Leptospirosis | *Leptospira* spp.

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 Leptospirosis.

Version	Status	Authorisation	Consensus Date
1.1	Full revision and new template. Movement of NAT from suggestive to definitive criteria. Additional section to include SNOMED CT terms.	PHLN	17 September 2024
1.0	Initial PHLN Laboratory Case Definition	PHLN	25 May 2007

1 PHLN summary laboratory definition

1.1. Condition

Leptospirosis due to infection with *Leptospira* spp.

1.1.1. Definitive criteria

- Isolation of pathogenic *Leptospira* spp. from a clinical specimen; or
- Fourfold or greater increase of *Leptospira* agglutination titre by Microscopic Agglutination Test (MAT) between acute and convalescent phase serum obtained at least two weeks apart or
- A single *Leptospira* agglutination titre by MAT greater than or equal to 400 in one or more serum specimens; or
- Detection of *Leptospira* DNA by nucleic acid test (NAT) from a clinical specimen.

1.1.2. Suggestive criteria

- Detection of IgM antibodies against *Leptospira* by serology (e.g. Immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA)).

2 Introduction

Leptospirosis is an emerging infectious disease and one of the most widespread zoonoses in the world[11]. It is estimated over 1 million cases occur worldwide each year. Early diagnosis and the ability to differentiate leptospirosis from other diseases is important to reduce the risk of more serious infection or mortality[4]. Leptospira spp. are endemic to native, feral and domestic animals that may serve as reservoirs, with rats and other rodents recognised as the most important sources[5]. Human infections result from contact with contaminated soil, vegetation or water, or with the body fluids of infected animals[14].

In Australia, leptospirosis in humans is a notifiable disease with the incidence in humans close to 1.0 per 100 000. The hospitalisation rate varies between 50–60% with an average reported hospital stay of 5–7 days. There is a broad range of occupations associated with the disease but the most common are the animal associated industries such as meatworkers and dairy farmers and agriculture workers, such as in the banana industry[17].

The genus Leptospira belongs to a group of distinctive bacteria called spirochaetes. They are a helical Gram-negative aerobic bacteria 6–12 µm long and 0.1 µm in diameter. They are highly motile, spinning on their long axis[5].

There currently are 17 species of Leptospira as determined by DNA-DNA hybridisation. These species can be further divided into pathogenic, non-pathogenic and opportunistic/possibly pathogenic. Pathogenic Leptospira species include; *L. interrogans, L. kirschneri, L. santarosai, L. weilii, L. alexanderi, L. borgpetersenii, L. genomospecies* 1 and *L. noguchii*. Non pathogenic Leptospira include: *L. biflexa, L. meyeri, L. wolbachii, L. vanthielii, L. terpstrae, L. yanagawae* [18] among others,and opportunistic/intermediate Leptospira include *L. broomi, L. fainei and L. inadai*[10,12,13]. The lowest taxon is serovar of which there have been over 300 serovars of Leptospira identified.

Transmission to humans occurs through penetration of the organism into the blood stream via cuts, skin abrasions or mucus membranes. Urine excretion of the organism by carrier animals, in particular rodents, is the primary environmental source of infections for humans. In humans, the disease manifests with symptoms similar to that of other diseases such as dengue, rickettsia, malaria and hepatitis. Specific symptoms of leptospirosis may include chills, malaise, headaches and abdominal pain and severe cases may involve acute renal failure (ARF), jaundice and pulmonary haemorrhage[13]. The disease presentation is biphasic with an acute or septicaemic phase of around one week followed by antibody production in the immune phase.

3 Laboratory diagnosis

The selection of diagnostic method is dependent on the phase of the infection. Leptospires usually circulates in the blood, and appear in the CSF, urine and penetrate internal organs of the patient for about 10 days after the onset of disease. Shedding of leptospires in the urine is intermittent and can be transient, lasting days or weeks. During this time, visualisation by microscopy, culture and isolation and detection of leptospiral DNA are all applicable in this acute phase[10]. Detectable titres of antibodies appear in the blood about 5-10 days after disease onset, but sometimes later if antibiotic treatment has been instituted. In this convalescent/immune phase serology is the primary diagnostic tool.

The microscopic agglutination test (MAT) remains the reference serological method for diagnosis of leptospirosis with isolation providing the gold standard for definitive evidence of infection.

3.1. Culture

Culture and isolation requires the need for specialised media and resources to maintain the cultures for up to six (6) weeks for weekly viewing by darkfield microscopy. In the acute phase, which lasts up to about 10 days, leptospires may be cultured from the blood or cerebrospinal fluid[5,11]. Urine generally has proven a difficult specimen for culture due to contamination problems.

It may be difficult to see growth in fluid media until the concentration of leptospires has become very high, and then opalescence is discernible when the tube is gently agitated.

Growth is also difficult to see in semi-solid media unless one or more distinct rings (Dinger's rings) appear at varying intervals from the surface (usually from 1–4 cm). These rings of heavy concentrations of leptospires persist after the organisms are dead, their persistence not necessarily indicating viable organisms. Therefore, it is vital that cultures are checked by darkfield microscopy.

Primary cultures should be examined for contamination on days 1–3 after inoculation; then for the presence of leptospira weekly.

When leptospires are seen in primary culture, subcultures should be made into 2–3 tubes with inocula of approximately 0.5 ml. The original primary culture is retained for further observation of growth and in case further samples are required for subculture.

Rate of growth will vary. Some strains grow well and multiply rapidly from the start; some appear to multiply fast for a few days, and then become static and inert; in others small numbers of lively leptospires appear but seem to multiply very slowly.

3.1.1. Suitable specimen types

Suitable specimen types include body fluids such as uncoagulated whole blood, urine and CSF. Blood is the most suitable material. Urine for culture isolation can be challenging due to the high contamination rate and poor success rate.

3.1.2. Specimen collection and handling

Specimens must be collected prior to antibiotics being administered.

Blood should be collected using aseptic technique and ideally 2–5 drops of uncoagulated whole blood should be inoculated into specialised media (Ellinghausen McCullough Johnson Harris; EMJH) containing 0.5% agar as soon as possible. Large inocula will inhibit the

growth of leptospires. Care should be taken to ensure that the culture medium used is free from contaminants.

Fresh midstream urine should be collected as aseptically as possible and should be inoculated into culture medium within two hours after voiding, since urine is acid and decreases the viability of leptospires.

Specimens must be stored and transported at ambient temperature; low temperatures are detrimental to pathogenic leptospires.

3.1.3. Test sensitivity

Culture is the gold standard for detection of the organism but a negative culture does not exclude an infection with the agent. Leptospires are fastidious bacteria. Contaminants, incubation temperature, and the handling of specimens during collection and transport can affect viability.

3.1.4. Test specificity

Isolation of pathogenic leptospires is proof of an infection.

3.1.5. Predictive values

Negative: A negative result does not exclude leptospirosis for the diagnosis.

Positive: A positive result confirms the diagnosis of leptospirosis, but serology should be sought for identification of the infecting serovar or serogroup prior to typing of the isolate.

3.2. Nucleic Acid Assays

The Nucleic Acid Assays (NAA) or Polymerase Chain Reaction (PCR) has been used increasingly over recent years to detect a large number of microorganisms[6,8,12,14,16]. PCR for pathogenic leptospiral DNA can rapidly confirm the diagnosis in the early phase of the disease, when bacteria may be present and before antibody titres are at detectable levels. Moreover, using real-time PCR, it is possible to quantify the amount of template and therefore, the number of target organisms. The ability of the PCR to identify acute cases has clinical value for management of the patient. The drawback with PCR is the inability to identify the infecting serovar[11].

3.2.1. Suitable specimen types

Body fluids such as whole blood, serum, urine and CSF.

3.2.2. Specimen collection and handling

Specimens must be taken prior to antibiotics being administered, aseptically into sterile collection containers and kept cool during transport.

A minimum of 200 µl specimen is required.

3.2.3. Test sensitivity

3.2.4. Assay and target dependent but should be highly sensitive for *Leptospira*.Test specificity

PCR dependent but should be highly specific for *Leptospira.*

3.2.5. Predictive values

Negative: A not detected result does not exclude leptospirosis as the diagnosis.

Positive: A detected result confirms the diagnosis of leptospirosis but convalescent serology should be sought for identification of the infecting serovar or serogroup.

3.2.6. Suitable test acceptance criteria

As per *NPAAC Requirements for the Validation of In house In vitro Diagnostic Devices*.

3.2.7. Suitable test validation criteria

As per *NPAAC Requirements for the Validation of In house In vitro Diagnostic Devices*.

3.2.8. Suitable internal controls

As per the *NPAAC Requirements for Medical Testing of Human Nucleic Acids*.

3.2.9. Suitable external quality assurance program and proficiency testing

Leptospirosis Molecular (LEPN435) National Serology Reference Laboratory External Quality External Quality Assessment Scheme (LRN EQAS).

3.3. Serology

There is a broad range of serological methods used for diagnosing the disease in humans—the MAT, LEPTO Dipstick, LEPTO lateral flow, Indirect haemagglutination test, LETO Dri Dot and anti—IgM enzyme linked immunosorbent assay (ELISA). The most commonly used being MAT and ELISA.

3.3.1. Microscopic Agglutination Test (MAT)

The MAT is the gold standard for serology and is used to identify the most probable serovar or serogroup that has caused an infection. Other techniques such as the ELISA can detect different classes of antibody but may be subject to false positive reactions and will require confirmation of these results by the MAT[5]. In MATs, serial dilutions of the antiserum are made in tubes or trays and an equal volume of Leptospira suspension is added. After the mixture is incubated, it is checked under low-power darkfield microscopy by taking loop fulls of the mixture onto a slide or more efficiently by using direct viewing using flat bottom microtitre trays. The results are assessed by determining a 50% endpoint within the serial dilutions, that is, 50% of the leptospires are agglutinated. The MAT relies on the use of live cultures as the source of antigen, often performed using a panel of antigens representative of local serovars. A specific antibody response detectable by the MAT generally occurs at around 5–10 days after onset of the illness. The MAT is generally performed by reference laboratories due to the inherent safety risks of handling cultures of live leptospiral organisms, the high cost of commercial media, and the need for ongoing maintenance of representative serovars or serogroups. The diagnostic performance of the MAT is strongly linked to the content of serovars in the panel used for each locality.

3.3.1.1. Suitable specimen types

Serum. The use of lipaemic, haemolysed or contaminated sera is not recommended.

3.3.1.2. Specimen collection and handling

A minimum of 200 µl of serum is required. Serum needs to be stored at 4°C or, for longer term storage, at –20°C.

3.3.1.3. Test sensitivity

Test sensitivity depends on the standardisation of culture densities to meet the required 2-4 x 10^8 leptospires/ml with accurate reading to the 50% endpoint.

3.3.1.4. Test specificity

The diagnostic performance of the MAT is strongly linked to the content of serovars in the panel used for each locality, i.e. relevant local isolates or reference strains which provide a high level of cross reaction.

3.3.1.5. Predictive values

Positive and negative predictive values are high but limited by acute and convalescent specimen availability.

3.3.1.6. Suitable test acceptance criteria

Satisfactory performance of controls.

3.3.1.7. Suitable internal controls

Cultures and hyperimmune sera supplied by a reference laboratory.

3.3.1.8. Suitable external quality assurance program (proficiency testing)

Leptospirosis Serology module: Royal College of Pathologists Australasia Quality Assurance Program Pty Ltd (RCPA QAP P/L).

International Proficiency Testing Scheme for the Leptospirosis Microscopic Agglutination Test: International Leptospirosis Society (ILS).

3.3.2. Enzyme Linked Immunosorbent Assay (ELISA)

Rapid serological tests for the diagnosis of leptospirosis as alternatives to the MAT have been attempted both as “in house” and commercial tests over recent years. The most common being the enzyme linked immunosorbent assay (ELISA) and IgM dipstick assays. The ELISA assays directed against IgM class antibodies are generally more sensitive than agglutination type tests but may be subject to variations in specificity[4]. The ELISA remains the most commonly used screening test due to its simplicity and suitability for automation[2].

3.3.2.1. Suitable specimen types

Serum. The use of lipaemic, haemolysed or contaminated sera is not recommended.

3.3.2.2. Specimen collection and handling

Serum needs to be stored at 4°C or, for longer term storage, at –20°C.

3.3.2.3. Test sensitivity, specificity, predictive values

Follow the manufacturer’s guidelines if using a commercial kit. The specificity can be low so all positives should be confirmed by the microscopic agglutination test (MAT).

3.3.2.4. Suitable test acceptance criteria

Follow the manufacturer’s recommendations if using a commercial kit.

3.3.2.5. Suitable external quality assurance program (proficiency testing)

Leptospirosis Serology module: Royal College of Pathologists Australasia Quality Assurance Program Pty Ltd (RCPA QAP P/L).

4 Typing

Typing of isolates can be achieved by nucleic acid based technologies or by serology. With serology the taxonomy system of Leptospira is based upon the antigenic determinants on the cell wall of the organism. When reacted against reference hyperimmune sera, the serogroup or serovar can be determined by these reactions. Under the cross agglutination absorption test (CAAT) two strains are considered to belong to different serovars if, after cross absorption with adequate amounts of heterologous antigen, 10% or more of the homologous titre regularly remains in at least one of the two antisera in repeated tests[18].

Whole genome sequencing has become the new gold standard for Leptospira taxonomy[9], recently replacing DNA-DNA hybridisation as the most commonly used approach for standard bacterial species delineation. Multilocus sequencing typing (MLST)[1,3,20] and core genome MLST (cgMLST)[7] schemes have also been developed for identification of leptospiral isolates at the subspecies level.

5 Laboratory nomenclature for national data dictionary

SNOMED CT code	Concept name	Description
77377001	Disorder	*Leptospirosis*
26764003	Organism	*Leptospira species*

6 References

1. Ahmed N, Devi SM, Valverde Mde L, Vijayachari P, Machang'u RS, Ellis WA, Hartskeerl RA. Multilocus sequence typing method for identification and genotypic classification of pathogenic Leptospira species. Ann Clin Microbiol Antimicrob. 2006 Nov 23;5:28. doi: 10.1186/1476-0711-5-28. PMID: 17121682; PMCID: PMC1664579.

2. Bajani MD, Ashford AA, Bragg SL, Woods CW, Aye T, Spiegel RA, Plikaytis BD, Perkins BA, Phelan M, Levett PN, Weyant RS. 2003. Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. J Clin Microbiol 2003 Feb;41(2):803-9.

3. Boonsilp S, Thaipadungpanit J, Amornchai P, Wuthiekanun V, Bailey MS, Holden MT, Zhang C, Jiang X, Koizumi N, Taylor K, Galloway R, Hoffmaster AR, Craig S, Smythe LD, Hartskeerl RA, Day NP, Chantratita N, Feil EJ, Aanensen DM, Spratt BG, Peacock SJ. A single multilocus sequence typing (MLST) scheme for seven pathogenic Leptospira species. PLoS Negl Trop Dis. 2013;7(1):e1954. doi: 10.1371/journal.pntd.0001954. Epub 2013 Jan 24. PMID: 23359622; PMCID: PMC3554523.

4. Cumberland P, Everard COR, Levett PN. 1999. Assessment of the efficacy of an IgM-elisa and microscopic agglutination test (MAT) in the diagnosis of acute leptospirosis. Am J Trop Med Hyg 61(5):731-4.

5. Faine S, Adler B, Bolin C, Perolat P. Leptospira and Leptospirosis, 2nd Ed., MedSci, Melbourne, Australia.1999.

6. Guerreiro H, Croda JL, Flannery B, Mazel M, Matsunaga J, Mitermayer GR, Laue, T., P. Emmerick, and S. Schmitz. 1999. Detection of dengue virus RNA in patients after primary of secondary dengue infection by using the TaqMan automated amplification system. J Clin Microbiol 37:2543-2547.

7. Guglielmini J, Bourhy P, Schiettekatte O, Zinini F, Brisse S, Picardeau M. Genus-wide Leptospira core genome multilocus sequence typing for strain taxonomy and global surveillance. PLoS Negl Trop Dis. 2019; 13(4):e0007374. PMID 31026256.

8. Hawrami, K., and J. Breuer. 1999. Development of a fluorogenic polymerase chain reaction assay (TaqMan) for the detection and quantification of varicella zoster virus. J Virol Methods 79:33-40.

9. International Leptospirosis Society Taxonomic Subcommittee. Position Statement - Speciation of Leptospiral Isolates and Minimum Criteria for Species Definition. November 2023.

10. Levett PN, Ko AI, Haake DA. 2001. Leptospiral proteins recognized during the humoral immune response to leptospirosis in humans. Infect Immun 69(8):4958-68.

11. Levett, P. N. 2001. Leptospirosis. Clin Micro Rev. 14:296-326.

12. Nogva, K.H. and D. Lillehaug. Detection and quantification of Salmonella in pure cultures using 5'-nuclease polymerase chain reaction. Int J Food Microbiol 1999 Oct 15;51(2-3):191-6.

13. Slack A, Symonds M, Dohnt M, Smythe L. An improved multiple-locus variable number of tandem repeats analysis for Leptospira interrogans serovar Australis: a comparison with fluorescent amplified fragment length polymorphism analysis and its use to redefine the molecular epidemiology of this serovar in Queensland, Australian Journal of Medical Microbiology 2006 Nov; 55(Pt 11):1549-57.

14. Slack AT, Symonds ML, Dohnt MF and Smythe LD Identification of pathogenic Leptospira species by conventional or real-time PCR and sequencing of the DNA gyrase subunit B encoding gene. BMC Microbiology 2006 Oct 27; 6(1):95.

15. Smythe L. 2006 'Leptospirosis - a review of laboratory diagnostic methods' Australian Journal of Medical Science February Vol.27 No.1.pages 16-19.

16. Smythe L, Smith I, Smith G, Dohnt M, Symonds M, Barnett L, McKay D. A quantitative PCR (TaqMan) assay for pathogenic Leptospira spp. BMC Infectious Diseases 2002, 2:13.

17. Smythe L, Symonds M, Dohnt M, Slack A. National leptospirosis surveillance report number 14 (January-December 2006) WHO/FAO/OIE collaborating centre for reference & Research on leptospirosis, Queensland Health Scientific Services, Coopers Plains. 2006. http://www.health.qld.gov.au/qhpss/leptospirosis.asp. Retrieved 4 April 2007.

18. Smythe L, Adler B, Hartskeerl RA, Galloway RL, Turenne CY, Levett PN. Classification of Leptospira genomospecies 1, 3, 4 and 5 as Leptospira alstonii sp. nov., Leptospira vanthielii sp. nov., Leptospira terpstrae sp. nov. and Leptospira yanagawae sp. nov., respectively. Int J Syst Evol Microbiol 2013 May;63(Pt 5):1859-1862. doi: 10.1099/ijs.0.047324-0.

19. Stallman N. International Committee on Systematic Bacteriology, Subcommittee on the taxonomy of Leptospira: Minutes of the Meeting, 6 to 10 August 1982, Boston, Massachhusetts. International Journal of Systematic Bacteriology 1982;34:285-59.

20. Varni V, Ruybal P, Lauthier JJ, Tomasini N, Brihuega B, Koval A, Caimi K. Reassessment of MLST schemes for Leptospira spp. typing worldwide. Infect Genet Evol. 2014 Mar;22:216-22. doi: 10.1016/j.meegid.2013.08.002. Epub 2013 Aug 8. PMID: 23932960.

7 Glossary

Ag/Ab – Antigen/Antibody

AMR – Antimicrobial resistance

ARTG – Australian Register of Therapeutic Goods

BA – Blood agar

Biotype – Strain distinguished from other microorganisms of the same species by its physiological properties or a group of organisms with the same genotype

CCNA – Cell cytotoxicity neutralisation assay

(US) CDC – Centers for Disease Control and Prevention

CDNA – Communicable Diseases Network Australia

CDS – Calibrated dichotomous susceptibility

CIA – Chemiluminescent immunoassay

Clade – Group of organisms composed of a common ancestor and all its lineal descendants

CLSI – Clinical and Laboratory Standards Institute

CSF – Cerebrospinal fluid

Ct – Cycle threshold

DFA – Direct fluorescent antibody

DNA – Deoxyribonucleic acid

EDTA – Ethylenediaminetetraacetic acid

EIA – Enzyme immunoassay

ELISA – Enzyme linked immunosorbent assay

EUCAST – European Committee on Antimicrobial Susceptibility Testing

HI – Haemagglutination inhibition

ICT – Immunochromatographic test

IFA – Immunofluorescent antibody

IgA – Immunoglobulin A

IgG – Immunoglobulin G

IgM – Immunoglobulin M

IVD (device) – In vitro diagnostic medical device

In vitro – performed in a test tube, culture dish, or elsewhere outside a living organism

In vivo – performed or taking place in a living organism

ITS – Inter-genic spacer region

LAMP – Loop-mediated isothermal amplification

LPS – Lipopolysaccharide

MALDI-TOF – Matrix-assisted laser desorption ionization-time of flight

MAT – Microscopic agglutination test

MDST – Molecular drug susceptibility testing

MDR – Multidrug resistant

MIA – Microsphere immunoassay

MLST – Multilocus sequence typing

NAAT – Nucleic acid amplification test/ing

NATA - National Association of Testing Authorities, Australia

NGS – Next generation sequencing

NPAAC – National Pathology Accreditation Advisory Council

NRL – National Serology Reference Laboratory

PCR – Polymerase chain reaction

PC2 laboratory – Physical containment level 2 laboratory

PC3 laboratory – Physical containment level 3 laboratory

PC4 laboratory – Physical containment level 4 laboratory

PFGE – Pulsed field gel electrophoresis

POC – Point-of-care

QAP – Quality assurance program

QC – Quality control

RAPD – Random amplified polymorphic DNA

RCPA – Royal College of Pathologists of Australasia

SBT – Sequence based typing

Serotype – Pathogens of the same species that are antigenically different

SNT – Serum neutralisation

Strain – Variant that possesses unique and stable phenotypic characteristics

SQAP – Serology quality assurance program

Test sensitivity – Ability of a test to correctly identify patients with a disease

Test specificity – Ability of a test to correctly identify people without the disease

UTM – Universal transport medium

VTM – Viral transport media

WGS – Whole genome sequencing