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Combining On-site Rapid Testing and Connectivity for Better Management of Legionella Risks

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Abstract

Current lab culture methods for testing for the presence of Legionella are of insufficient accuracy to fully manage risks associated with Legionnaires' disease (Helbig et.al, 2002; Joseph, 2002; Yu, 2002; Fraser and Spiteri, 2011). The Hydrosense Legionella rapid field test utilises lateral flow technology to provide presence/absence of Legionella antigen at a specific analytical sensitivity in 25 minutes. The test detects all 10 subgroups of Legionella pneumophila serogroup 1 (LpSG1), the cause of practically every fatal outbreak of Legionnaires' Disease recorded globally.

The computing power of smartphones has been harnessed to read the Hydrosense test to provide a semi-quantitative result with connectivity than enables an accelerated risk management decision. An intensity ratio based on the relative optical density of the control and result measurements is used to create instant test results including semi-quantification. The online Portal records location, time and date; provides scheduling, and alerts and notifications of positive results or out of specification temperatures and biocide levels in real-time. Immediate notification of a positive result to the responsible duty holder reduces the significant risk associated with the typical minimum 1 week wait for lab culture results by supporting decision on remedial action in under 30 minutes, protecting employees, customers and the general public. Real Time on-site management of Legionella risk is now possible thereby reducing public health risks.

Keywords

Legionella, testing, risk management, public health, rapid-testing, connectivity.

INTRODUCTION

Legionnaires' disease is one of the most serious waterborne illnesses and is typically caused by Legionella bacteria in the built environment. The disease is predominantly caused by the bacterium Legionella pneumophila which is almost ubiquitous worldwide in rivers, ponds, lakes and other natural water features. The disease occurs when the bacterium enters the lungs via aerosolised water droplets and is fatal in ~10% of cases (Cdc.gov, 2018).

While the Legionella pneumophila bacterium seems to be low risk in the natural environment, it is capable of multiplying to dangerous levels in man-made water systems, typically those that are poorly managed or poorly monitored. This paper explains some of the inherent risks in standard testing procedures and shows how a new approach, similar to that used commonly in clinical settings, can provide useful additional information to water management professionals in order to better protect the public.

This paper is comprised of two parts; a content analysis conducted on the current literature surrounding Legionella and the most widely used testing procedure for detecting the bacterium, lab culture testing; and a study comparing the recovery rates of traditional lab culture testing methods and a new innovative lateral-flow test from Hydrosense. Both methods will be discussed in detail, with reference to a wealth of current Legionella testing literature. And a case will be built to support the use of lateral flow test technology, which can help businesses and water management professionals create safer environments for the public.

MANAGEMENT OF LEGIONELLA RISK

Wherever possible risks should be removed from water systems, however where a risk cannot be removed, risk management protocols should be followed. While regulatory systems vary from country to country, or even regionally, the common elements are:

- Ensuring that the release of water spray is properly controlled
- Managing water temperatures to avoid the Legionella growth range (20°C-50°C)
- Managing conditions that promote the growth of Legionella including restricting the availability of amoebae and nutrients in biofilm or sediments
- Eradicating or minimising the stagnation of water within the system by fully removing redundant pipework
- Cleaning the system as required
- Controlling the growth of Legionella (and other microorganisms) or limiting their ability to grow
- Testing to monitor the effectiveness of any control measures applied to the system
- Keeping an up to date schematic record of the water system as it is extended, repaired and maintained

These elements can broadly be broken down into maintenance, controls and testing. In this paper we will examine testing and its contribution to risk management.

In a comprehensive study of 27 Legionnaires' disease outbreaks, 23 had descriptions of failures that contributed to the outbreak and over half had more than one type of failure. 65% of outbreaks were due to process failures of some type (CDC, 2016). Given this background, it is clear that reliance on controls to prevent Legionnaires disease will, by themselves be insufficient and that detection of the failure of controls via an appropriate testing regime is necessary to protect individuals from this deadly disease.

CONTENT ANALYSIS: THE LABORATORY CULTURE METHOD FOR THE DETECTION OF LEGIONELLA

While procedures of maintenance and control can consist of differing applied techniques, laboratory culture testing is used as the primary method of detecting and quantifying Legionella in every major regulatory framework worldwide. The method, has some attractive advantages when compared to other methods, namely:

- It has a documented ISO standard (Water quality -- Enumeration of Legionella, 2017) which can be performed by accredited labs to give a more predictable accuracy
- It detects only culturable bacteria and thereby shows the bacterium's potential for growth
- It is a familiar technique which has been used in the majority of outbreaks to date. Because of this, there is a wide range of historical data available, which can be used for comparison purposes.

For many years the culture method has been described as the 'Gold Standard' for Legionella testing. Nevertheless, the method has some critical deficiencies which can significantly increase risk if the method is used in isolation.

Time to result - Obtaining a result via the culture method typically takes between 7 to 10 days. Studies have shown that Legionella pneumophila can proliferate very rapidly, potentially doubling population within a mere 24 hours (Buse and Ashbolt, 2011). 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 merely 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 (Jernigan et al., 1996).

Sample Integrity – 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 (Lee et al., 2011). Even a short 6 hour holding period of the sample prior to processing can affect results (McCoy et al., 2012). Furthermore, there is a risk that insufficient treatment of the water to neutralise biocides, a lack of control of temperatures for the sample whilst in transit, and errors in the Chain of Custody of the sample can all cause significant errors, resulting in either a low colony forming unit count or, worse, a false negative result (Lee et al., 2011). In many cases, with courier-shipped samples, it's difficult to guarantee that best practice has been followed and that the sample hasn't been altered. Processing the sample on-site can alleviate these risks.

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 when environmental factors alter (e.g. re-emergence of amoeba.) They have been shown to be viable even after one year of storage in ultrapure water, and this form of the bacteria can still infect human lung cells (Dietersdorfer et al., 2018). However, they cannot be cultured, resulting in low CFU counts or false negatives from the test (Alleron et al., 2018). It has been proposed that this form of the bacteria may account for the fact that L. pneumophila often cannot be cultured from cooling towers suspected to be a source of infection (Steinert et al., 1997).

Acid and Heat Treatment – The ISO standard specifies acid and heat treatments to kill colonies of bacteria that may subsequently dominate the plate inhibit 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 (Water quality -- Enumeration of Legionella, 2017).

Lab Culture Testing Achieves Low Recovery Rates – The ISO standard clarifies that in the inter-laboratory trial undertaken as detailed in the latest version of the standard the Recovery Rate was >64% (Water quality -- Enumeration of Legionella, 2017).

Considering these limitations of the lab culture method, it becomes clear that a more holistic risk management approach is needed in order to effectively prevent and control risks associated with Legionella bacteria.

DISCUSSION

Following this critical analysis of the lab culture method, it can be assumed that for superior water management, professionals would need a test that allows for immediate action, thereby removing the risks associated with 7 to 10 days of proliferation period. Secondly, a method which promotes instant communication with duty holders or responsible persons, so that action may be taken to immediately control risk, would not only be extremely useful, but preferred. Furthermore, a test which can be carried out on-site would alleviate any risks associated with poor maintenance of a sample in transport, and again would be preferred. Additionally, the risks associated with VBNC bacteria pose a serious health hazard to any water system and any company currently using the lab culture method as their primary and only method of detection. It can be assumed based on available research that VBNC Legionella cannot be detected by lab culture, that a method that can detect this temporarily dormant bacterium would be preferred and would constitute an improvement to water safety. Due to the aggressive nature of heat and acid treatment and this risk of this treatment killing off viable Legionella in a sample, a testing method that requires no treatment is recommended. And lastly, a method that has a higher recovery rate than that suggested by the ISO standard would help provide greater accuracy, peace of mind and would inform better risk management and decision making. In summary, the findings from the content study showed that lab culture is in many ways flawed and that there is not only a need but academic support for a new innovative method that can eliminate the risks discussed above.

INTRODUCTION TO RAPID ON-SITE LEGIONELLA TESTING TECHNOLOGY

One of the most widely-used clinical tests for Legionnaires' disease is based on antigen testing technology. An example of an antigen-based rapid Legionella test for water is shown in Figure 1. This is an example of a Lateral Flow Immunochromatographic Assay (LFICA). This technology is often used where it is important to rapidly identify the presence of specific bacteria.

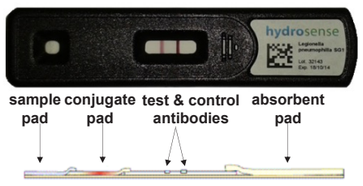

Figure 1. Picture of Hydrosense test device and schematic cross-section

Sample Pad - When a 100µl water sample is introduced to the test device, it is absorbed into the sample pad which has a number of treatments designed to stabilise any variability in the water sample cause by water type/source, biocides or other water treatments, and any other contaminants. This is a critical difference from the similar tests that are used in a clinical setting as clinical samples such as urine, blood or sputum are typically more homogeneous and predictable than water samples.

Conjugate Pad - Once the sample begins to absorb into the conjugate pad it encounters L. pneumophila serogroup 1 (LpSG1) antibodies that have been labelled with gold nanoparticles. At this size the gold particles reflect red light. The antibodies bind to surface antigens on LpSG1 bacteria in the sample, thereby colouring them red and the sample continues to flow by absorption along the lateral flow test.

Test and Control Antibodies - Next the sample flows onto the pad which has two lines visible in the test window of the device. These are a faint blue colour prior to the test being undertaken. The first line encountered is the test line itself. This line also has antibodies for LpSG1 and will thus capture the previously labelled LpSG1 bacteria in the sample. The second line encountered has a control antibody and is a classic 'control' line for a lateral flow test – providing assurance that the test has been run correctly and a sufficient amount of sample was applied to the test. The test result at the Limit of Detection (LOD) can thus be simply read as two red lines signifying a Positive result and One line (the control line) signifying a negative result. No lines or only the test line and not control line signifies an invalid test (typically due to insufficient sample being provided). The LOD for the test is 100,000 CFU/l when 100µl of water is applied directly to the tests and 100 CFU/l when a filtration protocol is followed. The test takes 25 minutes to run from application of the sample to read the result. This is required to be sure the test result is negative, though it is often possible to see a positive result much more quickly.

Absorbent Pad – This pad's function is merely to promote the flow of the sample across the lateral flow test.

STUDY 1: DOES LAB CULTURE HAVE A LOW RECOVERY RATE?

Rapid On-Site Test Vs Culture Method

The following study was carried out to support the following hypothesis, which was constructed following a thorough content analysis of the Legionella testing literature and critical discussion of the lab culture testing method:

H1: Based on the content analysis and the limitations previously mentioned in the content analysis, it was assumed that lab culture testing would have a lower recovery rate than the lateral- flow test from Hydrosense.

Method: 18 geographically diverse water samples with six of each from cooling water, domestic (hot and cold) water, and whirlpool/spa water we collected. Each was confirmed as negative with the Rapid On-Site test before spiking with one of fifteen different LpSG1 isolates – ten LpSG1 subtypes and five wild isolates confirmed as LpSG1 by two independent laboratories (Cooper et al., 2008).

Results: Table 1. Comparison of culture method and the Test results from spiked water samples.

Note - Culture results were considered Negative when <100 CFU/mL (Cooper et al., 2008).

	Rapid On-Site Test	Lab Culture Results
Positive	146	101
Negative	36	81
Total	182	182
Recovery Rate	80.2%	55.5%

The study found that the recovery rate for samples with greater than the LOD for the rapid test (100CFU/l) was considerably higher for the rapid on-site test.

It was not possible as part of this study to determine what percentage of the positives found by the rapid on-site test were due to the detection of VBNC bacteria. However, any of the previously described limitations of the lab culture method could have been responsible for the discrepancies.

IMMEDIATE TEST RESULT DATA CAPTURE AND DISSEMINATION: A CASE FOR CONNECTIVITY

One of the key disadvantages of the traditional methods for detection of L. pneumophila is the time it takes to receive the result. Furthermore, records of the test results may be paper based or electronic with manual processes often required to keep them in a single, easily referenceable area or to keep the results grouped by test location. In cases where multiple labs are used, and thus multiple information sources are involved, this may be even more complex. The lateral flow test technology, featured in study 1 above, provides a comprehensive alternative, with a simple and easy to use mobile device App. The |Hydrosense Pro App offers full real time reading of tests and connectivity between locations and individuals.

Methodology: After login the mobile App allows locations to be specified and tests to be read. See Figure 2 for the test reading screen with guides to ensure correct positioning. The reading algorithm uses patent-protected technology to adjust for varying camera angles and lighting conditions but still provide a reliable result.

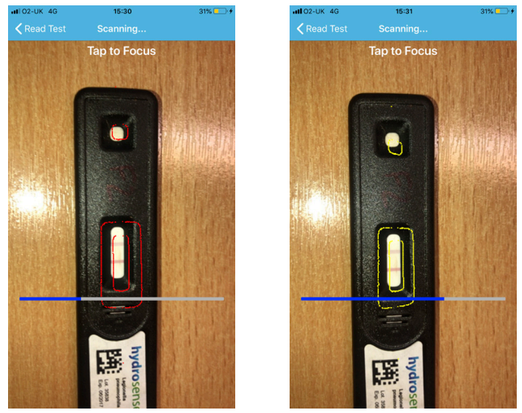

Figure 2. Reading the test device with the mobile device App – Showing the built-in guide that ensures correct reading of the test

Once the test is read the user is provided with the ability to add comments to the record (e.g. to note that cleaning should occur at the location despite a negative result) and the ability to share the result (for instance with a duty holder that can make a decision to close the facility to the public, or to take action to immediately clean or biocide the system.) See Figure 3.

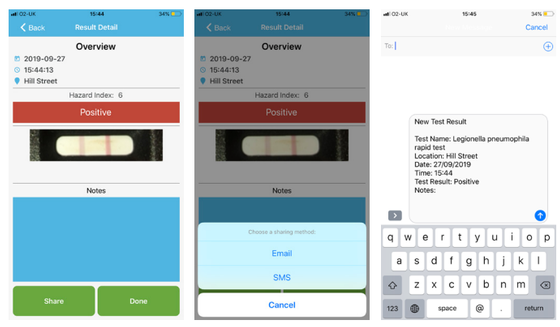

Figure 3. Screenshots of Hydrosense mobile device App showing result screen, sharing functionality and SMS option of share facility respectively

Aims: To complement the already significant time-to-result saving (minimum 7 days for lab culture tests versus 25 minutes for the rapid on-site test) and further accelerate the decision-making process when Legionella is detected in a water system. The Hydrosense On-Site Rapid Test and Hydrosense Pro App and Portal, when combined, provide the fastest way to progress from taking a water sample to providing an actionable result to the duty holder for the facility being risk managed for Legionella.

Crucially, data collected by the App and Portal can help to mitigate future risks and identify trends, as all results are held in a central database providing a better dataset for epidemiological and risk analysis, expanding understanding of risks associated with Legionella in the long-term. Furthermore, information on location and the type of system being tested (e.g. spa, shower, cooling tower, etc.) provides key data for subsequent research, allowing identification of areas of increased risk analysed by location, time and other factors in a way not typically available to users of lab culture tests.

The real-time connectivity and the inherent accuracy of the reader helps even inexperienced users to make better-informed and more responsible decisions while making it simple for them to gain assistance should it be required.

Lastly, the functionality available on the portal to track water temperatures and biocide levels, combined with the Hydrosense test provides a full image of a water system, which helps to improve public health safety and reduce liability.

With Legionnaires' disease prevalence increasing (Beauté and Robesyn, 2016; Messonnier and Breysse, 2017) it is clear that historical information provided by lab culture results are not sufficient to protect organisation's reputations and people's lives. Using the latest Legionella test technologies and connected applications to assist risk management frameworks currently reliant on the lab culture method, can allow for a more holistic and responsible approach, while building a dataset that can help researchers to propose new control methods and risk management techniques.

CONCLUSION

Legionnaires' disease is a serious and growing (Beauté and Robesyn, 2016; Messonnier and Breysse, 2017) health problem. Current risk management methodologies and practically every global regulatory framework use a useful but imperfect technique to detect failures in risk management controls and other causes of Legionella infection. In order to minimise risk, water management professionals should consider using other methods for Legionella detection that allow them to gain additional information on the presence of the bacteria in their water systems.

A rapid testing technology such as antigen testing can provide critical information on dangerous Viable but Non-Culturable (VBNC) bacteria, and give more timely results, minimising risks significantly. When this is used in concert with communications technology to rapidly disseminate call-to-action and other information relating to the water system, and its Legionella risk, this offers a significantly improved protection profile for water systems' employees, customers and the general public.

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