

Chapter 6: Environmental Sampling

6.1 Introduction

Examination of water samples can be a useful method for identifying potential sources of *Legionella* infection. The objectives of environmental water sampling are as follows:

- Confirmation or exclusion of the implicated site as a source of infection
- Risk assessment of the site's water system(s)
- Distinguishing between local or system-wide colonisation of water system(s)
- Identifying critical sites
- Checking the regulation of the temperature, pressure and flows in the plumbing system
- Selecting the right strategy for short-term control of *Legionella*
- Facilitating a proposal for the long-term control strategy for the whole facility.

Sampling for the purposes of routinely monitoring the effectiveness of control measures should only be undertaken on the basis of a comprehensive risk assessment. Whilst sampling for the routine monitoring of *Legionella* represents only one aspect of monitoring the effect of a water treatment programme, it can be useful for auditing control measures, and also to validate new disinfection regimes.³⁸ In addition, sampling and culturing for *Legionella* may be carried out for the purpose of tracing the source of an outbreak.

Sampling is not a substitute for good maintenance practices and water treatment.⁷⁵

6.2 Sampling criteria

A successful examination for *Legionella* depends on several factors:

- The quality of the sample(s)
- The location of sampling points in terms of being representative of the water system being tested
- The timing of the sampling in relation to the normal operating conditions and control measures of the system, including the timing and levels of biocide dosing
- Proper transportation and storage of the sample(s) to ensure that the sample(s) should undergo as little change as possible before the analysis begins.³⁸

6.3 Safety

Environmental samples for *Legionella* should be collected by people with knowledge of *Legionella* ecology and general risk assessment. People taking environmental samples require training to ensure that they select samples containing the highest numbers of bacteria and that they are aware of the risk to themselves and to others from potentially positive sites. Based on a written risk assessment, in some circumstances, it may be necessary to use respiratory protective equipment (see Chapter 7, Section 7.4 on PPE).³ Individual staff who may be particularly prone to an increased risk of *Legionella* infection due to underlying conditions or immunosuppression should not be involved in sampling operations.³⁸

6.4 Site assessment

The number and types of sites that should be tested to detect *Legionella* must be determined on an individual system basis because of the diversity of plumbing, heating, ventilation and air-conditioning systems in the various institutions that may be sampled.³ Samples should be representative of each separate water system. They should be taken from the proximal and distal end of the water system and a number of sentinel points in between, the number and location being based on a comprehensive risk assessment (Table 9). Selection of sampling sites also depends on whether the sampling is for routine monitoring or to investigate an outbreak.

Table 9. Sentinel points for sampling

System	Sample points
Cold water system	Storage tank Furthest outlet from the storage tank Other outlets in areas considered to represent a particular risk e.g. hospital wards with 'at risk' patients
Hot water system	Calorifier outlet or nearest tap to the calorifier outlet Return supply or nearest outlet to the return supply Base of calorifier where drain valves have been fitted Furthest outlet from the calorifier Other outlets in areas considered to represent a particular risk e.g. hospital wards with 'at risk' patients

It is essential to undertake a survey of the site to be investigated prior to taking any sample.³⁸ All surveys follow a basic pattern. The source and the quality of the water should be determined and the site should be examined to establish the location of all systems using water. These systems should then be reviewed and assessed to determine which systems contain water at temperatures likely to support the growth of *Legionella* bacteria. In addition, areas within the systems where growth of *Legionella* bacteria may be expected to be greatest should be reviewed, as should locations where potentially contaminated water might produce aerosols or where aerosols might be released into the environment. The route or pathway of the water through the system should be followed from its entry into the site to the point where it is used or discharged. If a schematic diagram does not exist or is not available, or is known to be or is suspected of being out-of-date, then an up-to-date diagram should be prepared indicating, for example locations of:

- The in-coming water supply, whether of mains or private source
- Storage tanks, expansion or pressure vessels, filters, booster vessel pumps and strainers
- Water softening filters or other storage or treatment facilities
- Calorifiers or water heaters
- The type and nature of materials and fittings, for example taps, showers, water closet cisterns, valves, thermostatic mixer valves, pressure release valves, bathroom radiators and towel rails connected to the domestic water supply (and associated pipework) and the presence of metals, plastics, jointing compounds
- Evaporative cooling towers and condensers or heating circuits
- Air conditioning systems or humidifiers within the building which are supplied with, and store water and which may produce aerosols
- Other equipment that contains water and which might be a potential risk, such as spa pools, humidified display cabinets, machine tools, fountains, etc.
- Equipment that is used infrequently or might not normally be of concern but presents a risk only when the system undergoes maintenance or repair
- The presence of dead-legs or blind-ends.³⁸

When all risk sites have been identified the appropriate samples can be collected. There should be discussion with the laboratory which will analyse the samples on the number and type of samples required.³⁸ Arrangements should also be made for the transportation of the samples to the laboratory.

Aseptic precautions during sampling

It is important to take appropriate precautions to eliminate cross-contamination occurring between sampling sites, especially when collecting dip samples from storage tanks, cisterns and cooling towers.³⁸

6.5 Sample types

Two primary sample types should be collected when sampling for *Legionella* - water samples and swabs of biofilm.³

Water samples capture the planktonic form of *Legionella* or any disturbed biofilm. Generally, a minimum

of one litre must be collected.³⁹ Samples should be collected in new, unused, capped or pre-sterilised polyethylene or similar containers containing sufficient sodium thiosulphate to neutralise any chlorine or other oxidising biocide. Temperatures should be measured using a calibrated thermometer, placed in the middle of the water stream.

Swab samples capture the sessile form of *Legionella* that is associated with biofilms.⁸⁰ Swab samples must be taken before water samples when collecting both sample types from the same outlet. Swab samples must be kept moistened with sterile water. Multiple samples can be collected from the same site. Sterile absorbent cotton wool swabs should be used.³⁸

6.5.1 Pre-flush sample

A pre-flush sample is water collected immediately after the tap or fitting is opened. The tap or fitting should not have previously been disinfected, or water run to waste. The pre-flush sample represents water held within the tap or fitting and ideally, should be taken when the tap has not been used for several hours.³⁸

6.5.2 Post-flush sample

A post-flush sample is water collected after the tap or tap fitting has been disinfected and water in the fitting has run to waste. The post-flush sample represents the quality of circulating water supplied to the tap or fitting.³⁸

A pre-and post-flush sample should be taken at all outlets sampled.

6.6 Additional information

Information should be gathered to help interpret the results. As a minimum, the following information should be included on the request form:

- The site and sample point
- The sample references and date
- The reason for sampling
- The temperature of the sample source (e.g. the temperature of a hot-water system at one minute after turning on the tap and at two minutes after turning on the cold tap)
- Any biocide used
- The timing of the dosage in relation to sampling
- The concentration detected at the time of sampling
- Any other risk factors of importance (e.g. closed system opened for maintenance)
- High risk of nutrient present, such as in plastics manufacturing plants
- Any cases associated with the site.³

During the sampling all details that may help the implementation of possible remedial measures should be recorded. For example, obvious pressure and temperature drops or rises in the water circuits, the presence of iron sediment or sludge, the condition of the aerator and taps, the occurrence of scale, and the presence of various rubber and plastic attachments.

6.7 Sample transport and storage

All samples should be transported to the laboratory in dark, insulated containers to protect them from extreme temperatures and from light.³ Analysis should begin as soon as possible after the sample has been taken, preferably on the same day. If analysis is delayed, samples should be stored so that concentration and incubation procedures can be commenced within 48 hours of collection. The maximum time from sample collection to culture of the concentrate is 14 days. Samples should be transported and stored at less than 18°C but not less than 6°C.³⁹ Storing the sample in a refrigerator at temperatures below 6°C may reduce subsequent recovery of *Legionella* bacteria since the bacteria may be induced into a non-culturable state. Although *Legionella* will not multiply significantly during this period, the organism may be adversely affected by the presence of biocides remaining in the sample. If biocides are likely to be present in the sample and cannot be neutralised prior to storage this information should be recorded, and the transport and storage times kept to a minimum.³⁸

6.8 Laboratory analysis

Analysis of water samples and swabs for *Legionella* should be carried out by an accredited laboratory which takes part in an external quality assessment scheme for the isolation of *Legionella* from water and is operating in accordance with the international standard ISO 17025:2005. Laboratory facilities for environmental testing should be available in each HSE area.

To meet international best practice requirements a national *Legionella* reference laboratory should be established for clinical and environmental sample testing, to act as a typing centre and to provide expert opinion on the microbiology of the organism.

For more detailed information on sampling procedures see 'The determination of *Legionella* bacteria in waters and other environmental samples (2005) – Part 1 – rationale of surveying and sampling' produced by the UK Environment Agency.³⁸

6.9 When to take an environmental water sample

It is essential that before a *Legionella* control programme is commenced that a risk assessment is undertaken on site (Chapter 4) and that a written control plan is developed to cover the actions required if sampling for *Legionella* is positive. The UK HSC guidelines on the control of *Legionella* bacteria in water systems outline when sampling should be performed and provide guidance on the appropriate action that should be taken.⁶⁴ A summary of its recommendations in relation to hot and cold water systems and cooling towers is outlined below.

6.9.1 Hot and cold water systems

Routine monitoring for *Legionella* in hot and cold water systems is not normally required unless problems arise in the system, for example:

- In water systems treated with biocides where hot water storage temperature is <60°C and distribution temperature is <50°C. Sampling should be carried out monthly initially. The frequency of testing can be reviewed after a year and may be reduced when confidence in the efficacy of the biocide regimen has been established
- In systems where control levels of the treatment regimen (e.g. temperature, biocide levels) are not being consistently achieved. As well as carrying out a thorough review of the system and treatment regimen, more frequent samples should be taken to determine the efficacy of control measures
- When an outbreak is suspected or has been identified.

Table 10 outlines the action level for *Legionella* sampling in hot and cold water systems.

Table 10. Action level following *Legionella* sampling in hot and cold water systems

<i>Legionella</i> bacteria cfu/litre	Action required
>100 but <1,000	<p>Re-sample and review control programme - if only one or two samples are positive the water 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</p> <p>If the majority of samples are positive, the system may be colonised, albeit at a low level, with <i>Legionella</i>. 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</p>
>1,000	<p>Re-sample, review programme, disinfect system - 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</p> <p>If the identified control measures including disinfection fail to achieve reduced levels of <i>Legionella</i> bacteria, the water distribution system should be examined in more detail. If the structure and fabric of the water distribution system is found to be the cause of continued failure to control the level of <i>Legionella</i> bacteria, the water distribution system or part of the system should be replaced as deemed appropriate to ensure control</p>

Source: Adapted from Table 4 in HSC UK – Legionnaires' disease: the control of *Legionella* bacteria in water systems: approved code of practice and guidance⁶⁴

6.9.2 Cooling systems

In cooling tower systems, in addition to routine sampling for aerobic bacteria, a routine monitoring scheme should include periodic sampling for the presence of *Legionella* bacteria. This should be undertaken at least quarterly unless sampling is necessary for other reasons such as to help identify possible sources of the bacteria during outbreaks. More frequent sampling should be carried out when commissioning a system and establishing a treatment programme or when conducting a review of the system/risk assessment to help establish when the system is back under control.

Sampling methods should be in accordance with the international standard ISO 11731 – *Water quality – detection and enumeration of Legionella*.³⁹ This standard provides advice on best practice for the collection, transportation and storage of samples. It can be purchased from the National Standards Authority of Ireland, Glasnevin, Dublin 9 (Phone: +353 1 8073874). Samples should be taken from the cooling tower water reservoir.

Table 8 in Chapter 5 outlines the action levels following microbial monitoring of cooling towers. Failure to detect *Legionella* bacteria should not lead to the relaxation of control measures and monitoring. Neither should monitoring be used as a substitute in any way for vigilance with control strategies and those measures identified in a risk assessment.

6.9.3 Healthcare facilities

Routine environmental sampling and culture for *Legionella* in healthcare facilities should be based on a comprehensive risk assessment and should be part of an overall management strategy.

The subcommittee recommends that routine water sampling should be done six monthly in healthcare facilities, including nursing homes and long-stay care institutions. In patient care areas for persons at high risk for *Legionella* infection i.e. transplant units,⁶⁹ monthly culturing for *Legionella* in water samples is recommended as part of a comprehensive strategy to prevent legionnaires' disease in transplant recipients. The addition of filter-heads to showers in transplant units should be considered.

The subcommittee also recommends that the Dutch guidelines be followed and the number of samples taken should be based on the number of outlets in the water system as shown in Table 11.⁸¹ This will require additional resources.

Table 11. Number of water samples recommended for healthcare institutions

Number of outlets	Number of samples
<50	2 samples
51-100	4 samples
101-200	6 samples
201-400	8 samples
401-800	10 samples
801-1,600	12 samples
>1,600	14 samples

6.9.4 Domestic premises when a case has possible domestic exposure

Health Protection Scotland (HPS) published their advice on water sampling for *Legionella* in domestic premises, based on a study carried out between 1994 and 1998 by the UK Building Research Establishment (BRE).⁸²

The study found that it was not unusual to isolate *L. pneumophila* from domestic water systems and its presence per se did not present an unacceptable risk to occupants. Host factors played a significant part in determining if exposure resulted in symptomatic illness. It is likely that most if not all of the population is periodically and even regularly exposed but that only in special circumstances do host factors, level of exposure and infectivity of the particular *Legionella* strain result in a clear case of disease. **Immunocompromised patients should be advised on the avoidance of risk.**

HPS concluded that, as there is a possibility of identifying *Legionella* in any domestic system, sampling of an individual's home should not be a routine response to a notification of a sporadic case unless there are other factors which can be taken into account. Such sampling may lead to isolation of the organism

with consequent pressure for its elimination, a process that is technically problematic and may well be unsuccessful. If domestic water sampling is contemplated there must be a clear rationale for doing so which considers in advance what action, if any, will be taken in the event of identifying the organism in the supply. Possible valid reasons for considering testing a domestic water supply include:

- Eliminating the house as a source of infection in an individual case for epidemiological purposes only
- Identifying a continuing risk of exposure in situations where there is reason to believe that another occupant of the property might be at increased risk (as opposed to a normal level of risk) of developing illness.

HPS also proposed that at least one of the following additional criteria should be fulfilled:

- Evidence that a *Legionella*-like illness, though not necessarily clinically or microbiologically confirmed, has occurred previously amongst occupants of the same house
- Evidence that sampling of the water system would contribute information to inform prevention and control of legionellosis in general terms and which could not otherwise be obtained.

6.9.5 Spa pools

Spa pools will also require regular monitoring for *Legionella*, as other routine microbiological parameters are not good indicators of the risk from *Legionella* (see Chapter 8, Section 8.5.9).³⁸