Difficulties encountered during the evaluation of a *Legionella* contamination level in a sanitary installation

K. Dinne (1), B. Bleys (2), O. Gerin (3)
(1) karla.dinne@bbri.be
(2) bart.bleys@bbri.be
(3) olivier.gerin@bbri.be

(1), (2), (3) Belgian Building Research Institute (BBRI), Belgium.

Abstract

Monitoring of Legionella bacteria is important for public health reasons in order to identify the environmental sources which can pose a risk of legionellosis, such as hot and cold water distribution systems and associated equipment. Different international standards describe how to take water samples and which technique can be used to analyze these water samples. Worst case scenario's for sampling indicate that the first liter, without a flush, should be taken to evaluate the risk.

During our research we evaluated different sampling protocols. The full- scale test facility at BBRI, contaminated with *Legionella pneumophila* bacteria served as case-study [6-7].

Samples were taken on a regular base from the sampling valves (Depart & Return) and from the faucets (shower or kitchen faucet). The sampling ball valves on the depart and return were mounted on T- pieces. This means that a small water volume (~4 ml) from the circulation loop is trapped in the connection, presenting a small "dead zone" suitable for biofilm development. The study included "first liter" samples, taken at once (volume 1liter), as well as fractionised water samples (collecting the first 200 ml water separately from the 800 ml water). Also biofilm measurements were included to evaluate the contamination level of the circulation loop [7].

This article will point out the influence of the sampling protocol on the interpretation of the 'Legionella contamination level 'within a sanitary installation. The study indicates the importance of a well described sampling strategy and protocol, in compliance with the information needed for a risk assessment.

Keywords

Drinking water installation, Legionella development, water sampling, analysis

1 Introduction

Much has been leaned about 'Legionnaires disease' ant its causative agent *Legionella* since the outbreak in 1976 meeting of the American Legion in Philadelphia. Because of the dedication of scientists worldwide, we now have advanced knowledge of the clinical and epidemiological aspects of Legionnaires disease, we know the sources and reservoirs for *Legionella* organisms and the environmental conditions under which they grow and die. We also have state-of the art technology to detect the *Legionella* bacteria in water systems and we have some knowledge on how to control *Legionella* organisms in water systems.

Different specific guidelines are available for the management of water systems associates with buildings. The "European Technical Guidelines for the Prevention, Control and Investigation of infections caused by Legionella species" [1] updates advice on risk assessment and the management of different sources of infection and offers a standardised approach to procedures for preventing and investigating *Legionella* infections. This guideline aims to further harmonise these procedures among Member States but national laws on specific aspects of control and prevention differs between these European guidelines and regulations in force in Member States.

Until now, no scientific information is available on the concentration of *Legionella* bacteria in water necessary to cause illness. There is no safe level of *Legionella* in water systems. The ultimate objective is to either not have any *Legionella* in the system or to rid a system of all *Legionella* bacteria. However, minimising the *Legionella* concentration in the water of the building system is one of the major challenges for building owners, maintenance staff, plumbers and service firms. The advisory report from the Superior Health Council of Belgium [2] for Health Care Premises, reported a maximum level of 1000 cfu/l *Legionella pneumophila* bacteria in order to minimise the risk of infections.

The major similarity in all the regulations and guidelines is the importance of the risk assessment. The risk assessment should provide adequate information for the user and the investigator about the risks from each system and the measures necessary to ensure that the water systems are safe and without risks to health. The risk assessment is a critical component, which aims to identify weak points in a system where waterborne hazards could enter, and which might increase within the system to levels which pose a risk to users and anyone else who could be exposed. The information generated by the risk assessment will be used to develop a management scheme to manage the hazards and mitigate the risks by implementing appropriate remedial works and control measures. If microbiological samples are to be taken as a part of the risk assessment process, the assessor should have been trained to know how and when to take samples and where from. The assessor must be aware of how the sampling protocol applied at different technical equipment and components in a system can influence the result of the *Legionella* concentration in the water, reported by the laboratory.

During our research we evaluated the importance of some technical details (as connections) present in the installation, responsible for stagnation of small water volumes. Different sampling protocols are analyzed in order to estimate / evaluate the

real concentration of *Legionella* bacteria circulating in the test facility. The full- scale test facility at BBRI, contaminated with *Legionella pneumophila* bacteria served as case-study. The test facility and the applied drawn-off profile are described in our previous articles [6-7].

2 Test setup

2.1 General description

Generally, water samples should be taken for routine sampling as the results are comparable over time and therefore useful for trend analysis. There are many published methods for the detection of *Legionella* from water samples, including those in both international and national standards. The International Standardization Organization (ISO) produces standard methods including for the detection of *Legionella* by culture (ISO 11731: 2017 [3]). Within Europe, CEN (Committee for European Standardization) has adopted this ISO 11731 standard, what means that different countries in Europe must adopt them for use.

It is important to understand that a sample taken from a water system is only a small portion of the total system volume and that a negative *Legionella* result does not necessarily mean the entire system is under control. Microorganisms are not uniformly distributed throughout the water system all the time, especially in areas with poor flow and stagnation or where controls are not effectively maintained.

Samples should be taken, transported and conserved in accordance with nationally and internationally accepted methods (Water Analyses Compendium A 001-004, and 10 [4]; ISO 5667 Part 1,3 and 5; ISO 19458 [5]).

In Belgium, worst case scenario's for sampling indicate that the first liter of water, just after opening the tap (without a flush, without disinfection of the tap) should be taken to evaluate the risk.

2.2 Sampling campaigns

During the study of the disinfection procedures on the test facility [6-7], the evolution of the *Legionella* concentration is analyzed at different sampling points (depart pipe, return pipe, kitchen draw-off pipe and shower draw-off pipe). The first liter of water, directly after opening the tap from the sampling point is collected in a sterile bottle and analyzed in the laboratory. The evolution of the *Legionella* concentration in the system during different disinfection procedures (thermal shocks) is presented in the previous articles.

Tapping the first liter of water could give higher concentrations of *Legionella*, due to a small volume of stagnation in the tap point, which will not be representative for the concentration circulating in the system.

A consecutive sampling is applied at two taps (depart and return pipes)

- the first liter, just after opening the sampling tap is collected in a sterile bottle.
- the next 4 liters water are flushed away,

- a second sample is taken (= 5th liter water).
- another liter is flushed away
- the last sample is taken (= 7th liter water).

Figure 1 shows the results of the *Legionella* concentration in the different water samples: the first liter, the 5th liter and the 7th liter as well as the mean value (all water samples from the return pipe).

The connections of the sampling tap on the return pipe host a small volume of water (~4 ml) which is stagnating and will not rise in temperature during heat chocks. In order to evaluate the possible *Legionella* concentration in this volume of trapped water, the tap is removed, and the volume was collected with a sterile pipet for further analysis. Table 1 shows the *Legionella* concentration in the dead zone of 4 ml water, and the *Legionella* concentration circulating in the return pipe.

Figure 1: Picture showing how the dead water volume is collected using a sterile pipet

As the *Legionella* concentration in the test facility fluctuates due to the consecutive heat chocks, the sample of the 'first liter' was analyzed more in detail. To evaluate if the small volume of water trapped in the sampling valve influences consequently the results, the first liter sample is collected in 2 portions (the first 200 ml separately from the following 800 ml water) and analyzed separately. Figure 2A and 2B show the results of the *Legionella* concentration in the different water samples, over a sampling period of 4 months with the hot water production at 45°C and heat shocks at 65°C, the depart pipe and return pipe.

The sensitivity of the method should be such that the laboratory can reliably recover 50 cfu/l.

3 Results

3.1 Concentration of *Legionella* bacteria in the water, circulating in the test facility

Figure 1 shows the results from consecutive water sampling at the return pipe. The first liter, the 5th and 7th liter, as well as the mean value of the *Legionella* concentration (of the 3 samples) are listed. Sampling the first liter, without flushing, as required in Belgium for

worst case scenario analyses, will indicate most of the time (72%) a higher concentration of *Legionella* bacteria.

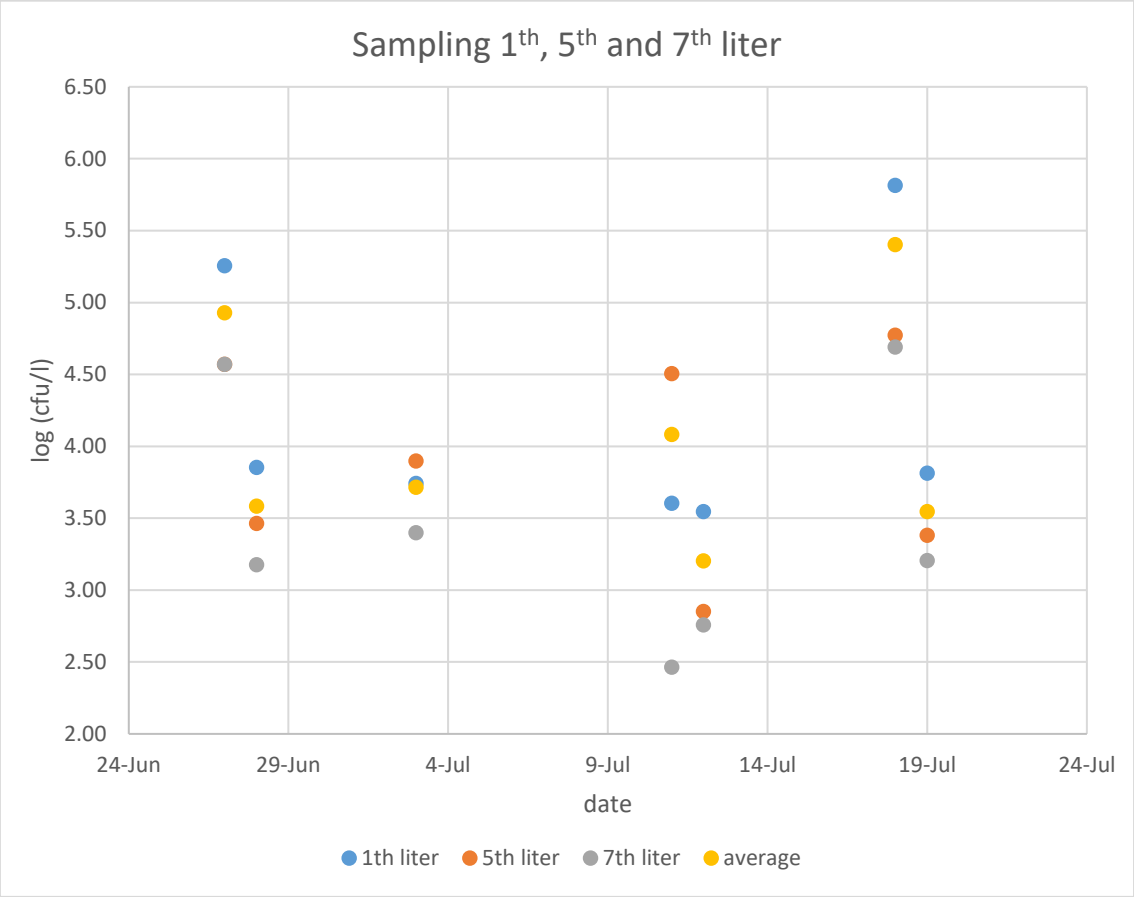


Figure 1: Consecutive sampling at the return pipe (the first liter, the 5th liter and the 7th liter)

3.2 Presence of *Legionella* in a small dead zone

In a sanitary installation, small water volumes can be trapped at connections zones. These dead zones won't be treated during automatic heat shocks without forced drain-off. After removing the tap, the trapped water volume is collected and analyzed. In order to evaluate the concentration of *Legionella* in the circulation system at that moment, the tap is reinstalled, and consecutive water samples are taken (1st liter draw-off, 5the liter draw-off and 7th liter draw-off). The analysis of the trapped water volume showed the presence of *Legionella pneumophila* in the small water volume. (see table 1).

Water Sample from the return pipe (03.07.17)	**Concentration (cfu/l) *Legionella pneumophila* (sg 1)**	**Log10 (concentration) *Legionella pneumophila* (sg1)**
volume of water (4 ml) trapped in the sampling tap	280	2.4
First liter draw-off	5100	3.7
5th liter draw-off	7900	3.9
7th liter draw -off	2500	3.4

Table 1: Legionella concentration in the system (return pipe) and in the trapped volume of water (4 ml) from the return pipe

In this test situation, the trapped water volume is very small, and should not influence/falsify the concentration of the first liter draw-off (in case the dead water volume was not removed). As a larger dead volume can be present in other situations, development in the dead zone might influence the result of a first liter draw-off.

3.3 First liter sampling

As the previous test on the homogeneity of *Legionella* bacteria circulating in the water indicates that the first draw-off liter represents most of the time (72%) the highest concentration of *Legionella* bacteria, the first liter water sample is collected in 2 portions (200 ml and 800 ml). Each portion is analyzed separately.

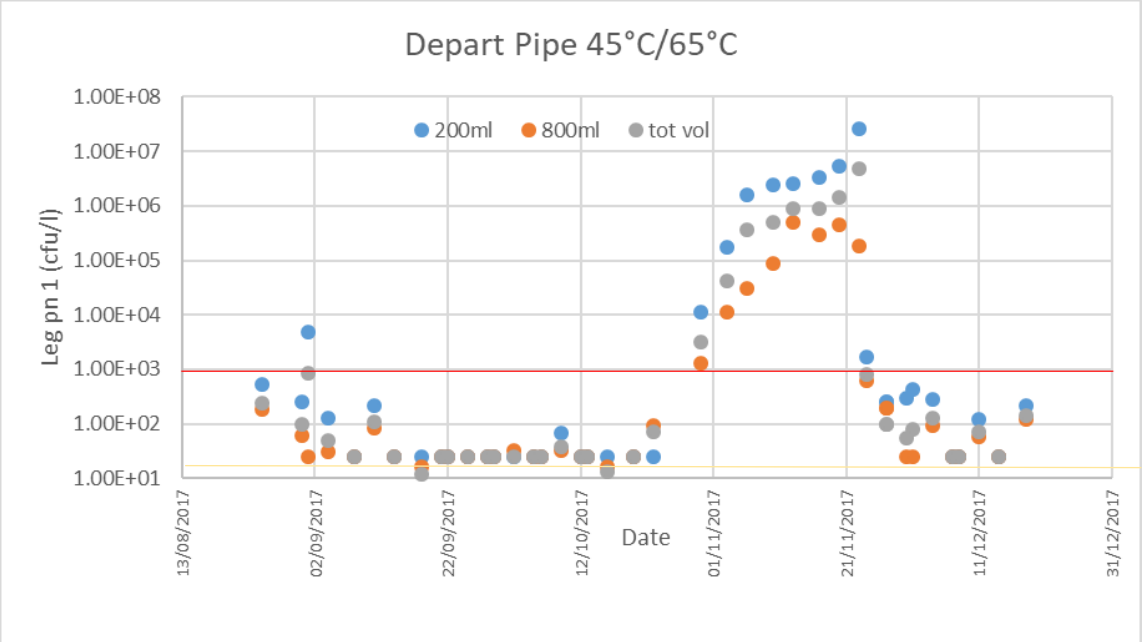


Figure 2A: Concentration of *Legionella pneumophila* in the first liter, sampled at the depart pipe, (sampling 200 ml / 800 ml)

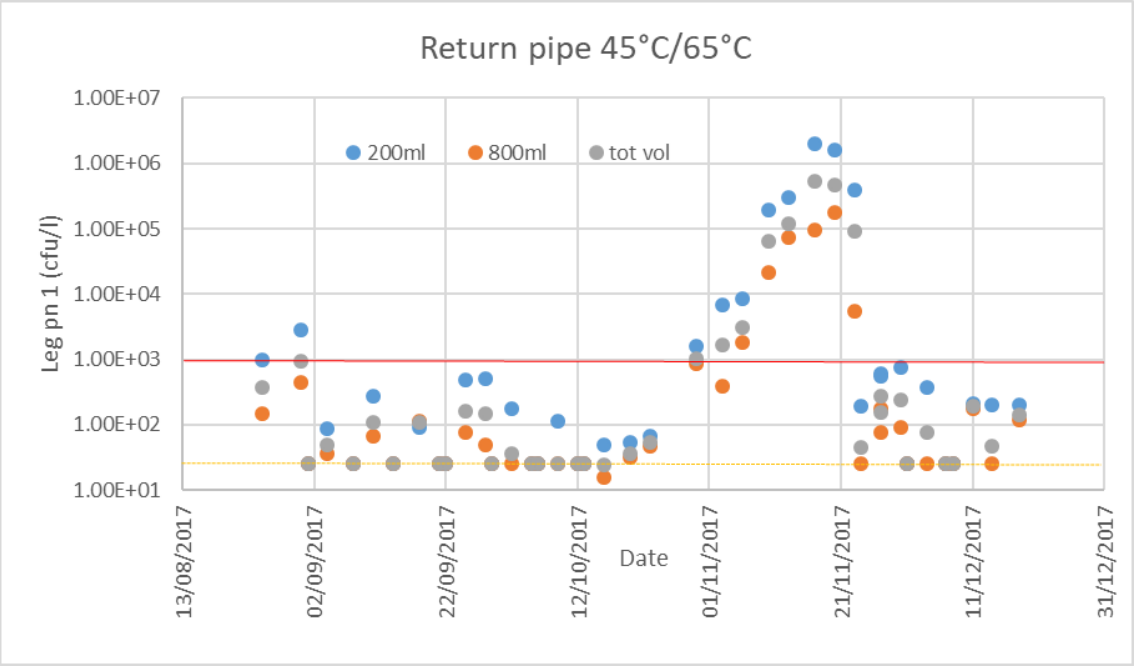


Figure 2B: Concentration of Legionella pneumophila in the first liter, sampled at the return pipe, (sampling 200 ml / 800 ml)

Figure 2A and 2B show the *Legionella* concentrations found in the first 200 ml water sample, in the 800 ml water sample and the *Legionella* concentration recalculated for the total water volume (1000 ml). These results indicate that the first 200 ml contains the highest concentration of *Legionella* bacteria compared to the next 800 ml of water.

3.4 Biofilm sampling

The return pipe contained 20 pieces of PE-x pipe with a surface ~ 20 cm² each. The DHW can flow through this littles pieces and the biofilm can grow on it. Before and after a disinfection shock, a piece of pipe was collected for biofilm analysis. As mentioned in a previous article no correlation was found between the concentration of *Legionella* bacteria in the water of the test facility and the ATP measurements on the pipe sections. The results from the ATP measurements showed that the bacterial flora on the tube was not affected by the different heat chocks [7].

4 Conclusions

Those carrying out risk assessments should understand the factors which lead to the colonization and growth of waterborne pathogens, including *Legionella*, and how these can be prevented or controlled. Those inspecting systems and taking samples should be familiar with all aspects of a water distribution system. The individual nature of each site should be considered.

If periodic sampling and testing for *Legionella* is part of the risk evaluation, a strict sampling plan should be introduced. Routine sampling at specific tap points, conform a

specific sampling protocol can lead to results comparable over time and are therefore useful for trend analysis.

Sampling the first liter, directly after opening the tap without any flush, reflects the worst-case scenario. Higher concentrations are detected in the first liter compared with *Legionella* concentrations in the bulk water flow in the system. In our test facility, even the first 200 ml are enough to evaluate the worst-case.

Small volumes of trapped water can contain *Legionella* bacteria. These places should be flushed during thermal disinfection in order to minimize the *Legionella* growth.

Biofilm monitoring and ATP measurements are no valuable alternative for routine *Legionella* sampling and analysis.

5 References

1. European Technical Guidelines for the Prevention, Control and Investigation of infections caused by Legionella species, June 2017.

2. Recommandations pour la prévention des infections à Legionella dans les établissements de soins, groupe de travail Legionella, N° CSH 7509, édition janvier 2002.

3. ISO 11731: Water Quality-Enumeration of *Legionella*, 2017

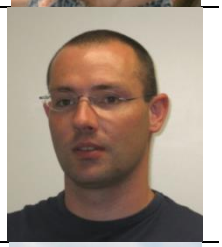
4. Water Analyses Compendium, WAC/A 001-004, and WAC/A/10

5. ISO 19458: Water Quality - Sampling - General guide for sampling, transport, preservation and handling of samples for bacteriological analysis, 2006

6. Evaluation of the risk of *Legionella spp* development in sanitary installations, K. Dinne, O. Gerin, B. Bleys, 2017 Symposium CIB W 62 Haarlem, the Netherlands

7. Evaluation of the risk of *Legionella spp* development in sanitary installations (Part 2), K. Dinne, O. Gerin, B. Bleys, 2018 Symposium CIB W 62 Azores, Portugal

6 Presentation of Author(s)

Karla Dinne is biochemical engineer and head of the laboratory Microbiology and Microparticles of the Belgian Building Research Institute (BBRI).	
Olivier Gerin is bioengineer and researcher in the laboratory of water technologies of the Belgian Building Research Institute (BBRI).	
Bart Bleys is bioengineer and head of the laboratory water technologies of the Belgian Building Research Institute (BBRI).