
hydrosense
Testing Methods e-book

Introduction

According to the US Centre for Disease Control and Prevention (CDC), the deadliest Legionnaires' disease outbreaks could have been prevented with better water system management[1].

Managing the risk of Legionella in water systems, in order to prevent bacteria from growing and spreading, is essential for protecting public health. It is also far more cost-effective than responding to an outbreak.

Almost 70% of health and safety professionals agree that Legionella testing must be done more frequently, in order to protect businesses and the general public from Legionnaire's disease[2] and there is no doubt that a comprehensive Legionella risk management and control plan - which includes appropriate testing - is the best way to mitigate risk, reduce illnesses and save lives[1].

Comprehensive water management programs involve a careful examination of the inherent risks found in man-made water systems, including the identification and assessment of the possibility of Legionella exposure[3]. In most well-run organizations, the team responsible for managing water safety will understand the risk areas presented by the system and how to manage them.

But because Legionella is so pervasive, and complete eradication of the bug is almost impossible, it is vital that strong control measures are supplemented with regular testing to ensure that the plan is effective and that it remains so.

CDC confirms that the effectiveness of Legionella controls in a water system can be evaluated by carrying out routine sampling for Legionella[3].

Not only does routine Legionella testing ensure that water quality and safety is maintained, it also reduces the potential health risks presented by negligence, the unplanned absence of water management staff, failure of biocide dosing systems and a host of other unforeseeable events that inevitably occur within organizations. Testing also protects responsible persons from the catastrophic impact an outbreak can have, from both an operational and litigation standpoint.

To ensure that water systems on your premises are safe, Legionella testing should be conducted regularly. In some industries, testing is required by law[4,5]. However, choosing the best testing method for your facility can be difficult, especially when regulations in some industries or countries can be unclear or fragmented.

A recent study conducted with health and safety professionals, responsible for the water safety management[2], showed that **84% of respondents would like to be more educated on the available tests for Legionella.**

In response to this outcry, our Legionella experts have prepared this e-book, to help duty holders and responsible persons make faster and better-informed decisions about testing for Legionella.

Legionella Testing

Up until a few years ago, the only method available on the market for the detection of Legionella in water samples was the culture method, which is performed in a laboratory. However, significant limitations have now been identified for this method.

Water management professionals have since started looking for new, innovative testing techniques that allow for a more flexible and individually tailored approach that can promote better public health outcomes.

While the lab culture method continues to be considered the 'Gold Standard' for Legionella detection, new, exciting and more rapid alternatives are making their way onto the market. The most popular being PCR (a DNA-based test technology) and the Rapid Antigen Test.

This e-book will discuss the pros and cons of using each of these techniques.

Popular Methods

Culture method – Culture is the oldest method in the industry for the detection and quantification of Legionella bacteria. The lab culture method can isolate and quantify Legionella found in water samples.

PCR - PCR (Polymerase Chain Reaction) testing is a molecular biology technique in which the DNA of a microorganism is extracted and then amplified. This enables the laboratory to determine the presence and quantity of that organism's DNA in a water sample.

Antigen Test - Antigen testing utilizes lateral flow technology and is optimized to quickly detect the specific cell surface antigens of Legionella on-site, in environmental water and biofilm samples, for all phases of the Legionella life cycle.

Lab Culture Method

The lab culture test is a traditional method, used in laboratories worldwide, and is a part of a standard regulatory framework in most industries. It has a documented International Standards Organization's ISO 11731 standard[6], which can be performed by accredited labs to give a more predictable accuracy. It also detects culturable bacteria, thereby showing the bacterium's potential for growth.

Nevertheless, the method has some critical deficiencies which can significantly increase risk if the method is used in isolation.

Key features of the Lab Culture method:

Good quantification and speciation – The method can provide good quantification of Legionella, even when very low levels of bacteria are present in the sample. A laboratory, armed with specialist lab equipment and Legionella experts, can perform additional testing to provide detailed species identification. In other words, **culture can give you a good indication of the levels of Legionella present in your water system and what strains or species they are.**

Universally familiar biological technique - As the oldest method in the industry, it is very familiar for microbiologists. The method has also been used to study the majority of outbreaks to date and there is a large body of historical information with which to compare current data.

Low Recovery Rates – The ISO standard states that in the inter-laboratory trial undertaken, as detailed in the latest version of the standard, the Recovery Rate was only >64%[6]. That means there is a **possibility for up to 36% inaccuracy in any culture test**, even when following the ISO standard in an ISO accredited laboratory. Non-ISO laboratories are likely to have even lower recovery rates.

Slow 'time to result' - Obtaining a result via the culture method typically takes between 7 to 14 days. Studies have shown that Legionella pneumophila can proliferate very rapidly, potentially doubling in population within a mere 24 hours[7]. Consequently, any results received from a lab culture test could be a positive 'call to action' that is a week too late, or a negative result which may give a false sense of security for the system being tested. **Slow time to result is a very high-risk factor, especially since the risk of acquiring Legionnaires' disease can increase by 64% for every hour spent near the source of an outbreak[8]**.

Damage from transport – A sample typically has to travel to the laboratory for testing, often via a 3rd party courier. En-route the accuracy of the test can be compromised due to excessive heat, radiation or other bacteria in the sample that may dominate the Legionella present[9].

Lab Culture Cannot Detect Viable but Non-Culturable Bacteria - When shocked due to extreme temperature, exposure to biocides, lack of nutrients, or other stress, Legionella may enter a Viable but Non-Culturable (VBNC) state. In this state, the bacteria are temporarily dormant but can reactivate. These bacteria are dangerous and can cause Legionnaires' disease, however, they cannot be cultured. Lab culture tests are therefore incapable of detecting them, resulting in significant risk from low CFU counts or false negative results[10]. It has been suggested that this form of the bacteria may be why cooling towers investigated after outbreaks often appear to not be infected.

Acid and Heat Treatment – The ISO standard specifies acid and heat treatments to kill colonies of bacteria that may dominate the plate and inhibit the growth of any Legionella present. However, this process may also kill some of the Legionella present, leading to a low CFU count or a potentially false negative result[6].

PCR

PCR testing is used to detect the DNA of Legionella bacteria.

Key features of the PCR method:

Sensitivity and Accuracy – PCR can achieve a recovery rate of 90%[11]. However, this high sensitivity has to be calibrated to reflect the fact that Legionella bacteria are naturally occurring and are present in all water systems at some level. The PCR method thus requires careful interpretation to ensure the relative risk of Legionellosis presented by the system is fully understood. It is also **important that each water type being tested is pre-screened for impurities and biocides as they can cause interference with the test and produce false positive results.**

Does not Provide a Call to Action - PCR returns results in a number of genomic units (GU) per litre, but an equivalence to Colony Forming Units (CFU) count (used worldwide to establish call to action) has not been established[11]. Moreover, **PCR enumerates DNA of both live and dead cells**, potentially leading to an overestimation of the actual health risk[12]. Therefore, a positive result might be hard to interpret, and risk management protocols still need to be established around the technique or the results will be unable to support the decision-making process.

Fast Results - The PCR method is a useful tool for establishing risk under emergency or outbreak conditions because it can produce a positive or negative result in hours rather than days[13], as long as an appropriate lab is close and accessible. It is more typical however, for **PCR tests to take between 24 - 48 hours**, particularly when shipping to the lab is required.

Damage from Transport - As with the lab culture method, PCR is not carried out on-site and bacteria **damage can occur during the transportation process**, leading to a less reliable result. On top of that, it can increase the total time required for analysis and acquisition of results.

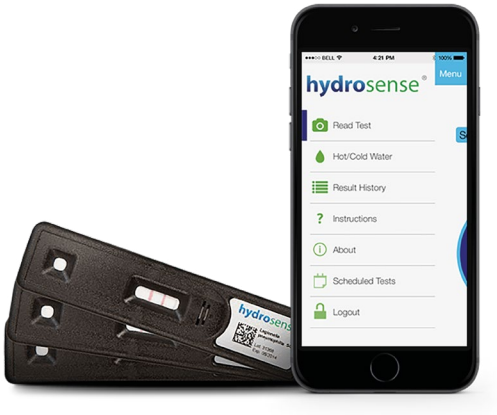
Expensive - Because the test is carried out in the laboratory, where several steps must be completed, such as concentrating viable cells through membrane filtration; sonication and heating of the concentrated cells to lyse the cells and free DNA; and purification of the DNA for the Polymerase Chain Reaction, the test is very labor intensive and complicated and must be conducted by trained and experienced professionals. Consequently, **the prices of the PCR assays are relatively high, when compared to other testing methods**.

Detects VBNC bacteria - The PCR test **can detect Legionella bacteria in a Viable but Non-Culturable state**, in which it is still extremely dangerous to humans, but cannot be detected by the culture method[13].

Antigen Testing

Antigen-based rapid Legionella testing uses well established Lateral Flow Immunochromatographic Assay (LFICA) technology. This type of test is most widely used in clinical settings for the detection of Legionnaires' disease in humans, and according to CDC, is the preferred diagnostic test for cases of Legionnaires' disease [14].

The same methodology has now been optimized and applied to detect Legionella in environmental water samples by Hydrosense.



Key features of the Antigen method:

On-site - The test can be performed on-site, which allows detection of the bacteria in its natural environment. Field analyses also remove the risks associated with transportation of water samples and does not require additional treatments, such as those performed in the lab. **Currently, the antigen test is the easiest and fastest method for Legionella detection that can be carried out on-site.**

Immediate Results - The biggest advantage of the rapid antigen test is time to result. Antigen testing is the **fastest method currently available in the world**, for the detection of Legionella in water samples. Results are provided in 25 minutes, compared to 7-14 days in the case of lab culture, or ~24 hours in the case of PCR.

Ease of use - **The antigen test requires no training or experience and can be carried out by anyone, anywhere.** The ease and simplicity of the test can eliminate the costs associated with sending samples to the lab and is an incredibly convenient way to test for Legionella in remote areas or out at sea. Moreover, the ease of use of the antigen test enables simple, periodic sampling of water systems for the reduction of risk and is a useful addition to any water management program.

Detects VBNC bacteria - The antigen test detects bacteria in all stages of its lifecycle, including dangerous Viable but Non-Culturable Bacteria, which cannot be detected by the culture method.

Provides a clear call to action - The test is specific to Legionella serogroup 1, the strain of Legionella that is responsible for 70 to 92% of laboratory-detected cases of Legionellosis, in the United States and Europe[15]. Therefore, a positive result with **the antigen methods is a clear call to action for the strain of Legionella which has caused almost all known cases of Legionnaires' disease.**

Flexible Limit of detection - The wide range of antigen test kits allows users to choose the test with the Limit of Detection (LOD) that meets their specific requirements. The most sensitive ones provide a LOD as low as 100 CFU/L.

High Sensitivity - The antigen test was independently validated in a paper presented at the Industrial Water Conference in 2008. **The study confirmed that the recovery rate of the rapid antigen test was 80% whereas the culture method only achieved a recovery rate of 55%** during the same study.

What About Accreditation?

While accreditation is widely available for laboratory-based methods all around the world (e.g. UKAS, CDC Elite accreditation) rapid on-site methods cannot be accredited through the same process because they are performed in the field (which is, of course, their biggest advantage).

Moreover, in the EU, there is no directive on Legionella water tests, therefore no rapid Legionella tests can currently receive a CE marking. This situation is expected to change as the market continues to demand faster, more accurate information from testing methods focused on significant Legionella risks.

Which Method is Right for me?

Lab culture tests remain widely specified in regulations. If you must test your water system for regulatory reasons, then a lab culture test is likely to be required.

However, when considering the significant increase in cases of Legionnaires' disease worldwide[16,17] and the various limitations associated with this method, it is becoming clear that doing the bare minimum is no longer enough to protect your business and people's lives.

Many companies have been fined hundreds of thousands or even millions for their negligence, despite following regulatory frameworks.

Many of these organizations were understandably under the impression that ticking boxes and updating their paperwork would be sufficient protection from Legionella bacteria. However, a number of variable factors can put pressure on the effectiveness of an organizations water management plan. While it is the duty holder who is ultimately liable for prosecution (in certain jurisdictions) in the event of an outbreak, the reality is that current regulatory frameworks do not fully equip responsible persons with the tools and solutions to fully mitigate their risks.

Most regulatory frameworks recommend carrying out the lab culture test as part of a compliant risk management program. However, this method supplies duty holders with historical information only and can take 7-14 days to provide results, which then have to be interpreted to be fully understood.

This long process leaves employees and the general public exposed to a contaminated source for a prolonged period. And to make things worse, for reasons mentioned above, the lab test may provide a false negative result, leaving people vulnerable to infection until the next risk assessment is due.

Therefore, the lab culture method should not be used in isolation. Although it is likely to remain a core part of risk management programs for compliance reasons, the industry is now moving away from relying solely on this method. Recent research shows that 85% of industry professionals agree that compliance frameworks should adopt new technologies to better counter Legionella threats[2]. Furthermore, 54% of them stated that they would prefer to use methods which can be performed on-site with more immediate results[2].

New, innovative rapid testing methods can give more timely results and provide critical information on dangerous Viable but Non-Culturable (VBNC) bacteria, minimizing risk significantly. PCR has been gaining a lot of attention in recent years and while its high accuracy and fast results make it an attractive alternative for the lab culture method, its inability to present results in CFU/L makes it suboptimal for fast and effective decision making.

The antigen test is the only on-site test available in the world today. It provides an immediate picture of Legionella contamination risk and facilitates quicker, better- informed decision making about water quality.

This simple, real-time call to action gives users a clear YES/NO answer based on the Limit of Detection of the test kit, putting power back in the hands of the duty holders and empowering them to make fast and responsible decisions about water safety.

The method is innovative and supports a completely new and holistic approach to risk management, that focuses on prevention rather than delayed action. By recording test information, using the Hydrosense App, you can create a complete picture of Legionella contamination risk and a roadmap to prevent future outbreaks, thus reducing public and employee health risk and avoiding lawsuits, huge fines and reputational damage.

Unfortunately, there is no simple answer to the question – "What testing method is right for me?". Every company and every water system has its own complexities and regulatory body to comply with. But with a comprehensive risk assessment and water management plan in place, you will be able to ask yourself the right questions. And the testing methods you choose should be those that best fit to answer them.

Find out more about Hydrosense in our Hydrosense ebook.

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