Comparison of the analytical sensitivity of two rapid point-of-care (POC) urinary antigen diagnostic tests for Legionella Serogroups 3, 4, and 6

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Introduction

Current diagnosis of Legionnaire's disease (LD) relies predominately on detection of antigen in a urine specimen during the acute phase of the illness. Many of the urinary antigen tests are intended to identify only the dominant Legionella pneumophila serogroup: serogroup 1 (Lp1). However, additional serogroups have been reported to cause LD in certain patient groups. Indeed, certain reports of LD among immunocompromised patients in hospitals have identified serogroup 6 as constituting nearly half of the disease burden in some circumstances where case fatality rates were as high as 48%. This has produced a diagnostic "gap" for LD caused by non-Lp1 serogroups.

Although a number of the commercially available antigen tests do exhibit limited cross-reactivity for various non-Lp1 serogroups, their reactivity is highly variable, and the analytical sensitivity of the tests for non-Lp1 L. pneumophila is generally much lower. This lower analytical sensitivity of traditional visual lateral flow tests has made the use of these urinary antigen tests unreliable in non-Lp1 LD diagnosis.

The purpose of this study was to compare the fluorescence-based Sofia Legionella FIA with the Alere BinaxNOW for analytical detection of Serogroups 3, 4 and 6.

Materials and Methods

Testing of each serogroup was performed using freshly prepared bacterial dilutions, which were tested at room temperature immediately after preparation.

One at a time, an aliquot of the Legionella pneumophila stock was rapidly thawed in a 35-37°C water bath and serially diluted in pooled negative urine. A sufficient volume of each dilution was prepared to support the entire study.

A total of six (6) 10-fold dilutions were made. Testing began from the first 10-fold dilution and continued in triplicate until a negative Sofia Legionella FIA or BinaxNOW Legionella Urinary Antigen result (1/3) was determined. Two-fold dilutions were then prepared from the last positive dilution and tested in triplicate until a negative result (1/3) was determined. Once the dilution resulting in a negative measurement was found, testing continued at the last positive 2-fold dilution.

Testing proceeded at this level until either 2 negative results were obtained or a total of 20 replicates were completed and the C95 was reached (19/20 positive results). (Assuming β=5%, the C95 is the lowest concentration of the analyte that yields a positive signal in the assay 95% of the time-also referred to as Limit of Detection.) The same procedure was followed for each Legionella serogroup tested.

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Results

L. pneumophila SG-3, ATCC 30657 was detected by the Sofia down to a concentration of 9.00 x 10^4 CFU/mL. The Alere failed to detect the strain, visually or with the Reader, at the starting concentration of 3.60 x 10^7 CFU/mL.

L. pneumophila SG-4, ATCC 13398T was detected by the Sofia down to a concentration of 7.60 x 107 CFU/mL. The Alere failed to detect the strain, visually or with the Reader, at the starting concentration of 1.52 x 109 CFU/mL.

L. pneumophila SG-6, ATCC 13400 was detected by the Sofia down to a concentration of 7.40 x 10^5 CFU/mL. The Alere failed to detect the strain, visually or with the Reader, at the starting concentration of 7.40 x 10^7 CFU/mL.

Analytical Performance

Table 1: Reported Limits of Detection for Tested Legionella pneumophila Serogroups for the Sofia Legionella Assay

L. pneumophila		Minimum Detectable Level (CFU/mL)
SG-3 ATCC 30657	Serogroup 3	9.00 x 10^4
SG-4 ATCC 13398T	Serogroup 4	7.60 x 10^7
SG-6 ATCC 13400	Serogroup 6	7.40 x 10^5

Table 2: Testing Summary – L. pneumophila SG-3, ATCC 30657

Concentration (CFU/mL)	Average S/CO	Replicate CV%	Positivity Rate	Alere BinaxNOW Visual Result	Alere Reader BinaxNOW Result
3.60 x 10^7	3.8	NA	1/1	NA	NA
3.60 x 10^6	8.8	1.80%	2/2	0/3	0/3
3.60 x 10^5	2.8	0.80%	2/2	0/3	0/3
1.80 x 10^5	1.9	5.30%	2/2	0/3	0/3
9.00 x 10^4	1.3	12.60%	20/20	NA	NA
4.50 x 10^4	0.8	7.60%	0/5	NA	NA
3.60 x 10^4	0.8	2.80%	0/2	NA	NA
3.60 x 10^3	0.4	27.00%	0/2	NA	NA

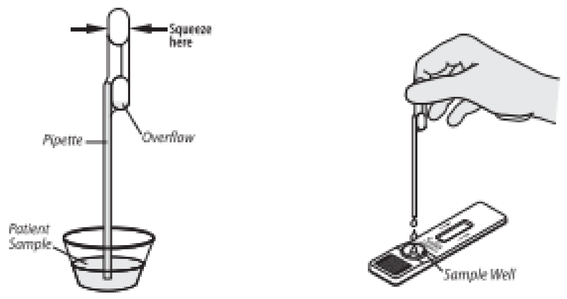
Table 3: Testing Summary – L. pneumophila SG-4, ATCC 13398T

Concentration (CFU/mL)	Average S/CO	Replicate CV%	Positivity Rate	Alere BinaxNOW Visual Result	Alere Reader BinaxNOW Result
1.52 x 10^9	0.6	NA	0/1	NA	NA
1.52 x 10^8	2.2	2.30%	2/2	0/3	0/3
7.60 x 10^7	1.4	8.20%	20/20	0/3	0/3
3.80 x 10^7	0.7	4.00%	0/2	0/3	0/3
1.90 x 10^7	0.8	22.80%	0/2	NA	NA
1.52 x 10^7	0.7	33.20%	0/2	NA	NA
1.52 x 10^6	0.4	19.30%	0/2	NA	NA
3.60 x 10^3	0.4	27.00%	0/2	NA	NA

Table 4: Testing Summary – L. pneumophila SG-6, ATCC 13400

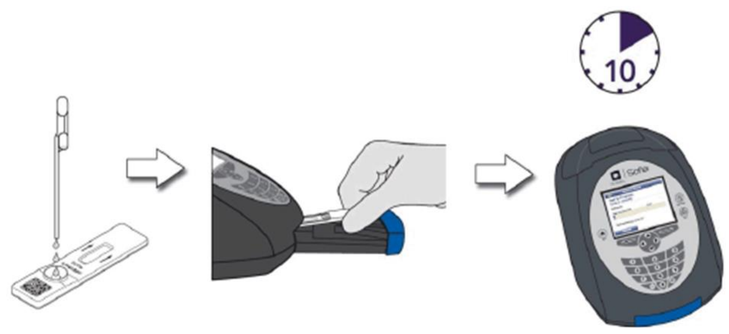
Concentration (CFU/mL)	Average S/CO	Replicate CV%	Positivity Rate	Alere BinaxNOW Visual Result	Alere Reader BinaxNOW Result
7.40 x 10^7	8.3	NA	1/1	NA	NA
7.40 x 10^6	6.8	NA	1/1	0/3	0/3
7.40 x 10^5	1.7	12.20%	20/20	0/3	0/3
3.70 x 10^5	0.8	19.70%	0/2	0/3	0/3
1.85 x 10^5	0.8	28.70%	0/2	NA	NA
9.25 x 10^4	0.4	18.90%	0/2	NA	NA
7.40 x 10^4	0.5	2.70%	0/2	NA	NA
3.60 x 10^3	0.4	27.00%	0/2	NA	NA

Quidel Sofia S. pneumoniae Assay Workflow & Sofia Analyzer



WALK AWAY MODE

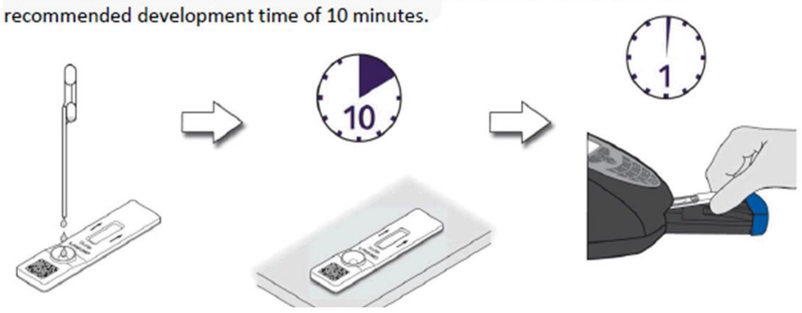
In WALK AWAY Mode, the user **immediately** inserts the Cassette into Sofia. The user then returns after 10 minutes to get the test result. In this mode, Sofia will automatically time the test development before scanning and displaying the test result.



READ NOW MODE

Allow the test to develop for the FULL 10 minutes BEFORE placing it into Sofia.

The user must first place the Cassette onto the counter or bench top for 10 minutes (outside of Sofia) and manually time this development step. Then, the user inserts the Cassette into Sofia. In READ NOW Mode, Sofia will scan and display the test result within 1 minute. **Note:** Results will remain stable for an additional 10 minutes after the recommended development time of 10 minutes.



Sofia Specifications:

- 1 Step protocol
- 10 minute procedure
- Automatic Walkaway Mode (instrument-Timed)
- Read Now Mode (Operator -Timed)
- LIS connectivity
- Virena Wireless Connectivity (Optional)

Conclusions

Results from this study indicated that the Sofia Legionella FIA is able to detect Serogroups 3 and Serogroup 6 at levels substantially equivalent to Legionella Serogroup 1 (8.43 x 10^4 CFU/mL). Detection of Serogroup 4 was substantially higher than the Legionella Serogroup 1.

The Alere BinaxNOW (visually or with the Reader) failed to detect any of the serogroups used in this study.

The results indicate that testing with Sofia Legionella assay could aid in the diagnosis of Serogroups 3 and 6 in addition to Serogroup 1.