

THE EVALUATION OF A COMMERCIAL EIA FOR THE DETECTION OF LEGIONELLA PNEUMOPHILA ANTIBODIES

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Introduction

Legionnaires' disease is a potentially fatal condition caused by the bacteria *Legionella*. Legionnaires' disease was first recognized in the USA in 1976. Currently there are at least 46 species and 70 serogroups of *Legionella*. *Legionella pneumophila* serogroup 1 (Lp1) is implicated in the majority of *Legionella* outbreaks worldwide. Lp1 was the causative agent in the largest *legionella* outbreak in Australia. The outbreak in 2000 at the Melbourne Aquarium resulted in 125 confirmed cases including 4 deaths.¹ *Legionella longbeachae* follows Lp1 as the next most common cause of Legionnaires' disease. The incidence of disease due to *Legionella longbeachae* is higher in Australia and New Zealand compared to other parts of the world. For the year 2006 the Department of Human Services in Victoria had 46 notifications for *Legionella pneumophila* and 13 notifications for *Legionella longbeachae*.² *Legionella pneumophila* is usually found in water storage systems where water is stored at 25-45°C. *Legionella longbeachae* is usually found in potting mixes and other moist soils such as compost heaps.

Transmission of *Legionella* is via inhalation of aerosols of water and dust. The incubation period is usually 1-2 weeks. Testing by Urinary antigen is the method of choice along with PCR and culture of respiratory tract specimens. Serology is usually performed by immunofluorescence (IFA) or by enzyme immunoassay (EIA). Seroconversion by immunofluorescence can take up to 6-8 weeks. The sensitivity of EIA kits is claimed to be greater than that of immunofluorescence.

Aim

The aim of the study was to evaluate a commercial EIA - Vircell *Legionella pneumophila* s1-s6 ELISA IgG+IgM for the detection of antibodies to *Legionella pneumophila* and to compare the results to our in-house indirect IFA.

Materials and Methods

72 serum samples were tested, this included 21 paired bleeds showing a seroconversion or a significant rise in titre (≥four fold rise) in our in-house IFA assay. Six of these pairs also had a positive legionella urinary antigen test on or around the time of the collection of the acute serum sample. A further 30 negative control samples were from case control participants collected during the Melbourne Aquarium outbreak. These samples were from people who were at the aquarium at the time of the outbreak but showed no clinical signs of legionnaires' disease.

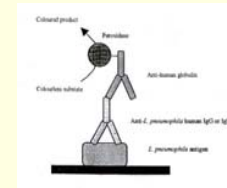
In addition to these samples we also included 8 samples which had a confirmed diagnosis of *Legionella* by PCR, these serum samples were convalescent bleeds timed 2-3 weeks post collection of PCR samples.

All serum samples had been in storage at -20°C.

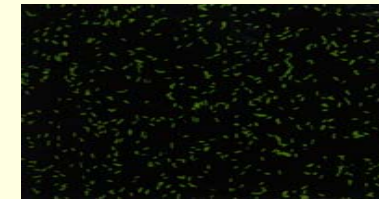
The in-house IFA in use at VIDRL consists of 2 pools. *Legionella pneumophila* s1-s6 in pool 1 and *Legionella longbeachae* (s1,s2) and *Legionella micdadei* in pool 2. Strongly reactive samples in the second pool are serotyped for *L.longbeachae* and *L.micdadei*.

The Vircell *Legionella Pneumophila* Serogroups 1-6 ELISA IgG+IgM is an Indirect enzyme immunoassay, testing was performed manually.

The Vircell assay is an indirect ELISA



Legionella pneumophila as detected by IFA. *Legionella pneumophila* can appear in various forms such as coccobacillus, bacillus and elongated forms.



Results

Table 1: Serology positives and controls

Seroconverters/ Significant rise (n=21 pairs)	EIA Positive	EIA Negative
L.pneumophila (n=14 pairs)	14	0
L.longbeachae (n=7 pairs)	2	5
Case Controls (n=30)	1 (equivocal)	29

Table 2: PCR Positives

PCR dx.	IFA Pos	IFA Neg	EIA Pos	EIA Neg
L.pneumophila (n=3)	3	0	2	1
L.Longbeachae (n=5)	5	0	0	5

Discussion

The sensitivity of the Vircell EIA as compared to our in house IFA assay was 66.7%, this increases to 94.1% when we exclude the *Legionella longbeachae* positive samples. The Vircell EIA showed a seroconversion in all 14 pairs of *Legionella pneumophila* IFA positive samples but only seroconverted for 2 of the 7 pairs of *Legionella longbeachae* IFA positive samples. Non-specific rises in titre have been known to occur in immunofluorescence assays for *legionella* often due to other gram-negative bacteria but as table 1 shows all 14 pairs of *Legionella pneumophila* seroconverters were also detected by the Vircell ELISA.

The 8 samples which had a confirmed diagnosis of *Legionella* by PCR had antibodies detected by IFA, 3 were *Legionella pneumophila* positive and 5 *Legionella longbeachae* positive. The Vircell EIA detected 2 of the *Legionella pneumophila* positive samples, the *Legionella longbeachae* samples were EIA negative.

The specificity of the Vircell EIA compared to our in house IFA was found to be 97.7%. The EIA returned 1 equivocal result out of the 30 case control samples.

The Vircell EIA is an easy to perform assay requiring a small amount of serum (5µl) and can be easily automated. We believe the assay is suitable for outbreak investigations and epidemiological studies. The kit performs well for the detection of *Legionella pneumophila* antibodies. Use of the kit as a diagnostic tool testing patients with atypical pneumonia would detect cases of *Legionella* caused by *Legionella pneumophila*, additional testing would be required for the diagnosis of *Legionella longbeachae*. *Legionella longbeachae* cannot be specifically detected by any commercially available EIA kit at this point in time.

References

- Victorian Infectious Diseases Bulletin Vol 9 Issue 4 December 2006.
- Notifications of Infectious Diseases Victorian Summary Report 1 Jan-4 May 2007.

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