



**Validation of the limit of detection of the Hydrosense® lateral flow test
with *Legionella pneumophila* Lenticules**

By Paul Moffat

ABSTRACT

Testing for *Legionella pneumophila* in water samples involves shipping samples to an accredited laboratory for culture of the organism. The culture method requires skilled technicians and a 7-14 day period to complete the testing. Other detection technologies are typically cumbersome, expensive and difficult to perform in the field. Therefore, a need for a cost effective, rapid on-site test that can be performed by unskilled operators. The Hydrosense® test is a lateral flow test (LFT) that provides detection of *Legionella pneumophila* serogroup 1 in 25 minutes. Testing with PHE Lenticules has shown that a limit of detection (LOD) of 100 colony forming units per liter (CFU/L) can be achieved with the Hydrosense® test when the kit provided filtration system is used.

INTRODUCTION

The Hydrosense® LFT kit allows for the rapid detection of *L. pneumophila* in environmental water samples. To achieve sensitivity levels that are practicable in terms of *Legionella* regulations such as HSG274 testing methods should be able to detect >100 CFU/L. The Hydrosense® test has a claimed LOD of 100 CFU/L when filtration is used. The testing set out to examine this claim using *Legionella pneumophila* Lenticules. Testing was performed by experienced scientists working at Express Micro Science (EMS).

The testing used *Legionella pneumophila* Lenticules as they provide an accurate and quantifiable quantity of bacteria in an easy to use format. Lenticule discs contain viable microorganisms in a certified and narrow defined quantity (ISO/IEC 17025), produced under reproducible conditions (ISO Guide 34). The bacteria are prepared directly from strains selected from Public Health England's (PHE) National Collection of Type Cultures (NCTC). The discs consist of bacteria in a solid water-soluble matrix. Microorganisms in this form are stable for at least one year and are in a viable stage (no lag phase or recovery time). Each batch is provided with a comprehensive certificate of analysis that specifies the mean number of CFU, an expanded uncertainty about the mean value, details about the method used to determine the product data and the number of passages (subcultures) from the original strain. They can be used in QC to assure the quality of test results (water, food, beverage, environmental etc.). Lenticule discs are sold by Sigma-Aldrich under license from PHE.

MATERIALS AND METHODS

Bacteria preparation:

Legionella pneumophila serogroup-1 Lenticules (Benidorm, Strain No.: NCTC 12821, Batch no. BCBT3820) was purchased from Sigma-Aldrich and stored at -20°C. Manufacturer's methods were followed in reconstituting the bacteria to ensure maximum recovery of the bacteria. One vial was removed from storage and left at room temperature for 15 minutes. After 15 minutes the Lenticule was tipped into 2mL of 1/40 Ringers solution (Manufacturer: Oxoid, BR0052). The solution was left for 10 minutes and then inverted 30 times. The solution was left for 5 minutes before further use.

The geometric mean value (GMV) of the Lenticule CFU was taken from the certificate of analysis provided by the manufacturer. GMV for the Lenticule batch was stated as 3.7×10^3 CFU. The *L. pneumophila* concentration of the 2mL Ringers solution was then calculated as 1.85×10^3 CFU/ml. Finally, a further 1 in 10 dilution was made in autoclaved tap water so the *L. pneumophila* solution was at a suitable concentration for preparation of the test solutions

(185 CFU/ml). To verify the concentration of this solution 0.5ml was plated in duplicate onto GVPC agar (Manufacturer: E & O Laboratories Ltd., Part No PP0870) and incubated at 37°C for 3 days prior to determining the CFU.

Test solution preparation:

Spike volumes of the *L. pneumophila* solution were calculated so that a range of concentrations from 0-500 CFU/L were achieved in a 300ml solution of autoclaved tap water. Spike volumes were calculated using the following formula:

$$S = \frac{C}{185000} * 300$$

Where: C is the required test solution concentration in CFU/L
S is the spike volume

From this equation the following spike concentrations were tested:

Test solution volume (mL)	Test solution concentration (CFU/L)	<i>L. pneumophila</i> solution spike volume (mL)	Number of replicates
300	0 (Negative control)	0.000	1
300	50	0.081	3
300	100	0.162	3
300	150	0.243	3
300	200	0.324	3
300	250	0.405	3
300	500 (Positive control)	0.810	1

Table 1: Table displaying the full range of test solution concentrations made and tested including the spike volume and the number of replicates performed per concentration.

Procedure:

Each test solution (250mL) was pumped through the Hydrosense® kit hollow fibre filter using a peristaltic pump. The liquid volume that was filtered was measured via collection into a measuring cylinder. The bacteria were recovered using the Hydrosense® kit pre-filled syringe with 0.25ml of recovery buffer (Manufacturer: Albagaia Ltd.) and performed as per the Hydrosense® kit instructions for use. As per the Hydrosense® protocol 0.1mL of the recovery buffer containing the concentrated *Legionella* bacteria was then run on the Hydrosense® LFT (Manufacturer: Albagaia Ltd., Batch No.: 38794). After 25 minutes the LFT Test line signal was visually assessed by the operator for the presence of a Test line and also scored using a visual score card (Figure 1) (Manufacturer: BBI solutions, Part No. 0198). Figure 1 details the lines present on the score card and how the intensity of the colour corresponds with each score. Any line intensity judged to be between two of the reference lines was given the score in-between them (see Figure 2).

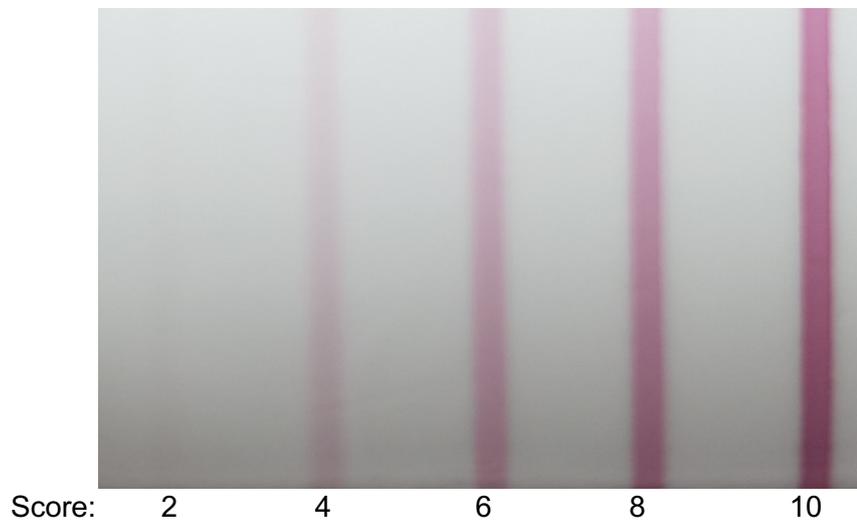


Figure 1: LFT score card displaying the assigned scores depending on the strength of the test line reaction which correlates positively with the concentration of *L. pneumophila* sg1 in the sample.

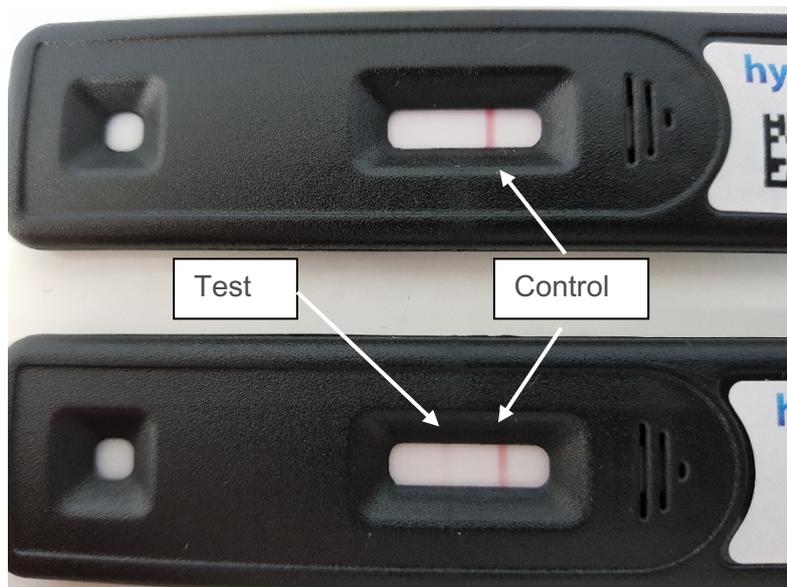


Figure 2: Hydrosense® LFT strips displaying a negative result (top) and a positive result (bottom). The left-hand line is the Test line which contains antibodies sensitive to the particular analyte – in this case antigens present on *L. pneumophila*. The right-hand line is the Control line designed to show that the reaction conditions and technology is working on the strip. The positive result shows a score of ~3 as an example.

A single negative control was run at 0 CFU/L (spike volume: 0ml) and a single positive control was run at 500 CFU/L (spike volume: 0.81ml). All remaining test concentrations were tested in triplicate at 50 CFU/L intervals from 50 to 250 CFU/L.

RESULTS

Test solution concentration (CFU/L)	Test Line Signal					
	Rep 1		Rep 2		Rep 3	
	Score	Visually Detected	Score	Visually Detected	Score	Visually Detected
0	0	No	n/a			
50	1	Yes	0	No	0	No
100	1	Yes	1	Yes	1	Yes
150	2	Yes	1	Yes	2	Yes
200	2	Yes	2	Yes	2	Yes
250	2	Yes	2	Yes	2	Yes
500	3	Yes	n/a			

Table 2: Summary of the results showing the scores from each run at each test solution concentration

From Table 2, both experiment controls passed. For the negative control of 0 CFU/L, the filtered sample gave a score of 0. For the positive control of 500 CFU/L, the filtered sample gave a score of 3.

The replicates of the 50 CFU/L test concentration did not give consistent positive results. A score of 1 was obtained for the first replicate, but a score of 0 recorded for the subsequent two replicates.

All of the replicates for 100 CFU/L and above test concentrations gave positive scores, with the score gradually increasing as the test concentration increased. At 100 CFU/L all scores were recorded as 1, whilst at 200 and 250 CFU/L all scores were recorded as 2.

Lenticule plate culture counts:

Plate 1	Plate 2	Mean	Dilution Factor	Concentration (CFU)
280	293	286.5	1 in 40	1.15×10^4

Table 3: *L. pneumophila* concentration of the Lenticule calculated by plating out onto GVPC and incubating for 3 days at 37°

From Table 3, the concentration of the Lenticule was within the geometric mean of the lenticule batch of ± 3 standard deviations ($1.1 \times 10^3 - 1.3 \times 10^4$ CFU).

CONCLUSIONS

The Hydrosense test kit can detect 100CFU/L in samples spiked with *L. pneumophila* Lenticule in sterile tap water when the kit provided filtration and recovery system is used. Achieving this LOD demonstrates that the Hydrosense test performs to a similar level to other methods such as lab culture but with the added benefit of providing results within 30 mins of sampling and being able to be used by unskilled operators. This is in stark contrast to the 10 days it can take to achieve results via the currently used culture method.