, however, it cannot differentiate between viable and non-viable *Legionella*. Additionally, it does not allow for linkage of clinical specimens with environmental samples to identify an outbreak source.²¹ Therefore, although it can be a valuable pre-screening tool for quickly identifying and remediating potential sources of *Legionella*, it should be used in conjunction with culturing during an outbreak situation to help identify the outbreak source.²⁵

Public Health Ontario's (PHO) laboratory does not perform PCR on environmental samples; however, it does use real-time PCR technology to detect *Legionella* in clinical respiratory specimens. If *Legionella* is detected by PCR, culture is attempted. Ultimately, the genetic sequences of clinical and environmental isolates are compared to provide laboratory support for the epidemiological investigation. The [Public Health Inspector's Guide](#)²⁶ offers advice on sampling procedures for *Legionella*.

5. What are the recommendations regarding routine environmental sampling for *Legionella* in hospitals, long-term care facilities and retirement homes?

Patients of hospitals and residents of long-term care facilities (LTCF) (e.g., nursing homes, assisted living facilities) and retirement homes may be at higher risk for Legionnaires' disease due to the following:

- declining immune function due to aging.
- pre-existing medical conditions (e.g., chronic lung disease, lung cancer, hematologic malignancies, renal disease, diabetes mellitus).
- swallowing difficulties, which can increase the risk of developing legionellosis through pulmonary aspiration.^{13,19}

Outbreaks of legionellosis have been frequently reported among residents of hospitals, LTCF and retirement homes.^{19,27-29} One of the most serious of these outbreaks occurred in Toronto in 2005 when 135 residents of a LTCF became infected with *Legionella*, 23 of whom died.³⁰

In addition to the facilities mentioned above, Aji et al. recommends that in order to account for our aging population, risk reduction strategies to prevent Legionnaires' disease are needed in residential buildings that take into account the proportion of people over 65 years old.³¹

Routine environmental sampling is sometimes performed in higher-risk facilities as a proactive measure when there are no suspected or confirmed cases of Legionnaires' disease associated with a facility or water source. The decision on whether to conduct routine testing of water systems for *Legionella* as a preventative measure is a contentious subject with differing approaches being advocated by various public health organizations and researchers.^{21,32}

The argument against routine water testing

Opponents of routine water testing do not recommend including routine environmental sampling in public health guidelines based on the following reasons:

- the ubiquitous nature of *Legionella* in potable water systems.
- the lack of evidence-based threshold levels for corrective action.
- the difficulty interpreting *Legionella* test results (see box below).³³

Instead, it is argued that the presence of *Legionella* should be assumed and appropriate infection prevention and control measures, including actively screening for cases of Legionnaires' disease, should be instituted and appropriately managed.^{21,34} Additionally, false negative results—which are common with culture-based techniques—can lead to misplaced confidence regarding the safety of the water system and may potentially lead to a public health risk.^{21,33}

Difficulty interpreting *Legionella* culture results are due to the following reasons:^{22,33,35,36}

- Culture methods (considered the gold standard) can underestimate the number of *Legionella* organisms present due to some *Legionella* bacteria being viable but unable to grow on routine *Legionella* culture media.
- *Legionella* growth can be inhibited or masked by overgrowth of competing microorganisms present.
- There is an inability to culture *Legionella* if they are housed within their amoeba hosts.

The argument for routine water testing

Despite the acknowledged limitations of *Legionella* monitoring and the difficulty interpreting test results, several public health organizations and researchers advocate its usefulness, provided it is performed within the framework of a water safety plan. The Center for Disease Control (CDC), Veterans Health Administration, American Industrial Hygiene Association and World Health Organization all note that routine environmental testing is a useful means of confirming the effectiveness of control measures to prevent Legionnaires' disease (i.e. the presences of *Legionella* is detected before cases occur).^{6,13,37,38} Silk et al. identified the potential for outbreaks among the vulnerable population in retirement homes and suggested these facilities should consider routine sampling of the potable water.¹⁹ This recommendation was also echoed by Cristino et al. who noted the successful control of *Legionella* by four LTCFs who implemented routine environmental sampling.²⁹

Routine Sampling based on risk assessment

Various public health organizations suggest that routine testing for *Legionella* be implemented on an individual system-by-system basis, informed by a local risk assessment to reduce unnecessary testing and expenditure.^{6,25,39,40} Based on the assessment, a decision concerning environmental testing can be

made. According to the CDC⁶ and the Health and Safety Executive⁴⁰, the circumstances when monitoring for *Legionella* may be appropriate are as follows:

- when a facility is having difficulty maintaining water systems within established control levels (e.g., temperature or disinfection concentrations).
- having a prior history of Legionnaires' disease.
- where there are people who are at increased risk for Legionnaires' disease (e.g., in healthcare facilities including long-term care homes).

PHO's laboratory does not provide testing of samples collected for routine environmental monitoring for *Legionella*. If this testing is desired, another laboratory should be selected. The chosen laboratory should be accredited by a provincial, national, or international accrediting body.

6. What action threshold levels should be applied *Legionella* is detected?

Several different agencies and organizations have published guidelines on interpreting *Legionella* levels,^{37,40-43} however, it must be understood none are scientifically based since no relationship between *Legionella* levels and infection has been established. For example, facilities may apply a "30% positivity action threshold" which means remediation of the water system is performed when *Legionella* is recovered from less than or equal to 30% of the samples tested.^{34,43} Another approach involves using numerical cut-offs for "acceptable" concentrations (in colony-forming units [CFU]/mL) of *Legionella* recovered in water. Examples of agencies providing recommendations for interpreting sample results and actions based upon the level of *Legionella* in the water are listed below:

- The American Industrial Hygiene Association: Recognition, Evaluation, and Control of *Legionella* in Building Water Systems³⁷
- Health and Safety Executive: Legionnaires' disease—Technical guidance:
 - Part 1: The control of *Legionella* bacteria in evaporative cooling systems⁴¹
 - Part 2: The control of *Legionella* bacteria in hot and cold water systems⁴⁰
 - The control of *Legionella* and other infectious agents in spa pool systems⁴⁴
- US Department of Labor, Occupational Safety and Health Administration—Technical Manual, Chapter 73⁴².

The Center for Disease Control (CDC) and Veterans Health Administration's view is that although it may not be feasible to fully eliminate *Legionella*, there is little scientific evidence to support a "safe level" of

Legionella and, if it is found, no matter what the level, an investigation should be launched and appropriate corrective action taken to eliminate Legionella, or at least strive to maintain levels as low as possible at all times.^{34,35} The CDC notes that outbreaks occurred despite building water systems being below action threshold limits. Others argue that the individual facility should ultimately have the responsibility to select action threshold limits that are appropriate to the risk being evaluated^{37,45}

7. What sites should be sampled during a *Legionella* outbreak?

Before samples are collected for testing at PHO's laboratory, it is essential to consult with the laboratory to discuss considerations of clinical and environmental testing and ensure that criteria for testing of environmental samples by the laboratory have been met. The [Public Health Inspector's Guide](#)²⁶ offers advice on sampling procedures for *Legionella*.

Selecting sites to sample following an outbreak requires a detailed understanding of the buildings water system. This is generally achieved by performing a thorough environmental risk assessment (ERA) of the water system to identify and assess sites that pose a health risk.^{8,46} Factors to consider during the ERA include:^{40,47}

- locations where water aerosols can be created such as showers and taps, decorative fountains, whirlpool spas, cooling towers and humidifiers.
- areas that contain water at temperatures likely to support the growth of *Legionella* (20° to 50°C).
- sites with stagnant or poor water flow including dead legs.
- infrequently used outlets or fixtures.
- construction, renovation or maintenance sites that may have disrupted the building's water system or required water flow to be stopped and started again.

Based on the findings of the ERA and the locations implicated by the epidemiological investigation (potable water system, fountain or whirlpool etc.), sampling sites can be selected on a priority basis. For example, aerosol sources that the case(s) may have been exposed to should be sampled first, followed by other high-risk sources (i.e., sites that potentially contain the highest numbers of *Legionella* bacteria).^{26,48,49} Sampling points should be continually reassessed as the investigation progresses and as more results and information become available to locate the source of the *Legionella*.⁴⁸ If the outbreak under investigation is associated with *L. longbeachae*, sites where individuals may have been exposed to composts and potting soils should be identified and sampled.

The Center for Disease Control offers [a downloadable environmental assessment form](#)⁴⁷ that may be useful for conducting an environmental assessment.

The tables below identify possible sampling sites for *Legionella* during an outbreak investigation.^{50,51}

Table 1: Possible sources and sampling sites for *Legionella* in potable water systems

Sample sites	Type of sample	Comments
Incoming water main (From municipality) Well/water tower	Bulk water	<ul style="list-style-type: none"> Where the temperature of the source water is greater than 20°C or there is a high organic content, or the heterotrophic bacteria count (HBC) is high, there is a potential for <i>Legionella</i> to be present in the water entering the system. A high HBC simply warns that the nutrients and temperature conditions may be conducive to <i>Legionella</i> growth
Every tank and cistern	Bulk water	<ul style="list-style-type: none"> Dip samples can be taken where the fresh incoming water enters the tank/cistern.
Water heater	Bulk water and biofilm swab if drained	<ul style="list-style-type: none"> Bulk water samples can be taken from the drain valve. Taking biofilm swabs requires complete draining of the tank.
Water softener	Bulk water	<ul style="list-style-type: none"> Samples should be collected both before and immediately downstream of the softener to determine whether the softener is colonized.
Expansion vessel	Bulk water	<ul style="list-style-type: none"> Should have a valve at the bottom of the vessel to allow sampling.
Shower and faucets	Bulk water and biofilm swab of shower head or inside the faucet. A biofilm swab may be taken from the aerator.	<ul style="list-style-type: none"> Pre-flush samples (water collected immediately after a faucet or shower is opened) are typically taken from hot and cold water systems. The pre-flush sample is intended to represent water held within the tap or fitting and should be taken when the tap has not been used for several hours. Facilities should remove the shower head and faucet aerator prior to sampling in order to minimize aerosol production. This will also help aid the inspection and sampling of any biofilm inside shower head or faucet aerator. A small number of post-flush samples may be taken to assess the degree of contamination within the pipework system as opposed to within individual outlets.
Thermal Mixing Valves (TMV)	Bulk water	<ul style="list-style-type: none"> Pre-flush samples of taps and showers downstream of TMVs will detect colonization of the TMV, faucet or showerhead, and if present, flexible hoses. If possible, samples should also be taken from separate hot and cold water outlets upstream of the TMV in order to be representative of the water flowing around the system.
Hot water return	Bulk water	<ul style="list-style-type: none"> Sample before recirculated water enters the hot water heater. If water is below 50°C, check to ensure return pump is operating correctly.

Table 2: Possible sources and sampling sites for *Legionella* in whirlpool spas

Sample sites	Type of sample	Comments
Water in the pool	Bulk water	<ul style="list-style-type: none"> Pool water may not yield large numbers of <i>Legionella</i>; however, solid material samples and biofilm samples tend to have large numbers of <i>Legionella</i>. If the pool is drained, a water sample can be collected from the overflow tank.
Biofilm above the water line	Biofilm swabs	<ul style="list-style-type: none"> Areas above the water line may not be subject to disinfection.
Water jets	Biofilm swabs	<ul style="list-style-type: none"> Several jets should be swabbed. If possible the inner pipe should be swabbed as well.
Filter	Solid material (sand, diatom powder, or polyester filling in cartridge filters)	<ul style="list-style-type: none"> Even if the pool is drained, filter material can still contain <i>Legionella</i>. PHO's laboratory should be consulted prior to sampling.

Table 3: Possible sources and sampling sites for *Legionella* in cooling towers

Sample sites	Type of sample	Comments
Make up water	Bulk water	<ul style="list-style-type: none"> Cooling towers can produce aerosols that can travel over considerable distances. If the healthcare facility where the outbreak has occurred has air intakes that are located near a cooling tower, then several solid samples of the air intake filters should be taken. PHO's laboratory should be consulted to determine the amount of filter material that will be needed for analysis. It is advised that a maintenance technician/engineer familiar with cooling towers assist with access to points where samples are taken.
Collection basin (area below the tower for collection of cooled water)	Bulk water and biofilm sample at the water line	
Sump (section of the basin from which water is pumped back). Silt and sludge may also be collected here	Bulk water and biofilm swabs at the water line	
Return service to the cooling tower located near the heat source	Bulk water	
Drift eliminator	Biofilm swab	<ul style="list-style-type: none"> Must be clean and in good state. They must cover the whole air outlet surface.

Table 4: Possible sources and sampling sites for *Legionella* in other water sources

Source	Sample sites	Type of sample	Comments
Decorative fountains	Fountain reservoir Fountain trough Underwater lighting Decorative material such as foam stones in the fountain	Bulk water Biofilm swabs Biofilm swabs Solid material	In one outbreak, foam material used to prevent splashing was heavily contaminated with <i>Legionella</i> .
Sprinkler system	Sprinkler jets	Bulk water and biofilm	Water could be stagnant.
Safety shower and eyewash stations	Shower and eye wash	Bulk water and biofilm	Water could be stagnant.
Humidifiers	Water used for humidification	Bulk water and biofilm	Take at least one biofilm swab of moist surface.
Nebulizers, hand powered resuscitators, Breathing ventilators	Water used for cleaning the device	Bulk water and biofilm	Take at least one biofilm swab of moist surface.
Soil	Soil	Bulk	PHO laboratory should be consulted on prior to sampling.

At each sample location, taking water parameter measurements (e.g., temperature and disinfectant residual) will help determine the potential risk for *Legionella* amplification.

8. What type of samples (water or swabs) should be taken when performing routine testing for *Legionella*?

The question of when to collect both water samples and swabs is an uncertain one. Biofilm shields *Legionella* and enhances its multiplication and research shows that the number of *Legionella* organisms from biofilm swabs is greater than the number of *Legionella* organisms sampled from water.⁵² As such, it is recommended that swabs are 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.^{13,52} However, a recent large study involving paired water and swab cultures found that water cultures were significantly better in detecting *Legionella* compared with concurrent swab cultures. The authors recommend (given the greater sensitivity and the simplicity of water collection over swabs) that only water samples be collected when performing routine environmental sampling for *Legionella*.⁵³

9. What is the re-testing protocol for *Legionella* after remediation of an outbreak source?

When a source has been identified and remediated following an outbreak, there is a clear need for re-testing for *Legionella* to ensure that the implemented control measures were effective. There is no established rule or good evidence for determining the frequency and duration of re-testing and how the results should be interpreted—it will vary from facility to facility and should be based on the assessed risk to the public’s health. The CDC recommends that after remediation, re-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.⁵⁴ Any *Legionella* detected during this time frame must be re-remediated (using modified remediation procedures) and the test cycle restarted.⁵⁴

It is generally agreed that once *Legionella* has colonized a water system, eradication through disinfection is very difficult.⁵⁵ One reason is that biofilm, which provides a habitat for *Legionella*, protects it from external stressors such as disinfectants and increases in temperature.^{13,56} Additionally, *Legionella* can incorporate themselves within protozoa also found in biofilm, some of which are very resistant to high temperatures and disinfection procedures.¹³ Long-term control (not eradication) of *Legionella* can only be achieved through proper treatment and management of water systems following strict guidelines for the prevention of *Legionella* growth.⁵⁷

Clinical aspects

10. How long should the exposure history look back?

There is uncertainty around the range of the incubation period. Initially, the incubation period for *Legionella* was thought to be 5 to 6 days, with a range of 2 to 10 days.^{13,58} However, evidence from some outbreaks shows that the range can be from 1 to 19 days.⁵⁸ 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.^{13,58,59} This will cover the potential exposure source for 90% of cases.⁵⁸

11. What factors must be in place to create a risk of someone developing Legionnaires' disease?

Legionnaires' disease is principally acquired by inhaling minute aerosolized droplets of contaminated water or aspiration of fluid from the mouth or stomach into the lungs. It is not considered to be transmitted from person to person or from ingestion unless aspiration occurs.⁶⁰ For Legionnaires' disease due to *Legionella longbeachae*, exposure to potting soil or compost (through gardening activity) is regarded as a risk factor, although the exact mechanism of exposure is not fully understood.⁶¹

The mere presence of *Legionella* bacteria in a building's water system is not sufficient to cause Legionnaires' disease. Certain factors that increase the risk of someone acquiring Legionnaires' disease include:⁶²

- conditions suitable for growth of the organisms such as stagnation, suitable water temperature (20°C to 45°C) and deposits (e.g., sludge, scale, rust, algae, other organic matter and biofilms) that are a source of nutrients for the organisms.
- A means of creating and spreading inhalable droplets such as cooling towers, humidifiers, showers, sinks, whirlpool spas, decorative fountains or supermarket misters.
- exposure of vulnerable persons (e.g., elderly, dialysis patients, smokers, newborn infants, persons with underlying medical conditions, or persons taking medications that weaken the immune system) to colonized water that is inhaled or aspirated into the lungs.

12. Is there a urine antigen test for *Legionella*?

The *Legionella* antigen can be found in the urine and commercially available urine antigen tests for *Legionella pneumophila* serogroup 1 can provide results after 15 minutes. Because of its ease of use and rapidity (which permits early diagnosis and treatment), the urine antigen test is by far the most frequently used diagnostic test.⁶³

A rapid review of the literature by Health Quality Ontario found a pooled sensitivity of 74% (i.e. just over seven out of ten people with Legionnaires' disease will test positive) and a specificity estimate of 99.1% (i.e. if they test positive for *Legionella pneumophila* serogroup 1 then it is likely a true positive finding).⁶⁴

A negative result does not rule out legionellosis. The urine antigen test can only detect the presence of *L. pneumophila* serogroup 1, so even though most reported human cases of legionellosis are associated with this serogroup, sole use of the urine antigen for diagnosis can result in a significant number of cases caused by other serogroups of *L. pneumophila* or other species of *Legionella* to be missed (e.g., patients infected by *L. longbeachae*).⁶⁵ A second disadvantage is that it does not allow for the comparison of clinical and environmental isolates to identify the source of the outbreak.⁶⁶

Because of the inherent limitations of urinary antigen detection, more than one testing method should be used for the identification of *Legionella* (see question 13).^{66,67}

13. Should lower respiratory specimens for *Legionella* testing be ordered during a *Legionella* outbreak investigation?

A range of laboratory tests exist for *Legionella* including urine antigen (UAT), genetic methods (PCR) and/or culture of respiratory specimens.^{66,68} These tests, while complementary, have important distinctions as it relates to diagnosis and treatment versus managing an outbreak.⁶⁸ Proper diagnosis and treatment of Legionnaires' diseases does not require identification beyond the fact that it is a *Legionella* species (genus level) whereas outbreak investigation and management requires a more precise characterization of the bacteria (type and strain) in order to identify the outbreak source.^{66,68}

The UAT is by far the most used laboratory method for diagnosing Legionnaires' diseases representing over 80% of the diagnostic tests used.^{66,68} This is due to less invasive nature, simplicity, speed, relatively low cost commercial availability, and Health Canada licence. Despite its popularity, UAT comes with two major limitations.

First, the UAT is unable to detect non-*Legionella pneumophila* serogroup 1. Clinicians must be aware of this diagnostic limitation when ruling out Legionnaires' diseases. A negative UAT does not necessarily exclude Legionnaires' diseases. Clinicians may wish to pursue alternative testing such as PCR if UAT results are negative but Legionnaires' diseases is still suspected.⁶⁸

Secondly, the UAT does not allow for identification of the *Legionella* exposure source which generally requires genetic matching between lower respiratory specimens (e.g., sputum, bronchoalveolar lavage) taken at the time of the patient's/resident's illness and environmental samples that test positive for *Legionella*. Therefore, ideally obtaining a respiratory specimen for culturing concurrently along with the urinary antigen test should be the desired goal.⁶⁸ This is of critical importance to public health investigations because it allows genetic typing comparison of clinical isolates with environmental isolates. This dramatically improves the likelihood that a confirmed environmental source of *Legionella* can be identified and remediated to prevent additional cases.⁶⁹

PCR is performed on lower respiratory tract specimens. The method identifies *L. pneumophila* and detects all *Legionella* species generically; it does not identify individual *L. pneumophila* serogroups or individual *Legionella* species. Therefore, culture of all PCR positive samples should still be done routinely to aid in identifying the source of the *Legionella* exposure.⁷⁰

Environmental samples collected for *Legionella* testing will not be accepted by PHO's laboratory in the absence of a confirmed clinical case by culture.

For further information on taking environmental samples, please contact PHO laboratory, Environmental Microbiology.

Infection prevention and control

14. 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 reduce exposure to water aerosols. Precautions should include:⁶²

- avoiding water temperatures between 20°C and 45°C which favour the growth of *Legionella* bacteria.
- avoiding water stagnation and low flow, the conditions that encourage the proliferation of the growth of biofilm (which can protect *Legionella*).
- 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.
- disinfecting regularly to control biofilm formation and the *Legionella* population.
- ensuring that the system operates correctly and is well maintained.
- ensuring cooling tower emissions are not drawn into the building air intakes. The Canadian standard (CAN/CSA-Z317.2-15—Special requirements for heating, ventilation, and air-conditioning (HVAC) systems in health care facilities), requires that prevailing wind and building air intakes be considered when choosing the location of cooling towers.⁷¹
- mitigating the risks associated with thermal flushing (aerosolization and scalding), restarting the water system (dislodging of biofilms due to water pressure), and construction activity (dislodging of biofilms due to vibration).

15. Apart from thermal disinfection, are there any alternative techniques of water treatment available to healthcare facilities for controlling biofilms/*Legionella*?

Traditionally, thermal control has been the primary means of controlling *Legionella* in water systems—hot water stored at 60°C and distributed so that it reaches the outlets at a minimum of 50°C (55°C in healthcare premises) within a minute.¹⁰ However, because of the use of thermal mixing valves to blend hot and cold water to prevent scalding and the complexity of water systems found in healthcare facilities, it is difficult to maintain the required temperature for controlling *Legionella*, especially at extreme ends of the water distribution system.¹⁰ Consequently, other disinfecting methods known to be capable of controlling *Legionella* colonization and growth may need to be considered as an additional infection prevention and control measure.

It must be emphasized that any newly installed or modified water disinfection method must be validated to ensure that it is effective in controlling *Legionella*. The HSE recommends when moving from thermal disinfection to another disinfection method that any reduction of hot water temperatures be carried out in stages as reducing hot water temperatures will leave the system vulnerable.⁴⁰ At each stage, the hot water temperatures can be reduced when the efficacy against *Legionella* is confirmed by monitoring for *Legionella* and disinfectant levels.⁴⁰ Additionally, after implementation, ongoing verification and meticulous monitoring of disinfection levels are essential to ensure that the control measures remain effective over time.⁴⁰

For a good review of water treatment technologies for *Legionella* control please see United States Environmental Protection Agency: Technologies for *Legionella* Control in Premise Plumbing Systems.⁷²

Copper-silver ionization

Copper and silver ions are typically generated by passing an electrical current between two copper/silver electrodes to stimulate continual release of copper ions and silver ions into the flowing water. The application of copper-silver ionization (CSI) results in the *Legionella* cell wall breaking down resulting in the death of the cell (leads to lysis).⁷³ A review by Lin et al. suggests that CSI is an effective control method for *Legionella* in hospital water systems.⁷⁴ Efficacy is not affected by high water temperatures and it provides good residual disinfecting protection throughout the system.⁷³ However, in the presence of biofilm and amoeba, *Legionella* can be protected from CSI.⁷² Additionally, studies have shown CSI resistant *L. pneumophila* after a few years of application.⁷² Outbreaks of legionellosis have been reported despite CSI treatment.^{3,34} The following points are noted about the application of CSI:

- The vendor will generally specify the required concentration of copper-silver ions and monitoring frequency required.⁷²

- Water hardness, which causes scaling of the electrodes, and increased levels of chloride can make it difficult to maintain the required silver ion concentrations.⁷⁵
- CSI is pH sensitive and it is difficult to maintain ion concentrations above pH 7.6 due to scaling on the electrodes.⁷²
- Use of CSI may result in pipe corrosion.⁷²
- Use of anticorrosion chemical containing phosphates can make it difficult to maintain the required silver ion concentrations.⁷²

Ultraviolet light radiation

Ultraviolet (UV) irradiation systems (254 nanometers) have been shown to be effective in controlling *Legionella* at the point of use as water flows through the UV unit.^{60,76} It has the advantage of being relatively easy to install and is not corrosive to the plumbing system. However, because it has no residual effect, it is commonly used in conjunction with thermal or another disinfection method which can maintain a residual to control *Legionella* and biofilm that may be established downstream of the UV unit.⁷²

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.⁴¹ UV units require proper maintenance to ensure their effectiveness for inactivation of pathogens.

Ozone

Laboratory studies have shown ozone to be effective in rapidly inactivating *Legionella*.^{72,76,77} However, ozone is very reactive and is only effective at the point of use; it does not remain in water long enough to offer residual disinfection against *Legionella* throughout the whole water distribution system.^{60,78} Because of this disadvantage, ozonation is best instituted with another disinfection method which can sustain a residual. An additional disadvantage due to its reactivity is the potential for damage to the piping system.⁶⁰

Chlorine

[See question 17.](#)

Monochloramine

Monochloramine (MC) is considered more effective than free chlorine in controlling *Legionella*, in part due to its in vitro ability to better penetrate biofilms and to inhibit *L. pneumophila*.

Experimentally, MC has been shown to be more effective than chlorine against biofilm associated *Legionella*.^{24,60,79} A two year study to determine if a conversion from chlorine to monochloramine in the municipal water supply would reduce *Legionella* levels showed a reduction from 60% colonization of the hot water to 4% colonization after the conversion.⁷²

At MC concentrations above 1.5 mg/L, *Legionella* is well controlled but can promptly recolonize the water system if there is an interruption of MC disinfection.²⁴ Some potential issues include the following:^{60,72,74}

- MC can cause corrosion and attack rubber and plastic components of pipes.
- MC usage can contribute to the formation of disinfection by-products such as nitrosamines.
- When using MC, the formation of nitrite and nitrate (nitrification) is a potential problem. This is due to excess ammonia and low chloramine residual in the treated water. Nitrification makes it hard to maintain MC disinfection residual, and can result in taste and odour issues.
- MC disinfection is reported to allow colonization of the treated systems with Mycobacteria and total coliform.

Chlorine dioxide

Chlorine dioxide has been used effectively by numerous hospitals for controlling *Legionella* and biofilm in their water systems^{74,79-81} although one study has found it to be ineffective.⁸² 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.⁷² Chlorine dioxide disinfection has limitations that include the following:^{72,74}

- The major challenge for chlorine dioxide is maintenance of an effective residual concentration throughout the whole water distribution system.
- A prolonged duration of treatment 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. Chlorite can cause anemia and affect the nervous system of infants, young children and the fetus. Chlorate can lead to an enlarged thyroid.

Hydrogen peroxide

Hydrogen peroxide has not yet been used extensively to control *Legionella* in hospital water distribution systems and there is little literature on its effectiveness. Studies using hydrogen peroxide formulated with silver have shown results ranging from complete control⁸³ to an approximate 2-log reduction in *Legionella* contamination.²⁹ By itself, hydrogen peroxide has been noted to produce satisfactory results (less than 30% positivity rate) in reducing *Legionella* contamination when the biocide level was ≥ 20 mg/L.⁸⁴ At 25 mg/L, when mixed with polyphosphates, hydrogen peroxide achieved almost complete disappearance of *Legionella* colonization in the long term.⁸⁵

Hydrogen peroxide looks to be a promising control method for decreasing *Legionella* colonization; however, further field studies are required to confirm its effectiveness in healthcare settings.

Point-of-use filters

Commercially available bacterial filters can be fitted to faucets and showers heads [called point-of-use filters (PUF)] to prevent *Legionella* and other waterborne pathogens from being released. Given the difficulty with eradicating *Legionella*, PUF are generally used to prevent infections in high risk areas as well as during an outbreak situation. Filters can be installed quickly and immediately and in certain outbreak situations may be a better alternative than restricting water use.⁷²

Numerous studies testify to the effectiveness of PUF for removal of *Legionella* and other waterborne pathogens from hospital water systems.⁸⁶⁻⁸⁸ For example, a hospital study found that PUF completely eliminated *L. pneumophila* and *Mycobacterium* from hot water samples through eight days of use. The authors concluded that PUF could prevent exposure of high-risk patients to waterborne pathogens.⁸⁸ Another study involving a cancer centre evaluated a faucet filter at five sinks and found that the filter was able to effectively remove *Legionella* over a 12 week period.⁸⁶

Before PUF are used, it must be recognized that the filters do not eliminate *Legionella*, thus it is possible for the organisms to multiply and contaminate other parts of the water distribution system. Additionally, PUF need to be changed routinely, depending on usage of the outlets and the manufacturer's instructions for use.

16. To what temperature should the heater source be set prior to water distribution to the rest of the health care facility?

Water temperatures between 20°C and 45°C tend to promote the growth of *Legionella*.¹³ Therefore, the best way to prevent colonization of *Legionella* in a water distribution system is to maintain the temperature outside of this temperature range.¹³

Ideally, for health care facilities, the temperature at the water heater outlet should be $\geq 60^{\circ}\text{C}$, and the distributed hot water temperature above 55°C.^{8,13,40}

Exposure to stress can induce *Legionella* to enter the viable but non-culturable (VBNC) state as a survival strategy.⁸ While in the VBNC state some species of *Legionella* can survive high temperatures (between 55°C and 70°C for 30 to 60 minutes) and given a lapse in the required thermal regime for controlling *Legionella*, can recuperate their ability to multiply and cause a potential public health hazard.^{8,84} For this reason, some researchers stress the importance of maintaining constant vigilance over the required thermal regime to control (but not necessarily eliminate) *Legionella*,⁸ while others advise not using a high temperature regime as the only means for controlling *Legionella*.⁸⁴

Where a scald risk is identified, thermostatic mixer valves (TMVs) need to be fitted as close as possible to the POU. The TMVs will blend cold water with the hot water, thereby lowering the temperature of the water reaching the outlet. Where TMVs are fitted, they should be as close to the point of use as possible to minimize the storage of blended water. Ideally, TMVs should be incorporated directly in the tap fitting. Blended water downstream of a TMV may provide an environment in which *Legionella* can multiply thereby increasing the risk of exposure. Thermal disinfection will not disinfect downstream of TMVs. TMVs should be maintained, cleaned and disinfected in accordance with the manufacturer's instructions for use.⁸⁹

17. What is the required chlorine residual in the water system?

For continued protection against potentially harmful organisms in distribution systems or premise plumbing systems, some level of chlorine needs to remain—referred to as free chlorine residual - after initial disinfection by the municipality.

The recommended optimum target for free chlorine residual concentration in a water distribution system is 0.2 mg/L before it reaches the consumer—a minimum residual level of 0.05 mg/L is required in Ontario.⁹⁰ This level of residual is generally insufficient to help control biofilm and *Legionella* in a building's water distribution system.⁴⁰ Where necessary (e.g. a hospital housing immunocompromised patients), supplemental chlorination with automatic continuous chlorinators are typically used to maintain a 0.5 to 1 mg/L residual through the distribution system to aid in the control of biofilm and *Legionella*.⁴⁰

The following points are noted concerning chlorination:

- Free chlorine residual can be quickly depleted by high turbidity levels, corrosion and elevated water temperatures.^{40,60} Therefore, chlorine levels must be closely monitored, especially at hot water taps at far ends of the hot water distribution system to ensure there is an effective chlorine residual concentration.⁴⁰
- The biocidal effectiveness of the chlorine is severely affected by pH levels; the water pH should be less than 7.5.⁶⁰
- If large amounts of scale, sediment or corrosion are present in the system, then biofilm is likely to be present which can make chlorination less effective. Biofilm may need to be controlled before effective disinfection can be achieved.⁴⁰
- Ontario has set a maximum target free chlorine residual of 4.0 mg/L within the drinking water distribution system.⁹⁰ Continuous chlorination at high levels can lead to odour and taste issues, skin and eye irritation and leaks due to corrosion.^{40,60}

- Free chlorine residual can react with organic matter such as biofilm within water systems to form a variety of disinfection by-products including chloroform—a trihalomethane which is classified as a possible human carcinogen.⁹¹
- Lapses in continuous chlorination can quickly lead to an increase in the *Legionella* population. It is critical that all disinfection systems—not only chlorinators—be monitored continuously since none of them eradicate *Legionella* from the water distribution system.⁵⁵

18. How often should the building's water system be tested for chlorine levels?

There is no standard for how often the free 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, sediment, corrosion, turbidity, history and pattern of the residual levels, the type and level of contamination and the population at risk.

For most water systems, the HSE recommends the following routine inspection and maintenance to ensure control:⁴⁰

- Weekly—check the chlorinator and the amount of chlorine product in the reservoir.
- Monthly—measure the concentration of free chlorine residual at taps nearest to and furthest from the chlorinator; the concentration should be 0.5 to 1.0 mg/L.
- 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 residual.

19. Water may remain stagnant in pipes until the water outlet is 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. In healthcare settings, there are areas which may have water outlets that are not used for significant periods of time as wards or floors are closed for renovation and then reopened. Outlets in these areas are more likely to harbour *Legionella* than those in areas where outlets are in regular use.⁸⁹

When outlets are not in regular use, the HSE recommends weekly flushing for several minutes.⁴⁰ 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 a susceptible population (e.g. the immunocompromised) is present. It is important that flushing be carried out with minimum creation of aerosols (i.e., the water flow should be increased gradually to a full flow to

minimize the production of aerosols) since inhalation is the main route of exposure. Workers performing flushing should not be at high risk for Legionnaires' disease.

Public health management

20. Why is it difficult to find a common environmental source of Legionnaires' disease if typing can be conducted through culture testing?

While genetic typing of culture isolates 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 significantly over time.⁴ Because of this fluctuation, the time difference between an outbreak becoming evident and samples being taken (which can be weeks or months) may make it difficult to interpret the results and establish an epidemiological link between the causative agent and environmental source.
- *Legionella* can reside within protozoa therefore standard culture methods can produce false negative results.¹
- There is difficulty in isolating *Legionella* if the samples contain high background levels of other microorganisms.⁸⁹
- Under stressful environmental conditions *Legionella* can enter a viable but non-culturable *Legionella* (VBNC) state in which standard culture methods will not detect it.⁹²
- Some *Legionella* species capable of causing disease do not grow well using the traditional culture method for the isolation of *Legionella*.⁸⁹
- A potential environmental exposure source may be missed during the environmental investigation, resulting in the actual source not being sampled.
- Environmental samples may have been taken after the environmental source was disinfected, yielding negative results.
- For large outbreaks involving environmental sources such as cooling towers, when using genetic typing, it is possible to identify the strain responsible for clinical cases in multiple environmental sources. In such situations, it is only through more sophisticated testing involving whole-genome sequencing and good epidemiological evidence that the most likely source can be identified.⁹³
- If the urine antigen test is used to diagnose Legionnaires' diseases it is possible that the case may be treated and released without a respiratory specimen being taken to identify the strain. From a public health standpoint it is critically important that a respiratory specimen on which culturing can be attempted be obtained concurrently with a positive urinary antigen test. The specimen

should be taken before treatment is initiated because it will be difficult to culture since most of the *Legionella* bacteria may be killed.

21. For the cases that have occurred in Ontario, were they of the same species or serogroups?

In Ontario from January 2010 to April 2014, 2.5% (725/28,965) of clinical specimens tested at the Public Health Ontario laboratory were positive for *Legionella* species.⁶⁶ Of all *Legionella* species identified, 700 (96.6%) were *L. pneumophila*, and 25 (3.4%) were *L. non-pneumophila*. *L. pneumophila* serogroup 1 represented 680 (93.8%) of all *Legionella* identified to the species or higher level by PHO laboratories. These results should be interpreted with caution as they may overestimate the prevalence of *L. pneumophila* serogroup. Urine testing is the predominant testing method for *Legionella* at PHO and this method only detects *L. pneumophila* serogroup 1.⁶⁶

22. Are there any recommendations for investigation of travel-related cases?

The investigations associated with travel related cases consist of two distinct components: 1) the clinical investigation and 2) the environmental investigation.

Ideally, the home address for the case will determine the jurisdiction responsible for interviewing the case and collecting relevant clinical and exposure information. However, considering the incubation period can be 2 to 14 days or longer,⁹⁴ the case may seek medical care with clinically compatible signs and symptoms in any jurisdiction.

The environmental investigation is usually done by epidemiological link (if supported) and appropriate clinical specimen (e.g., respiratory culture results) by the jurisdiction where the potential source of exposure was identified.

Travel is identified as a risk factor for Legionnaires' disease and has been associated with cruise ships,⁹⁵ hotels,⁹⁶ resorts,⁹⁷ whirlpool spas,⁹⁵ showers,⁹⁷ standing near a decorative fountain in a hotel,⁹⁶ or touring in cities with buildings that have cooling towers.⁹⁸

Depending on the location and source of infection, detection of travel-associated cases can be challenging due to:¹³

- the long incubation period (2-14 days or longer).
- cruise ships disembarking passengers at different ports permitting infected asymptomatic persons to disperse from the location where they acquired the infection.
- individuals that may stay at hotels or other accommodations prior to or after leaving the ship.
- physicians that are unlikely to see more than one case associated with a specific location.

- travel related Legionnaires' disease surveillance being poor (there is no international surveillance system to link cases), thus making it difficult to connect cases.

Public health units should question confirmed and probable cases of *Legionella* about travel exposures. Cases linked to exposure locations outside of Canada should be reported to Public Health Ontario (PHO) through iPHIS (integrated public health information system) referral with the following:

- investigating health unit
- iPHIS number
- date and country of travel
- places visited/stayed (full address, if possible)
- symptom onset date
- laboratory test results, including if respiratory culture done

23. When should one start an environmental investigation—after only one case or several?

The Ministry of Health and Long-Term Care's (MOHLTC) Infectious Disease Protocol states that reported cases should be investigated to determine the source of infection.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.⁵⁹

When two or more cases are epidemiologically linked by location and time of exposure, an investigation should be conducted to determine the existence of a cluster/outbreak. If an outbreak is deemed to exist, the MOHLTC's protocol outlines the steps needed to manage the outbreak.⁵⁹

According to the protocol, environmental sampling should be reserved only for investigations involving facilities and disease clusters/outbreaks where a potential common exposure has been identified.⁵⁹ Be sure to engage the PHO laboratory prior to specimen collection.

24. 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?

Once samples are taken, facilities can immediately stop the use of potential sources (e.g., closing a hot tub to bathers) and begin disinfection and remediation procedures. The closing (or elimination) and remediation of the potential source must often be expedited without waiting for results of environmental sampling in order to prevent further cases of legionellosis.

25. Is there a formal definition for nosocomial Legionnaires' disease for epidemiological investigations?

Ontario does not have a definition for nosocomial Legionnaires' disease. Both the WHO¹³ and the CDC⁹⁹ have definitions.

World Health Organization (WHO)

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.¹³

Depending on length of stay in hospital before onset and environmental investigation results, cases are defined as definitely, probably or possibly nosocomial.

Definite/confirmed 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 genetic typing methods) from isolates obtained from the hospital water system around 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).

Centers for Disease Control and Prevention (CDC)

The CDC's case report form defines a case of definite/confirmed nosocomial Legionnaires' disease as if the patient was hospitalised continuously for 10 or more days before the onset of illness, and as a possible Legionnaires' disease if the patient had exposure to a healthcare facility (inpatient or outpatient) for a portion of the 10 days prior to onset.⁹⁹

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