EVALUATION OF AN IMMUNOCHROMATOGRAPHIC URINE ANTIGEN ASSAY FOR DETECTION OF *Legionella pneumophila* SEROGROUP 1

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Introduction – In humans *Legionella pneumophila* can cause Legionnaire's disease (LD), a severe form of pneumonia indistinguishable from other types of pneumonia or Pontiac fever, a milder influenza like syndrome. Infection is due to inhalation of aerosols or aspiration of water containing the bacterium from a variety of water systems and most susceptible individuals include the immunocompromised and elderly population. *L. pneumophila* comprises 15 serogroups however it is L.p Serogroup (Sg) 1 that is the most commonly associated with LD. The clinical outcome of patients with Legionnaires' disease is vitally dependent on the speed of diagnosis so effective rapid antibiotic therapy can be initiated to reduce mortality rates.

Objective – To evaluate a new commercially available urine antigen assay (Pro-Lab Diagnostics Proflow™ LUA) for the detection of *L. pneumophila* Sg 1 antigen in urine samples.

Materials & Methods – 48 clinical urine samples confirmed as *L. pneumophila* Sg 1 positive by either/or combination of serology, PCR, urinary antigen and culture and 200 hundred clinical urine samples confirmed as *L. pneumophila* Sg 1 negative by urinary enzyme immunoassay were included in the trial.

Urine antigen detection – *Legionella* urinary antigen assay (Pro-Lab Diagnostics, Proflow™ LUA) was used following manufacturer's instructions for the detection of *L. pneumophila* Sg 1 in human urine.

IFAT – Instructions were followed as described previously by Wilkinson *et al* 1981

16s RNA PCR ELISA for the detection of Legionella-specific DNA in respiratory secretions – As previously described by Lindsay *et al* 2006

Culture – Method was followed as previously described by Fallon, 1981

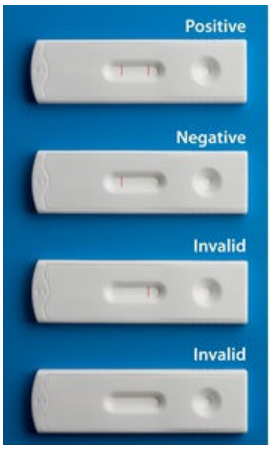
Results – The Proflow™ LUA assay uses *L. pneumophila* Sg 1 capture antibodies and conjugate antibodies. The sample is dispensed onto a sample pad which diffuses up a nitrocellulose membrane where the dried capture antibodies are located at a specific test line for *L. pneumophila Sg 1*. If the sample is positive the antigen-conjugate complex is bound to the capture antibody yielding a pink-red line. An internal control line is located in every test to determine whether the test has been implemented properly and all reagents are working appropriately. If this control is absent the test is deemed invalid

To estimate the specificity and sensitivity of Proflow™ LUA for *L. pneumophila* Sg 1, urine samples from 48 patients positive for infection caused by *L. pneumophila* Sg 1 and 200 urine samples from patients with suspected LD and *Legionella* infection were tested against the currently established Bartels EIA, Binax EIA, and BinaxNow ICT assay.

Sensitivity was estimated at 100% and Specificity was evaluated at 99.5% for the Proflow™ LUA assay (see table 1). All 48 urine samples positive for *L. pneumophila* Sg 1infection were positive with the Pro-Lab Proflow™ LUA assay. One sample was negative by the other three urine antigen assays but positive in the Proflow™ therefore was classed as a false positive.

Results Table 1.

	Positive	Negative	Sens. (%)	Spec. (%)
Proflow™ LUA	**48(1)**	**199**	**100**	**99.5**
BinaxNow ICT	48	200	100	100
Bartels EIA	48	200	100	100
Binax EIA	48	200	100	100



Discussion – The rapid detection and minimal expertise of urinary antigen assays makes them the most extensively used tool for the diagnosis of LD from *L. pneumophila* Sg 1. The Proflow™ LUA test showed 100% sensitivity and 99.5% specificity to *L. pneumophila* Sg 1.

Although the Proflow™ LUA assay specifies detection of *L. pneumophila* Sg 1 specific, meaning LD caused with *Legionella spp.* other than *L. pneumophila* Sg 1 would not be detected, there is further work being conducted to investigate possible non-Sg1 *Legionella* positive reactions. The false positive may have been due toi this, or a foreign substance or contamination not associated with *Legionella spp.*

Diffuse bands may pose some difficulty in reading of results, however the manufacturer specifies any pink/red band in the test line region should be interpreted as a positive result. Urinary antigen testing should be implemented with other methods or further urinary antigen testing with EIA.

From this study Pro-Lab Diagnostics Proflow™ LUA is suitable for the detection of *L. pneumophila* Sg 1 showing good performance characteristics in both sensitivity and specificity. Results were rapid, with strong reactions being reportable in ≤5 minutes. This study has shown the new commercially available ICT assay from Pro-Lab Diagnostics is an efficient, rapid, and leading example in diagnosing Legionnaire's disease.

References – Wlkinson, W. H., Cruce, D. D., & Broome, V. C. (1981) Validation of *Legionella pneumophila* Indirect Immunofluorescence Assay with Epidemic Sera. Journal of clinical Microbiology. Vol. 13, number 1, January 1981 pp139-146
- Fallon, R. J. (1981) Laboratory diagnosis of Legionnaires' disease. Clinical Pathology, Vol. 99 pp1-5
- Lindsay D. S. J., Abraham, W. H., Brown, A. W. & Edwards, G. F. S. (2006). Detection of *Legionella spp* and *Legionella pneumophila*-specific DNA in respiratory secretions by PCR ELISA and comparison with conventional methods. In *Legionella*: state of the art 30 years after its recognition, ASM press, edited by N. P Cianciotto *et al.* pp 55-57.