Appendix 4. NSW specific advice for interpreting serological tests results for *Legionella pneumophila and longbeachae*

Criteria for diagnosis by serology

In NSW:

- laboratory **definitive** evidence includes "seroconversion or a significant increase in antibody level defined as a fourfold or greater rise in titre to *Legionella*".
- laboratory **suggestive** evidence includes "single high antibody titre to *Legionella* as defined by testing laboratory".

Types of serological tests for Legionella

Serum antibodies produced in response to *Legionella* infection can be measured by:

1. Indirect immunofluorescent assay (IFA): used by the majority of laboratories in Australia.
2. Enzyme immunoassay (EIA): used overseas, not presently used in Australia.

Sensitivity and specificity

The sensitivity of paired serology for Legionella is between 80-90% and specificity is >99%. Higher sensitivity if convalescent serology is delayed to six weeks after onset of symptoms.

Interpreting serology results

INTERPRETATION		**NEXT STEPS**
Antibody titres <512 or where results report a titre of ≥256* South Eastern Area Laboratory Services (SEALS) and Pacific Laboratory Medicine Services (PaLMS) including Pathology North antibody titre ≤320	May represent past infection.	Request **convalescent serology** if clinically compatible illness. No case follow up is required at this stage unless: • the notified person resides in an area where there is a known outbreak occurring; or • the notified person is a resident in a residential care facility or an inpatient in hospital; or • where two or more cases have occurred in the same geographical location within a twelve week period Where there is no further serology collected, the notification should be classified as **possible or excluded based on clinical notes**
Single high antibody titre ≥512* SEALS and PaLMS antibody titre including Pathology North antibody titre ≥320	This is rarely seen in healthy controls, and if detected in a patient with suspected Legionnaires' Disease, may be significant although may still represent past infection.	**Consider convalescent serology**, if an alternative diagnosis is not identified. Meets laboratory suggestive evidence criteria. Case interview for likely sources of infection should be conducted

* Includes and results from ICPMR and QML Pathology (including Dorevitch Pathology, South Western Sydney Pathology Service and Laverty)

Note - for all serological results from other private laboratories including Sullivan Nicolaides Pathology, Douglas Hanley Moir (DHM) Pathology, Southern IML Pathology and Capital Pathology please consult with the Clinical Microbiologist

Important considerations:

- "Single high titre" refers to a serology result at a single point in time.
- There is known serological cross reactivity between *Legionella* serogroups. A specimen with titres for more than one *L. pneumophila* serogroup may still support a *Legionella* infection in

the presence of a clinically compatible illness (i.e. elevated results for more than one serogroup does not exclude infection).

- If *Legionella* infection is suspected based on baseline serology, convalescent serology and/or alternate tests (sputum culture/PCR, urinary antigen) should be sought to a) confirm infection and b) determine the serogroup that is causing the infection.
- If an alternative organism or cause of disease is found (with high diagnostic certainty), *Legionella* infection diagnosed based on serology-only is less likely

Convalescent serology results

RESULT	INTERPRETATION	NEXT STEPS
< Four fold rise in antibody titre	Does not exclude Legionnaires' diseases. Some culture-positive cases of *Legionella* do not develop *Legionella* specific antibodies. There may not have been sufficient time between collections for a rise in titre.	**Consider repeat convalescent serology** if Legionnaires' disease is still suspected. Case interview does not need to be conducted.
≥ Four fold rise in antibody titre	Highly predictive of Legionnaires' disease. No need to undertake repeat testing	No need to undertake any further testing. Meets laboratory definitive evidence criteria. Case interview should be conducted.

Important considerations:

- Assessment for seroconversion should be conducted 3-6 weeks after onset of illness.
- A convalescent specimen should be collected and tested in parallel with the first specimen (i.e. by the same laboratory).
- It may require 4-8 weeks to develop a detectable antibody response.

Interpreting serology results in the context of urinary antigen testing:

- A negative urinary antigen (UA) test in the presence of serology with elevated titres for any *Legionella* serogroup does not rule out *Legionella* infection.
- Urinary antigen testing is specific only to *L. pneumophila* serogroup 1 (LP1) and some assays also to *L. longbeachae* serogroup1 (LL1). Urinary antigen tests will not identify legionella infection cause by another species or *L. pneumophila* serogroups other than serogroup 1.
- Urinary antigen results may be negative in the presence of true LP1 or *LL*infection and repeat U/A or alternate tests (sputum PCR and culture) should be sought where serology and clinical symptoms indicate true infection.

Additional considerations:

- Antibody levels can fall to undetectable levels within a month of infection.
- Early antibiotic therapy may suppress antibody response.
- Some individuals may not develop antibodies above detectable limits.
- Cross reactivity is noted between *L. pneumophila* serogroups.
- Cross-reactivity with antibodies generated as a result of non-Legionella infection has been reported with *Pseudomonas, Haemophilus, Mycobacteria, Bordetella, Chlamydia, Rickettsia, Campylobacter and Bacteroides* species*, Enterobacteriaceae and Coxiella burnetii.*
- Some reports indicate that apparently healthy individuals may carry antibodies to *Legionellae*. Additional testing to directly detect the organism, either through culture or nucleic acid

amplification tests is recommended to make a diagnosis of current infection, especially if there is a compatible illness with no alternate diagnosis.

REFERENCES:

1. PHLN *Legionella* Laboratory Case Definition *(LCD)*
2. CDC - *Legionella* – Diagnosis, Treatment and Prevention
3. NSW Health Legionnaires' disease control guideline