Legionellosis (*Legionella sp.*) Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions to inform the diagnosis of key diseases in Australia. This document contains the laboratory case definition (LCD) for Legionellosis.

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1.3	Updated diagnostic methods	PHLN	February 2023
1.2		PHLN	July 2019
1.1		PHLN	February 2011
1.0	Initial PHLN Laboratory Case Definition	PHLN	April 2007

1 PHLN Summary Laboratory Definition

1.1 Condition

Legionellosis due to infection with *Legionella* species.

1.1.1 Definitive Criteria

1. Isolation by culture of *Legionella* species from any clinical specimen; or

2. Detection of *Legionella* species-specific target by Nucleic acid amplification (NAA); or

3. Detection of *Legionella* species-specific antigen in urine; or

4. Seroconversion or significant increase in serum Legionella antibody level.

1.1.2 Suggestive Criteria

1. Single high antibody level to *L. pneumophila* (Lp), *L. longbeachae* or other pathogenic member of Genus *Legionella*.

2 Introduction

Legionellosis is an acute infection caused by bacteria belonging to the genus *Legionella* that usually occurs in adults and is often associated with underlying disease. Legionellosis encompasses Legionnaires' Disease, referring to severe pneumonia, originally described and attributed to infection with *L. pneumophila* serogroup 1 (Lp1)[7], and Pontiac fever, a milder form of illness attributed to *Legionella* species infection more broadly[24].

Legionellosis most often presents as a respiratory illness, however systemic features are common and may predominate, and *Legionella* wound infection occasionally occurs. Lp1 is the most common cause of legionellosis, however, in Australia up to half of all cases are due to *L. longbeachae* serogroup 1, which is more common in some Australian jurisdictions than others[10].

Acquired antimicrobial resistance in *L. pneumophila* is extremely rare. However, a clinical isolate resistant to ciprofloxacin due to a single point mutation in the *gyrA* gene, has been reported and further fluoroquinolone resistant strains have been isolated from patients, some during therapy[2].

The source of legionellosis is environmental, although an environmental source is rarely identified with confidence in sporadic cases. Outbreaks or case clusters of Legionnaires' Disease (usually due to Lp1) occur sporadically and may be traced to a common source, such as an air-conditioner cooling tower, showers or other potable water outlets[26]. The use of biocides may prevent culture of *Legionella* species from environmental samples collected in these case clusters, resulting in greater reliance on nucleic acid amplification tests[12]. Cases of infection with *L. longbeachae* serogroup 1 (Ll1) are usually associated with contaminated commercial potting soils or similar garden products[25]. Infections due to other serogroups and species are less common, usually sporadic and more likely to occur in individuals with underlying disease associated with immune deficiency or respiratory pathology. The diagnosis of legionellosis is made by:

   a. isolation of bacteria belonging to the genus *Legionella* from a clinical specimen; usually respiratory, but occasionally blood or wound swab;

   b. a significant change in the level of serum antibody (or seroconversion) against a *Legionella* species, using a suitably validated test;

   c. detection of specific urinary antigen (Lp1 or *L. longbeachae*); or

   d. specific *Legionella* nucleic acid amplification test in an appropriate specimen.

3 Tests

3.1 Culture1,19

Legionellae are fastidious bacteria requiring essential growth promoting factors including cysteine for their successful isolation from clinical material. *Legionella* grows aerobically and requires a high level of humidity.

3.1.1 Suitable Specimens

Bronchial washings and broncho-alveolar lavage specimens are the best choice and, if possible, should be collected before commencing antibiotic therapy. Pleural aspirates, lung or other relevant tissue specimens are suitable if available. Avoid using saline during collection of specimens since this inhibits the growth of *Legionella* species. Where this is unavoidable, such as with bronchoscopy specimens, the specimen should be cultured promptly. If delays setting up cultures are expected the specimen can be centrifuged and resuspended in a growth medium such as trypticase soy broth (TSB).

Expectorated sputum and tracheal aspirates are less satisfactory due to heavy contamination with oral flora and a relatively low *Legionella* content. These specimens should be cultured to both non-selective and selective media. Additional agar media can be inoculated after acid or heat treatment to reduce the growth of contaminants.

3.1.2 Media

Most laboratories use buffered charcoal yeast extract agar (BCYE) with added ferric pyrophosphate, L -cysteine and alpha-ketoglutarate. More selective agar media commonly used are BCYE with added vancomycin, polymyxin B and pimafucin (BCYE VPP), and BCYE with cefamandole, polymyxin B and anisomycin (BMPA). Another *Legionella* selective agar is modified Wadowsky and Yee medium (MWY) which contains anisomycin, polymyxin B and vancomycin. MWY agar is less selective for *L. pneumophila* but its use is likely to enhance the recovery non-pneumophila strains of *Legionella* including *L. longbeachae*.

Legionella species usually require 48 hours incubation before growth is visible. Colonies may not be clearly visible for up to five days or more. Culture plates should be incubated and examined daily for typical colonies for up to 10 days using a dissecting microscope with side lighting. This technique highlights the iridescence of early colony growth and reveals the ground glass appearance of mature colonies.

3.1.3 Test sensitivity

The quality of the specimen determines the diagnostic sensitivity of culture methods. Patients with milder disease may have a lower bacterial load in their lower respiratory tract. Delayed inoculation will reduce the efficiency of culture. The presence of contaminating commensal bacteria may inhibit the growth of *Legionella,* particularly when the specimen contains low numbers. Antibiotic treatment may also inhibit growth.

3.1.4 Test specificity

The isolation of any *Legionella* species from a clinical specimen is considered to be clinically significant.

3.1.5 Predictive values

A negative culture does not exclude the diagnosis of legionellosis.

3.1.6 Suitable test acceptance criteria

Legionella species is a pale staining Gram-negative bacillus, often pleomorphic, which grows only on BCYE (cysteine-supplemented) and not on Blood Agar or BCYE without cysteine (if available).

3.1.7 Suitable internal controls

It is necessary to check that each batch of media used for isolation of *Legionella* species supports their growth under suitable incubation conditions by inoculating samples of each media batch with control strains *of L. pneumophila* and *L. longbeachae.*

3.1.8 Suitable test validation criteria

Isolation of *Legionella* species is the reference standard.

3.1.9 Suitable external QC program

None in Australia.

3.1.10 Special consideration

L. oakridgensis requires cysteine only for primary isolation. On subculture it will grow without cysteine. *Francisella tularensis* has similar growth characteristics but remains an unusual isolate in Australia.

3.2 Identification of *Legionella* species

In routine laboratory practice serotyping is used to identify presumptive *L. pneumophila* and *L. longbeachae* but is unreliable for other species owing to the high degree of cross-reactivity. For these species, rapid *Legionella* species identification from culture can be performed by reacting whole bacterial cells with antibody raised in rabbits to known *Legionella* species. The reaction can be performed on a slide using sera bound to latex particles to demonstrate agglutination, or by UV microscopy with fluorescent labelled antisera (DFA). While serological identification by latex agglutination is acceptable for most sporadic clinical isolates of *L. pneumophila* and *L. longbeachae,* all isolates should be kept and/or sent to a reference laboratory and fully identified if

    a. there is a suspected outbreak or

    b. the isolate is unusual and can be of clinical or public health significance or

    c. the diagnosis of legionellosis is uncertain but clinically relevant, or

    d. there is a possible medicolegal issue not covered in (a) – (c).

Legionella species identification can be completed quickly from suspected isolates on selective media by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) procedures[8,9,18], which can be performed in clinical laboratories with MALDI-TOF mass spectrometer bacterial identification capability.

Legionella strains, and number included in Bruker MALDI Biotyper software library[18].

L. anisa (*n* = 9)	*L. feeleii* (*n* = 8)	*L. longbeachae* (*n* = 9)	*L. rubrilucens* (*n* = 1)
L. beliardensis (*n* = 1)	*L. geestiana* (*n* = 1)	*L. maceachernii* (*n* = 3)	*L. sainthelensi* (*n* = 3)
L. birminghamensis (*n* = 6)	*L. gormanii* (*n* = 2)	*L. micdadei* (*n* = 5)	*L. santicrucis* (*n* = 3)
L. bozemanii (*n* = 10)	*L. gratiana* (*n* = 5)	*L. moravica* (*n* = 1)	*Legionella* sp. (*n* = 1)
L. brunensis (*n* = 2)	*L. hackeliae* (*n* = 1)	*L. oakridgensis* (*n* = 2)	*L. tucsonensis* (*n* = 2)
L. cherrii (*n* = 4)	*L. impletisoli* (*n* = 1)	*L. parisiensis* (*n* = 1)	*L. wadsworthii* (*n* = 1)
L. cincinnatiensis (*n* = 2)	*L. israelensis* (*n* = 1)	*L. pneumophila* (n = 3)	*L. waltersii* (*n* = 1)
L. dresdenensis (*n* = 1)	*L. jamestowniensis* (*n* = 1)	*L. pneumophila* ssp *fraseri* (*n* = 4)	*L. worsleiensis* (*n* = 1)
L. dumoffii (*n* = 7)	*L. jordanis* (*n* = 2)	*L. pneumophila* ssp *pascullei* (*n* = 2)	*L. yabuuchiae* (*n* = 1)
L. erythra (*n* = 1)	*L. lansingensis* (*n* = 1)	*L. pneumophila* ssp *pneumophila* (*n* = 6)	

Legionella, like most bacteria, are taxonomically classified by genomic analysis[22] and new species are regularly identified and added to genomic databases. Therefore, identification of cultured isolates can be achieved through 16S rRNA gene amplicon sequencing, *mip* gene or whole genome sequencing. This selective sequencing can achieve species level identification of common species, although the full description of unusual species still requires an extended range of definitive tests[21]. Whole genome sequencing of cultured isolates can achieve coverage of the whole bacterial genome including both genes used in species identification and is a useful method to characterise rare isolates not identifiable by MALDI-TOF or phenotypic methods.

Genomic identification methods have now superseded the classical definitive identification of *Legionella* species based on DNA/DNA hybridisation, gas chromatography (GC) for long chain fatty acids and high-performance liquid chromatography (HPLC) for ubiquinones.

3.3 Antigen Detection Tests

3.3.1 Urinary antigen test

Antigen to *L*Lp1 and *L. longbeachae* in urine may be detected by immunoassay and rapid format immunochromatographic (ICT) kits[10,15,16]. In Australia the most commonly used tests are qualitative immunochromatographic tests. These include the BinaxNOWICT for detection of Lp1 antigen in urine .and the ImmuView combined urinary antigen ICT test to detect both *Legionella pneumophila* (not serotype specific) and *Legionella longbeachae.*

Reference laboratories may also perform a *Legionella* Urinary Antigen Enzyme Immunoassay (Binax), which uses microtitre trays coated with polyclonal rabbit antibody specific for a heat stable lipopolysaccharide antigen. For this test, urine specimens can be

concentrated by ultrafiltration to enhance low or borderline results. The ICT is faster than the EIA format (15 vs 90 minutes) and more suitable for use with smaller numbers of specimens.

3.3.1.1 Suitable specimen

Urine.

3.3.1.2 Test sensitivity

A recent meta-analysis (published in 2022) found the pooled sensitivity of Legionella urinary antigen assays in suspected legionellosis was 79% (95%CI 71%-85%)[14]. Sensitivity is higher (86% 95% CI 78%-91%) in *L.pneumophila* serogroup 1 disease[14]. Whilst the Binax NOW ICT has excellent specificity and good sensitivity for detecting *Legionella* infection, occasional borderline or false negative results occur. If a Lp1 ICT result is in doubt, laboratories can refer the urine specimen to another laboratory which also performs the Binax EIA. As antigen excretion can be intermittent and may persist for months a repeat urine specimen can be requested, following the infection.

3.3.1.3 Test specificity

Close to 100% for the detection of *Legionella* spp. (95% CI 99%-100%)[14].

3.3.1.4 Predictive values

Positive: ~ 100% for *Legionella pneumophila*.

Negative: Negative predictive value varies with the prevalence of *Legionella* in the population However, in an analysis of 187 proven cases (culture positive for Lp1 or seroconversion) and 289 negative controls, the negative predictive value of the BinaxNOW ICT was 88%[10].

3.3.1.5 Suitable test acceptance criteria

Refer to the manufacturer's instructions.

3.3.1.6 Suitable internal controls

Positive and negative controls are supplied in the test kit

Or positive control: human urine containing Lp1.

Consider additional positive control of human urine containing *L. longbeachae* antigen when using a combined antigen test.

Negative control: normal human urine

3.3.1.7 Suitable validation criteria

As described by the test kit manufacturer.

3.3.1.8 Suitable external QC program

An EQA programme is available in Australia through the RCPAQAP. Additional QA is available from Europe through the European Legionnaires' Disease Surveillance Network (ELDSNet) - formerly European Working Group on Legionella infections (EWGLI):

(ecdc.europa.eu/en/about-us/partnerships-and-networks/disease-and-laboratory-networks/eldsnet).

3.3.1.9 Special considerations

Peak urinary antigen occurs 5–10 days after onset of symptoms and usually diminishes rapidly to barely detectable 21 days after onset of symptoms. In rare cases antigen may continue to be excreted for months after infection has resolved[16]. These kits are very sensitive for detecting most subtypes isolated from community-acquired Lp1 infections, which are usually caused by Pontiac strains. However, they are less sensitive when infections are caused by non-Pontiac strains, particularly the Bellingham subtype. This may have implications for their use in hospital settings[16]. These tests cannot detect species other than *L. pneumophila* and *L. longbeachae*. Furthermore the BinaxNOW test is specific for the detection of Lp1. Therefore, positive urinary antigen results should always be verified by culture or NAA if possible. In addition, if legionellosis is strongly suspected on clinical grounds, additional tests such as culture or NAA should be performed because a negative urinary antigen test does not exclude legionellosis, particularly when infection is caused by species other than *L. pneumophila.*

3.3.2 Direct fluorescent antigen (DFA)

DFA may be used directly on specimens including paraffin sections after dewaxing. However, it is rarely used except during outbreak investigations or when unfixed tissue is not available because of the laborious nature of the test, its lack of sensitivity and the low rate of positives seen routinely. As DFA has given way to more rapid, specific and consistent test methods in major Australian laboratories, it will not be considered further.

3.4 Nucleic acid amplification (NAA) Based Tests

Highly sensitive NAA assays are capable of detecting *Legionella* in serum and urine.[4,5,16] NAA has the advantage over urinary antigen assays in detecting a wide range of *Legionella* species. Most in-house assays described in the literature target the genes for rRNA or *mip* (macrophage infectivity potentiator). Becton Dickinson Biosciences' BD ProbeTec ET *L. pneumophila* (LP) Amplified DNA Assay has been approved by the US Food and Drug Administration for sputum specimens but is not marketed in Australia. Multiple products for *Legionella* NAA tests are listed on the Australian Register of Therapeutic Goods (ARTG). Specific details can be obtained from vendors of ARTG-listed tests.

3.4.1 Suitable Specimens

Respiratory tract specimens, urine, and serum.

3.4.2 Test sensitivity

This is measured in patients who fit the laboratory case definition (culture positive or urinary antigen positive or seroconversion or significant increase in antibody level).

All targets[1]

Urine 49.7% (95% CI 26.5–73.0)

Blood 48.9% (95% CI 38.4–59.5)

Respiratory samples 97.4% (95% CI 91.1–99.2)

S rRNA: 54.4%–80% in serum, 80% in respiratory specimens, 46%–86% in urine specimens,[13]

16S rRNA: 30.9% in serum, 72 to 95% in respiratory specimens

mip: 52.9% in serum, 78 to 98% in respiratory specimens

Sensitivity is highest early in disease and the assay may be positive up to one month post disease onset. NAA in serum may be positive for longer than the urinary antigen test.

3.4.3 Test specificity

Specificity varies with primer and probe design and approaches 100% in some studies. There is marginal cross-reactivity of some Lp NAA assays with *L. fairfieldensis* and *L. worsleiensis.* A positive *Legionella* NAA assay, irrespective of intended target gene sequence, cannot distinguish viable from non-viable *Legionella* but false positive reactions due to laboratory sources of contamination can be excluded by internal quality control. In the presence of clinical features of legionellosis, a positive Legionella NAA test result on an extract from a clinical specimen satisfies the criteria for laboratory definitive evidence of infection.

3.4.4 Predictive values

Positive predictive value depends on primer and assay design and can be high. Negative predictive value depends on the site of specimen collection and time after onset of disease.

3.4.5 Suitable test acceptance criteria

Results for control samples obtained as expected.

3.4.6 Suitable internal controls

Controls should be designed to detect sample inhibitory activity and external contamination of reagents or clinical samples by environmental *Legionella*, as recommended in NPAAC guidelines: Laboratory Accreditation Standards and Guidelines for Nucleic Acid Detection and Analysis.

3.4.7 Suitable validation criteria

Consistent with NPAAC Guidelines:

Requirements for the Development and Use of In-House In Vitro Diagnostic Medical Devices (IVDs).

For commercial IVDs the assay should be listed with the Australian Register of Therapeutic Goods and the certificate and validation data should be available on request from the IVD sponsor.

3.4.8 Suitable external QC program

[Quality Control for Molecular Diagnostics (QCMD)](#) offer an external quality assessment and proficiency testing program for *L. pneumophila* that is available to Australian participants.

3.4.9 Special considerations

NAA tests can be used to detect *Legionella* in tissue in paraffin blocks after dewaxing and may be useful for diagnosis when unfixed specimens are not available. Since *Legionella* are ubiquitous in the environment, caution should be exercised in using these tests to confirm cases of legionellosis if there is any likelihood of environmental contamination of the specimen. Some commercial DNA (spin column) extraction kits have been contaminated with *Legionella* DNA, producing false positives when used for DNA extraction from specimens.[5]

3.5 Serological Tests[19,23]

Different public health and hospital laboratories use different test methods, with a correspondingly different range of interpretive cut off points. These reflect genuine and substantial differences in the epidemiology of legionellosis across Australia.

Serum antibodies produced in response to *Legionella* infection can be measured by a variety of methods including the indirect immunofluorescent assay (IFA) and enzyme immunoassay (EIA). In Australia, the majority of laboratories use either in-house or commercial IFA assays. A commercial, automated CFT for *L. pneumophila* serogroups 1-6 is also available in Australia.

IFA

In the IFA assay, dilutions of patient sera are added to killed *Legionella* cells fixed to microscope slides. Antihuman immunoglobulin conjugated with fluorescein isothiocyanate is then added. If *Legionella* antibody is present in the serum sample, the *Legionella* cells fluoresce when the slides are viewed with a UV microscope.[6]

EIA

There are commercial *Legionella* EIA assays registered on the ARTG, but published results[13] and local surveys show that commercial assays are generally inferior to in-house EIA assays. EIA assays are designed to provide a sensitive screen for legionellosis and detect IgM using sonicated whole Lp1 or Ll1 cells as their source of antigen. A single positive IgM assay should be interpreted with caution in sporadic cases because IgM to *Legionella* species can remain elevated for years. Numerous cross-reactions occur among the non-*pneumophila* legionellae and other organisms.

3.5.1 Suitable specimens

A minimum of paired serum samples should be collected as soon as possible after the onset of illness and then 3-6 weeks later. There may be up to nine weeks' delay before seroconversion can be detected.

3.5.2 Test sensitivity

IFA:

Seroconversion is defined as a four-fold increase in titre of immunofluorescent antibody against heat killed Lp1, to ≥ 1:128. Sensitivity is 70–80% overall, and up to 90% if collection of convalescent serum is delayed to six weeks after onset of symptoms.

Similar sensitivity has been found for antibody seroconversion to the heat killed Ll1 antigen. Four-fold rises to ≥ 1:512 were detected in 11/12 patients with culture-confirmed *L. longbeachae* infection[16].

A single high titre of 1:512 or higher in either Ll or Lp is a sensitive indicator of infection with *Legionella* but may represent past infection or more rarely, infection with another species (refer below).

EIA:

In published reports commercial EIA sensitivities vary widely from less than 20% when compared with IFA in randomly selected sera[17] to ~70% when tested in patients from a single outbreak[23]. EIA assays developed in house using high quality antigen preparations have higher sensitivities (82%)[15] than commercial test kits. IgM measured by EIA can become positive earlier in the course of illness than IFA.

3.5.3 Test specificity

IFA:

Specificity of IFA tests, using Lp1 antigens, is reported from 95 to 99.9%[16].

Specificity of IFA for other *Legionella* species is variable and results should be interpreted with caution because of the numerous cross reactions that may occur among *Legionella* and other bacteria. For example, cross reactions in IFA tests using non-Lp1 antigens have been reported in patients with pneumonia or bacteraemia caused
by *Pseudomonas*, *Haemophilus*, *Mycobacteria*,
Bordetella, *Chlamydia*, *Rickettsia, Campylobacter* and *Bacteroides* species, Enterobacterales and *Coxiella burnetii*[6].

EIA:

IgM EIA based tests have been reported to be slightly less specific (97%) than IFA when acute and convalescent sera are tested for Lp1[23]. There are no published data for specificity of IgM EIA for Ll*,* but, as with IFA, cross-reactions among *Legionella* and other organisms are highly likely to affect specificity. Therefore, single positive results should be interpreted with caution.

3.5.4 Predictive values

IFA:

Positive tests: four-fold rise in antibody titre is highly predictive of legionellosis (*L. pneumophila* to 1:128 or higher and *L. longbeachae* to 1:256 or higher). Serological tests for *Legionella* species other than Lp1 are predictive of legionellosis caused by the *Legionella* species antigen used **or** a related *Legionella* species.

Negative tests: In one study approximately 10% of patients with culture proven *L. longbeachae* failed to seroconvert after 3 months[23] and up to 20% may not seroconvert to Lp1 antigen in culture proven cases[23].

A single high antibody titre of ~512 or greater against *L. pneumophila* is rarely seen in control subjects and, if detected in a patient with suspected legionellosis, may be significant and should be reported.

Similarly, single high antibody titres against *L. longbeachae* of ~512 or greater are rare (1–2 %) in healthy controls and may be significant in a patient with compatible illness. Actual cut-off titres vary in different laboratories, based on local test evaluation using representative samples of control sera.

EIA:

Positive tests: Single high levels to Lp1 are highly predictive of disease but may represent past infection.

Single high levels of antibodies to *L. longbeachae* may represent past infection or cross-reaction. The significance of positive EIA results should be determined using another serological test or test method such as NAA or urinary antigen assays.

Negative tests: predictive values of commercial kits may be low, but much higher in well designed and validated in- house assays.

3.5.5 Suitable test acceptance/validation criteria

Consistent with NPAAC Guidelines:

[Requirements for the Development and Use of In-House In Vitro Diagnostic Medical Devices (IVDs)](.).

Commercial kits: according to manufacturer's instructions.

IFA: Positive control/s should be within one doubling dilution of the established endpoint.

3.5.6 Suitable internal controls

IFA: a positive control for each antigen tested should be titrated to at least two dilutions past the established endpoint.

3.5.7 Suitable external QC program

RCPA QAP Serology and Microbiology programs.

3.5.8 Special considerations

The endpoint titre for IFA is the reciprocal of the highest serum dilution giving 1+ fluorescence in at least 80% *Legionella* cells. A suitable means of determining the percentage of fluorescing cells is to use a fluorescent microscope equipped with a dark field condenser. The practice of running the IFA method using pooled antigens, containing multiple serogroups of *L. pneumophila* may give misleading results owing to the variability of fluorescent staining often seen with this species. The use of a fluorescence-labelled conjugate that detects all classes of antibody is recommended for optimum sensitivity. IgM antibody can persist for a long time and is not reliable for distinguishing new from old disease.

All in-house assays should be validated with an adequate number of culture-confirmed cases. Laboratories should establish *L. pneumophila* and *L. longbeachae* reactivity with sera from healthy controls in their region and if possible, from patients with bacteriologically confirmed pneumonia not caused by *Legionella* species. These studies may identify patients whose antibody levels suggest infection and require further action, including notification of the local Public Health Unit.

To avoid unnecessary testing and potentially false negative results, acute serum specimens (i.e. collected within 2 weeks of onset of illness) should be stored until convalescent sera are received for testing in parallel. An exception can be made for cases in which the first serum specimen is collected relatively late in the illness.

4 Genotyping and relatedness assessment

4.1 Methods

Various genotyping methods are in use for subtyping *Legionella* species including pulsed field gel electrophoresis (PFGE), restriction fragment length polymorphisms (RFLP) using Southern hybridisation, random amplified polymorphic DNA (RAPD), AFLP, MLST and consensus sequence-based typing (SBT), a method that uses 7 housekeeping and virulence genes. The choice of method depends on the preference of the laboratory performing the test.

Sequence-based typing (SBT), analogous to multilocus sequence typing (MLST), is the current "gold standard" typing method for investigation of legionellosis outbreaks caused by *Legionella pneumophila*[3]. A common SBT identity among isolates does not prove that they have originated from the same source but may provide helpful molecular epidemiological support for epidemiological and clinical surveillance data. Further determining genomic relatedness between isolates can be determined by whole genome sequencing using single nucleotide polymorphism (SNP analysis) in comparison to a reference genome. SNP analysis has proven feasibility and high discriminatory power and is increasingly playing a central role in *Legionella* epidemiological investigations[3].

It is important to note that whole genome sequencing analysis requires the availability of cultured isolates. For instance, the investigation of relatedness between isolates from clinical cases and from relevant environmental sources (e.g., cooling tower's water) is enabled by the availability of cultured *Legionella* isolates from both clinical samples and environmental samples that then undergo genomic analysis. This analysis is undertaken to determine the likelihood of an environmental *Legionella* isolate being the source of human infections[3].

A panel of seven monoclonal antibodies for subtyping Lp1 was developed through collaboration between British, US, Canadian and French laboratories in 1987 and is used extensively overseas[13]. The full panel distinguished ten main subtypes of Lp1: the Pontiac subtypes, Philadelphia, Knoxville, Benidorm, Allentown, France, and the non-Pontiac subtypes, Olda, Heysham, Camperdown, Oxford and Bellingham.

These place names were allocated according to where each subtype was first isolated. Only three monoclonal antibodies from this panel have been used in Australia and were developed at CDC by McKinney. These are available from SA Pathology on request. Monoclonal antibody 2 is particularly useful for identifying Pontiac strains, which have caused all known community outbreaks of Lp1 and most sporadic cases in Australia. Non-Pontiac strains do not react with Mab 2 but may cause nosocomial infection. Mab 2 can be used to identify Pontiac strains and therefore provide evidence for pathogenic subtypes in environmental samples from sources implicated in community outbreaks where there is not a human isolate to compare.

4.2 Special considerations

Subtyping to identify a possible environmental source of legionellosis caused by any *Legionella* species is only informative when supported by unambiguous epidemiological evidence to implicate that source. Most jurisdictions now have more than a decade's worth of Lp1 profiles. Comparison of these profiles demonstrates that each jurisdiction has its own distinct pattern of strain/s that causes the majority of sporadic and outbreak-associated infections.

There is evidence that commercial potting soils are a source of Ll1 infection. Potting soils from the homes of legionellosis cases have been shown to contain genotypically similar strains to isolates from the patients[25].

5 Laboratory nomenclature for national data dictionary

SNOMED CT code	Concept name
26726000	Legionella infection (disorder)
7527002	Legionella genus (organism)
80897008	Legionella pneumophila (organism)
103463005	Legionella pneumophila serogroup 1 (organism)
89605004	Legionella longbeachae (organism)
122216005	Legionella species culture (procedure)
9718006	Polymerase chain reaction analysis (procedure)
709241004	Legionella antigen (substance)
121186005	Legionella species DNA (substance)
120743008	Legionella species antibody (procedure)
720071002	Legionella IgM (substance)
710472002	Legionella IgG (substance)
252370006	Polymerase chain reaction analysis for genomic fingerprinting (procedure)

6 References

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