Expert Opinion

BioTechniques

Legionella water testing and the EU Drinking Water Directive: could potentially harmful *Legionella* bacteria slip through the gaps?

Sarah Delaney[1], Talia Arcari[1] & Orla O'Connor*,[1]

*[1]BioProbe Diagnostics, Room 218, Business Innovation Centre, Newcastle Road, NUI Galway, H91 NV29, Ireland; *Author for correspondence: orla.oconnor@bioprobedx.com*

BioTechniques 72: 00–00 (June 2022) 10.2144/btn-2022-0047

First draft submitted: 7 April 2022; Accepted for publication: 11 April 2022; Published online: 26 April 2022

KEYWORDS:

EU Drinking Water Directive ● *Legionella* pneumophila ● *Legionella* species ● Legionnaires' disease ● real-time PCR testing ● water quality ● water testing

The fallout of the EU Drinking Water Directive

Legionella bacteria are transmitted in contaminated water systems and infection can be caused through aerosol inhalation of this contaminated water. *Legionella* infection is the highest health burden of all waterborne pathogens in the EU [1]. The recent revision to the EU Drinking Water Directive (DWD) extends water quality risk analysis to include the monitoring of *Legionella* bacteria in all drinking water distribution systems, including large domestic water and industrial distribution systems [2]. The updated DWD entered into force in January 2021 and EU member states have 2 years to become fully compliant but can choose the testing method to use in accordance with the purposes specified in their national guidelines. The gold standard culture method (ISO 11731) is still required for full compliance with the DWD, but the new directive has become more flexible by incorporating the use of non-culture-based methods and molecular methods – in particular real-time polymerase chain reaction (ISO 12689), as a complement to the culture method and for risk-based monitoring. The fact that the DWD requires testing for *Legionella* spp. and not just *Legionella pneumophila*, the deadliest of the *Legionella* species, has sparked interesting discussions around national regulations and requirements for testing *Legionella* in water [3].

There appears to be discord in the implementation of the DWD [4] and different approaches are being adopted by different countries. France and Germany, for example, are predominantly focused on the detection of *L. pneumophila* [5]. In The Netherlands, a 'hybrid' approach to monitoring *Legionella* bacteria is advocated whereby the testing for a broader range of *Legionella* species is restricted to hospital settings as people with weakened immune systems are deemed more vulnerable [6]. Portuguese regulations allow real-time PCR testing of environmental water samples for the detection of both *L. pneumophila* and *Legionella* spp. when Legionnaires' disease cases have been reported and an investigation is carried out to identify the source of the infection [7]. To date, more than 20 species of the nearly 60 *Legionella* species identified have been implicated in human disease [8]. Some experts argue that the focus should be on testing for *L. pneumophila*, the species that accounts for most cases of the severe and potentially fatal Legionnaires' disease. The general view is that testing for other species that may not pose the same health risk can be expensive and time-consuming for testing laboratories and may lead to delayed test results to end users, ultimately having potential negative consequences for public health. However, assuming that the other wide range of *Legionella* species also potentially present in water systems will be benign can be problematic. The reality is that if one *Legionella* species is present, then clearly the growth conditions have been favorable – how long will it take before *L. pneumophila* rears its head?

Ignore non-pneumophila *Legionella* species at your peril

Legionella can cause either Pontiac fever, a mild infection with flu-like symptoms, or Legionnaires' disease, a type of pneumonia with a fatality rate of approximately 10% [1]. Although the majority of infections are attributed to *L. pneumophila* serogroup 1 (sg 1), cases of Legionnaires' disease caused by non-pneumophila *Legionella* species have been recorded. These include *Legionella longbeachae*, *Legionella bozemanii, Legionella anisa*, *Legionella dumoffii, Legionella micdadei* and *Legionella feeleii* [9]. Although these species may cause milder disease than *L. pneumophila*, people with underlying chronic conditions and immunocompromised individuals are at a higher risk. However, it must be noted that infections caused by non-pneumophila species in immunocompetent individuals have also been reported [10,11]. Depending on the geographic area, a great diversity of *Legionella* species and a high prevalence of non-pneumophila species have been observed. In some European regions there is a higher prevalence of non-pneumophila species compared with *L. pneumophila*, both in hospitals and domestic water systems [12]. In Australia and New Zealand, *L. longbeachae* causes equal cases of disease as *L. pneumophila* [13]. Although it is reported that non-pneumophila species account for only a small proportion of cases of Legion-

naires' disease, the number of these infections are undoubtedly underestimated worldwide [14]. Nonetheless, it would be imprudent to disregard these species. In addition to disease burden, there is the potential for non-pneumophila species to mask the presence of *L. pneumophila* within water systems. Because *L. pneumophila* is often found alongside other *Legionella* species in water [6], it may not be detected if the other species is in abundance.

True number of Legionnaires' disease cases largely underestimated

The predominance of *L. pneumophila* in reported infections is likely to be biased by the medical diagnostics method used to confirm suspected cases of Legionnaires' disease. Thus, the true number of *Legionella* infections is unknown and probably largely underestimated. Although culturing on selective medium remains the gold standard for the diagnosis of Legionnaires' disease, due to the slow growth of the organism, this method can take up to 7–10 days for results. Urinary antigen tests (UAT) are the most widely used method in clinical settings to obtain a diagnosis of Legionnaires' disease. This is due to its simplicity of use and rapid results within minutes. However, these UATs are limited as they are specific for *L. pneumophilia* sg 1, identifying 50–80% of cases. Therefore, if used as the sole diagnostic method, 20–50% of cases remain undiagnosed because disease caused by other serogroups or species are inevitably missed [15]. A study in Denmark found that patients infected with *L. pneumophila* sg 6 had an increased risk of death compared with those infected with other serogroups, with the lowest mortality observed for *L. pneumophila* sg 1 [16]. An outbreak of Legionnaires' disease in Italy in 2018 was attributed to *L. pneumophila* sg 2 [17]. In many countries, it is recognized that the incidences of Legionnaires' disease cases are underreported. In the US, it is thought that only 2.5–4.5% of actual cases are reported to the CDC [18]. The European Centre for Disease Control and Prevention noted in their 2019 epidemiological report that only 10% of Legionnaires' disease cases were culture-confirmed, meaning that infections caused by *Legionella* species other than *L. pneumophila* are likely underestimated [19]. This highlights the need for diagnostic methods that are fast, sensitive and that can reliably detect additional serogroups and non-pneumophila species – not only in healthcare settings but also within the environmental testing space.

Environmental *Legionella* testing & the importance of having a complete *Legionella* profile

With a complete profile of the *Legionella* species present in a water sample, better informed decisions and remedial actions can be taken, minimizing the risk of the bacteria spreading and causing human illness that could otherwise be prevented. Available methods for *Legionella* water testing include the plate culture method described in ISO 11731:2017, which is still the most widely used method for environmental testing, despite having several limitations: it does not recover all *Legionella* species or bacteria in the viable but non-culturable (VBNC) state, has a low sensitivity, a slow turnaround time (7–10 days to results), requires significant hands-on time and results can be inconclusive due to the overgrowth of non-target microorganisms [18]. The most probable number method is a liquid culture technique that allows identification and quantification of *L. pneumophila* [20]. It offers some advantages compared with the plate culture method, such as ease of use and a single protocol per water matrix, but a long incubation period is still required (7 days), and it only detects *L. pneumophila*; other *Legionella* species will not grow in the liquid medium, so additional testing will be required to determine the serogroup or obtain a full *Legionella* profile [18]. Another technique that can be used for *Legionella* detection in water samples is immunomagnetic separation (IMS). These tests are based on the use of anti-*Legionella* antibodies immobilized on magnetic microspheres combined with an enzyme-linked colorimetric detection [21]. Despite the manufacturer's claims that this is a fast and easy-to-use technology, it is not clear which *Legionella* species are detected, and the IMS method has not been widely adopted by *Legionella* testing laboratories [18]. Rapid lateral flow tests have also been adapted for environmental *Legionella* testing, but their utility is limited due to several disadvantages: they only detect *L. pneumophila* sg 1, are not quantitative and, depending on the sample type, their sensitivity is very low [22]. Multiplex real-time PCR assays offer a solution to most of these issues and have several advantages over the culture method: they are more sensitive, specific, easier to interpret and considerably faster, with a total turnaround time of 3–4 hours. The Bio Lp-1 assay (BioProbe Diagnostics) can not only determine if there are *Legionella* bacteria present in a water system, but also allow for differentiation between *Legionella* genus, *L. pneumophila* (sg 1–16) and *L. pneumophila* sg 1 within a water sample, and in a single PCR reaction. It helps to determine the significance of the problem and the extent of human risk. With this peace of mind, the urgency of the health scenario and the correct course of remedial action can be carefully assessed.

Conclusion & future perspective

Better diagnostic techniques and increased disease awareness have led to increased *Legionella* case reporting, but global challenges such as urbanization, aging populations, climate change and circular economy approaches will also have an impact on the number of Legionnaires' disease cases in years to come [23]. As EU member states scramble to become compliant with regulations, interpretations of the DWD may result in potentially harmful *Legionella* bacteria escaping detection. Traditional culture methods for detecting *Legionella* are no longer tenable, and policymakers and stakeholders should advocate for more rapid and efficient methods for environmental *Legionella* detection. The global environmental testing landscape is transforming and the role for real-time PCR testing is clear. This is amplified by the staggering growth rate of the global PCR market, with the demand for PCR machines more than doubling in 2020 [24]. As we look towards the future, the need for rapid and reliable *Legionella* detection methods will become increasingly necessary – if not to meet water safety regulations, then from a corporate social responsibility perspective. With the widespread use of real-time PCR machines around the world in the post-COVID-19 era, this will certainly be possible.

Financial & competing interests disclosure

The authors wish to acknowledge the European Union's Horizon 2020 research and innovation programme for its support under grant agreement no. 950822. The authors are employed by BioProbe Diagnostics. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1. World Health Organization. Legionellosis (2018). **www.who.int/news-room/fact-sheets/detail/legionellosis**
2. Directive (EU) 2020/2184 of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption (2020). **https://eur-lex.europa.eu/eli/dir/2020/2184/oj**
3. Hydrosense. New EU drinking water directive allows for more targeted approach to *Legionella* testing (2020). **https://blog.hydrosense-legionella.com/new-eu-drinking-water-directive-allows-for-more-targeted-approach-to-legionella-testing**
4. Debates – quality of water intended for human consumption – implementation of the EU water legislation (debate) (15 December 2020). **www.europarl.europa.eu/doceo/document/CRE-9-2020-12-15-INT-2-013-0000_EN.html**
5. *Legionella* Control. Water quality regulations to reduce *Legionella* outbreaks (2020). **https://legionellacontrol.com/legionella/water-quality-regulations-reduce-legionella-outbreaks/**
6. van der Mee-Marquet N, Domelier A-S, Arnault L *et al. Legionella anisa*, a possible indicator of water contamination by *Legionella pneumophila*. *J. Clin. Microbiol.* 44(1), 56–59 (2006).
7. Gregório de Freitas M de G. Doença dos legionários: diagnóstico laboratorial de doença dos legionários e pesquisa de *Legionella* em amostras ambientais (2017). **www.sip-spp.pt/media/33wky1i1/legionella-diagno-stico-laboratotial-2017-dgs.pdf**
8. Special Pathogens Laboratory. *Legionella* species. **https://specialpathogenslab.com/legionella-species/**
9. Muder RR, Yu VL. Infection due to *Legionella* species other than *L. pneumophila*. *Clin. Infect. Dis.* 35(8), 990–998 (2002).
10. Diederen BMW, van Zwet AA, van der Zee A, Peeters MF. Community-acquired pneumonia caused by *Legionella longbeachae* in an immunocompetent patient. *Eur. J. Clin. Microbiol. Infect. Dis.* 24(8), 545–548 (2005).
11. Lei C, Zhou X, Ding S *et al.* Case report: community-acquired *Legionella gormanii pneumonia* in an immunocompetent patient detected by metagenomic next-generation sequencing. *Front. Med.* 9 (2022).
12. Mazzotta M, Salaris S, Pascale MR *et al*. Occurrence of *Legionella spp.* in man-made water sources: isolates distribution and phylogenetic characterization in the Emilia-Romagna region. *Pathogens.* 10(5), 552 (2021).
13. Whiley H, Bentham R. *Legionella longbeachae* and Legionellosis. *Emerg. Infect. Dis.* 17(4), 579–583 (2011).
14. Chambers ST, Slow S, Scott-Thomas A, Murdoch DR. Legionellosis caused by non-*Legionella pneumophila* species, with a focus on *Legionella longbeachae*. *Microorganisms.* 9(2), 291 (2021).
15. Pierre DM, Baron J, Yu VL, Stout JE. Diagnostic testing for Legionnaires' disease. *Ann. Clin. Microbiol. Antimicrob.* 16(1), 59 (2017).
16. St-Martin G, Uldum S, Mølbak K. Incidence and prognostic factors for Legionnaires' disease in Denmark 1993–2006. *ISRN Epidemiol.* 2013, e847283 (2012).
17. Scaturro M, Rota MC, Caporali MG *et al.* A community-acquired Legionnaires' disease outbreak caused by *Legionella pneumophila* serogroup 2: an uncommon event, Italy, August to October 2018. *Eurosurveillance.* 26(25), 2001961 (2021).
18. Walker JT, McDermott PJ. Confirming the presence of *Legionella pneumophila* in your water system: a review of current *legionella* testing methods. *J. AOAC Int.* 104(4), 1135–1147 (2021).
19. European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC). The European Union One Health 2018 Zoonoses Report. *EFSA J.* 17(12), e05926 (2019).
20. Sartory Dp, Spies K, Lange B *et al*. Evaluation of a most probable number method for the enumeration of *Legionella pneumophila* from potable and related water samples. *Lett. Appl. Microbiol.* 64(4), 271–275 (2017).
21. Díaz-Flores Á, Montero JC, Castro FJ *et al.* Comparing methods of determining *Legionella* spp. in complex water matrices. *BMC Microbiology.* 15(1), 91 (2015).
22. Sun Z, Bai X, Chen X *et al*. A simple, specific, and rapid lateral-flow immunochromatographic test method for detection of *Legionella pneumophila* in water samples. *IPCBEE.* 58 (2013).
23. Pereira A, Silva AR, Melo LF. *Legionella* and biofilms – integrated surveillance to bridge science and real-field demands. *Microorganisms.* 9(6), 1212 (2021).
24. Polymerase chain reaction [PCR] market size, Growth, 2021–2028 (2022). **www.fortunebusinessinsights.com/polymerase-chain-reaction-pcr-market-102528**