EXPRESS MICRO SCIENCE

Validation of the limit of detection of the HydrosensePRO® lateral flow test filtration method with *Legionella pneumophila sg1 and Legionella pneumophila sg2-15* Lenticules

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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-to-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 HydrosensePRO® test is a lateral flow test (LFT) that provides detection of *Legionella pneumophila* serogroup 1 and *Legionella pneumophila* serogroup 2-15 in 25 minutes. Previous testing with Lenticules has shown that a limit of detection (LOD) of 100 colony forming units per liter (CFU/L) can be achieved with the HydrosenseONE® test when the kit provided filtration system is used. The purpose of this test was to assure that the HydrosensePRO® maintained the same LOD value (100 CFU/L) for both *Legionella pneumophila* serogroup 1 and *Legionella pneumophila* serogroups 2-15. Testing was conducted at Express Micro Science (EMS), a BS EN ISO 17025 accredited laboratory.

INTRODUCTION

The HydrosensePRO® LFT kit allows for the rapid detection of a wide range of *L. pneumophila* serogroups 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 HydrosensePRO® 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 EMS.

The testing used *Legionella pneumophila* Lenticules as they provide an accurate and quantifiable amount 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.).

MATERIALS AND METHODS

Bacteria preparation:

Legionella pneumophila serogroup-1 Lenticules (WDCM 00205 VT002057) were purchased from Vitroids and stored at -20°C. Manufacturer's methods were followed in reconstituting the bacteria to ensure maximum recovery of the bacteria. Three vials were removed from storage and left at room temperature for 15 minutes. After 15 minutes the Lenticules were tipped into 6mL of 1/40 Ringers solution (Batch: 1156, Exp: 09/03/2023). 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 *Legionella pneumophila SG1* Lenticule CFU was taken from the certificate of analysis provided by the manufacturer. GMV for the Lenticule batch was stated as 6.2×10^4 CFU. The *L. pneumophila* concentration of the Ringers solution

was thus calculated as 3.1 x 10^4 CFU/ml. Finally, a further 1 in 10 dilution was made in Ringers solution so the *L. pneumophila* solution was at a suitable concentration for preparation of the test solutions (3.1 x 10^3 CFU/ml). To verify the concentration of this solution, 0.1ml was plated in duplicate onto BCYE agar (Batch: 04164155, Exp: 19/04/2023) and incubated at 37 ± 1°C for 4 days prior to determining the total CFU.

Legionella pneumophila serogroup 2-15 Lenticule mix was manufactured by Glasgow Caledonian University (*method of preparation as per UK HSA (formerly Public Health England) supplied reference material)* and stored at -20°C. Manufacturer's methods were followed in reconstituting the bacteria to ensure maximum recovery of the bacteria. Three vials were removed from storage and left at room temperature for 15 minutes. After 15 minutes the Lenticules were tipped into 6mL of 1/40 Ringers solution (Batch: 1156, Exp: 09/03/2023). 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 2.5 x 10^4 CFU. The *L. pneumophila* SG2-15 concentration of the Ringers solution was thus calculated as 1.25 x 10^4 CFU/ml. Samples were also plated by pipetting 0.1mL in duplicate onto BCYE agar (Batch: 04164155, Exp: 19/04/2023) and incubated at 37 ± 1°C for 4 days prior to determining the total CFU.

Test solution preparation:

Spike volumes of the *L. pneumophila* solution were calculated to achieve concentrations of 0 and 100 CFU/L in a 1L solution of autoclaved tap water. Spike volumes were calculated using the following formula:

$$S = \frac{C}{L} * 1000$$

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

From this equation the following spike concentrations were tested:

Test solution volume (mL)	Test solution concentration (CFU/L)	*L. pneumophila* solution spike volume (mL)	Number of replicates
1000	0 (Negative control)	0	2
1000	100 (*Legionella pneumophila* SG1)	0.032	3
1000	100 (*Legionella pneumophila* SG2-15)	0.008	3

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

LFT Filtration 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: Hydrosense 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 HydrosensePRO® LFT. After 25 minutes the LFT Test line signal was visually assessed by the operator for the presence of a Test line and scored using a visual score card.

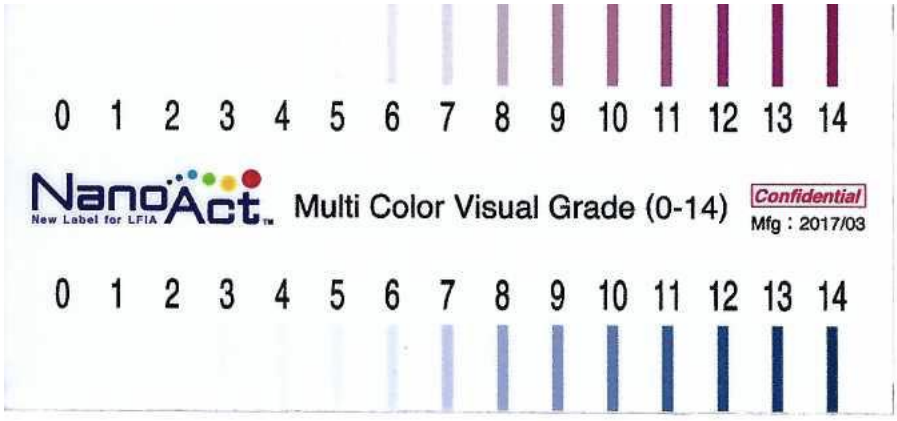

Image 1: Scan of NanoAct score card used to score the test strips

RESULTS

LFT Filtration Results:

Legionella pneumophila sg 1 and 2-15 mix estimated concentration (CFU/L)	Replicate 1	Replicate 2	Replicate 3
	Visual	Visual	Visual
0 (Negative control)	0	0	--
100 (*Legionella pneumophila* SG1)	1	1	1
100 (*Legionella pneumophila* SG2-15)	1	1	1

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

From Table 2, it can be seen that the test reached the LOD of 100 CFU/L giving scores of 1 visually for both *Legionella pneumophila* SG1 and SG2-15 mix. For the negative control of 0 CFU/L, the tests all gave a score of 0 (i.e., Negative).

Lenticule plate culture counts:

Lenticule organism	Agar Type	Plate 1	Plate 2	Mean	Concentration (CFU)
LP SG1	BCYE	28	20	24	2.4×10^3
LP SG2-15	BCYE	228	174	201	2×10^3

Table 3: L. pneumophila concentration of the Lenticules calculated by plating out onto BCYE and incubating for 4 days at 37°C. The concentration of the lenticules were within the geometric mean of the lenticule batches as stated on the certificates of analysis.

CONCLUSIONS

The HydrosensePRO® test kit has been demonstrated to detect its claimed LOD of 100 CFU/L in spiked samples for both *L. pneumophila* sg1 and *L. pneumophila* sg2-15 using lenticules in sterile tap water when the kit provided filtration and recovery system is used.