



Melioidosis | *Burkholderia pseudomallei*

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 melioidosis.

Version	Status	Authorisation	Consensus Date
1.1	Updates to definitive criteria. Addition of serology in suggestive criteria. Inclusion of nucleic acid amplification testing.	PHLN	31 October 2025
1.0	Initial PHLN Laboratory Case Definition	PHLN	June 2002

1 PHLN summary laboratory definition

1.1. Condition

Melioidosis due to infection with *Burkholderia pseudomallei*.

1.1.1. Definitive criteria

- Isolation of *Burkholderia pseudomallei* by culture from any clinical site; OR
- Direct detection of *Burkholderia pseudomallei* in a clinical specimen by nucleic acid amplification testing

1.1.2. Suggestive criteria

- A four-fold rise in antibody titre or a single high titre AND a compatible clinical illness AND suspected exposure to *Burkholderia pseudomallei* (e.g. residence in or travel from an area where *B. pseudomallei* is present).

2 Introduction

Melioidosis is caused by *Burkholderia pseudomallei*, an environmental bacterium endemic in tropical and subtropical regions globally¹. It is notifiable in the Northern Territory, Queensland, and Western Australia. Globally, most cases occur in Southeast Asia and northern Australia². With increased awareness, improved diagnostic capacity, and potentially changes in the climate, the known geographic distribution has expanded and cases are increasingly being reported from the Americas, Africa, and South and East Asia^{2,3}. Although uncommon, locally acquired cases have been reported in Central Australia⁴, Southeast Queensland^{5,6}, and in Southwest Western Australia^{7,8}.

There is a close association between melioidosis and rainfall, with most reported cases in tropical regions occurring during the monsoonal wet season, and in subtropical/temperate areas during periods of heavy rainfall⁹. Increased case numbers have been observed following severe weather events such as cyclones and floods^{10,11}. Case clusters have been reported in association with contaminated products (e.g. wound irrigation fluid, hand detergent and aromatherapy products)¹², unchlorinated water supplies¹³, and recreational activities¹⁴.

The majority of melioidosis cases are sporadic, arising from exposure to the bacterium via percutaneous inoculation, inhalation, or ingestion. Many patients recall a possible inoculating

event, such as a skin or soft tissue injury with contamination, or possible aerosol exposure outdoors during storms or through use of a lawn mower, whipper snipper or high-pressure hose⁹. Based on such reported events, the median incubation period is 4 days (range 1-21 days)⁹. Serological studies suggest that most individuals exposed to *B. pseudomallei* do not develop melioidosis. Risk factors for melioidosis include diabetes mellitus, hazardous alcohol consumption, chronic kidney disease, and various forms of immunosuppression^{9,15}. There is no evidence that HIV increases the risk of melioidosis.

Most melioidosis cases present acutely (88%), while chronic presentations (9%) and reactivation from latency (3%) are less common⁹. Mortality is mainly due to severe sepsis and ranges from 10-40% depending on access to diagnostics and treatment, including intensive care support^{9,16}. The most common clinical manifestation is pneumonia; imaging findings are nonspecific, and may include lobar consolidation, multifocal infiltrates from haematogenous spread, cavitation (mimicking tuberculosis), and hilar and/or mediastinal lymphadenopathy. Around half of patients are bacteraemic. Prostatic abscess is a common manifestation in men, presenting as fever with urinary retention. Other foci of infection can include abscesses of the liver, spleen or kidneys, and bone and joint infection. Skin infection may be localised and chronic (often in immunocompetent hosts), or multifocal resulting from haematogenous spread. Neurologic melioidosis is uncommon and can present as encephalomyelitis with cranial nerve deficits, brain abscess(es), and/or meningitis¹⁷. Cerebrospinal fluid is often culture-negative, and a presumptive diagnosis may need to be made based on patient epidemiology, clinical and radiological features, and serology.

Treatment is with at least 2 weeks of intravenous ceftazidime or meropenem, followed by at least 3 months of oral trimethoprim-sulfamethoxazole¹⁸. Doxycycline and amoxicillin-clavulanate are second line oral treatment options. Resistance to the agents used to treat melioidosis is rare, but can develop during treatment in those with cystic fibrosis or severe bronchiectasis, or those with a particularly high organism burden¹⁹. Clinical breakpoints for *B. pseudomallei* have been published by both CLSI and EUCAST^{20,21}. It is recommended to test susceptibility to ceftazidime, meropenem or imipenem, trimethoprim-sulfamethoxazole, doxycycline, and amoxicillin-clavulanate.

3 Laboratory diagnosis

3.1. Culture

B. pseudomallei grows well on standard media including horse or sheep blood agar and chocolate agar at 35-37°C in air. It may be overgrown by commensal organisms in

specimens from nonsterile sites. It will grow on MacConkey agar, however preferred selective media include Ashdown's agar (which contains gentamicin and crystal violet), *Burkholderia cepacia* selective medium (which contains polymyxin B, gentamicin, vancomycin, and crystal violet), and Ashdown's broth (which contains colistin and crystal violet). Gentamicin-susceptible *B. pseudomallei* (currently only known to be present in parts of Malaysia) will not grow on Ashdown's agar. *B. cepacia* selective agar is used in some centres as an alternative selective medium for *B. pseudomallei*, though it will not inhibit *B. cepacia* complex which may be difficult to distinguish from *B. pseudomallei*.

3.1.1. Suitable specimen types

Patients with suspected melioidosis should have blood cultures, urine, and sputum collected for culture. Depending on the presentation, tissue, skin swabs, pus, and fluids from normally sterile sites should also be collected. A throat swab placed directly into Ashdown's broth can be helpful in those unable to produce a sputum specimen. The numbers of *B. pseudomallei* colony-forming units can be low in tissue and exudate specimens and thus easily lost among heavy growth of commensal bacterial flora.

In addition, when a potential environmental source has been identified it may be necessary for a reference laboratory to attempt culture of soil or water specimens. Detailed guidelines for isolation of *B. pseudomallei* from soil and water samples have been published²². Culture of soil involves an initial extraction step with addition of a solution, mixing, and sampling of the supernatant which is then inoculated onto Ashdown's medium and incubated for 7 days. For water samples, bacterial concentration can be achieved by filtration; the filter is then incubated in an enrichment broth and subcultured onto Ashdown's agar.

3.1.2. Specimen collection and handling

Specimens should be kept cool and transported to the laboratory as soon as possible after collection.

3.1.3. Test sensitivity

The sensitivity of *B. pseudomallei* culture depends on clinical presentation, organism burden, and the type, quality and number of clinical specimens collected for culture. Body fluids and urine should be centrifuged and the pellet cultured. Some centres find an improved isolation rate of *B. pseudomallei* from non-sterile sites by using a preliminary incubation step in Ashdown's broth medium.

The diagnosis can be missed if the organism is misidentified as another *Burkholderia* sp.

3.1.4. Test specificity

Isolation of the organism from any site is diagnostic of melioidosis. Organism identification by any method should be corroborated with colonial morphology, Gram stain, bench biochemical testing, the antimicrobial susceptibility pattern, and patient clinical and epidemiological features. See 'Suitable test acceptance criteria' below.

3.1.5. Predictive values

A negative culture does not exclude the diagnosis of melioidosis.

3.1.6. Suitable test acceptance criteria

B. pseudomallei is a motile, Gram-negative bacillus which may have bipolar staining with Gram stain. It is oxidase positive and indole negative. Colonies may not be apparent until 48 hours of incubation, and initially appear creamy with a metallic sheen, subsequently becoming dry and wrinkly. On Ashdown's medium the colonies have a purple colour. The antimicrobial susceptibility pattern is a helpful adjunct for identification²³; *B. pseudomallei* is typically susceptible to amoxycillin-clavulanate, resistant to gentamicin (with the exception of isolates from parts of Malaysia which are gentamicin susceptible²⁴), and resistant to colistin.

Misidentifications (particularly as *Burkholderia cepacia* complex) using biochemical systems such as the Vitek® 2 GN ID (bioMérieux) card or API® 20 NE (bioMérieux) are common^{25,26}. *B. pseudomallei* can be identified using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) provided the organism is included in the database used. *B. pseudomallei* has been added to version 3.3 of the bioMérieux Vitek MS *in vitro* diagnostic (IVD) database²⁷, and is also included in the security-relevant library for use with the Bruker MALDI BioTyper. Misidentifications as *B. thailandensis* (a non-pathogenic close relative of *B. pseudomallei*) have occurred with the standard Bruker Microflex Biotyper IVD database. Reference laboratories may have developed in-house libraries for the differential identification of *B. pseudomallei*.

Agglutination sera can be obtained from Thailand and this is a useful bench test for identification of colonies²⁸. Nucleic acid amplification testing can also be used on culture isolates to confirm *B. pseudomallei* identification (see section below)²⁷.

3.1.7. Suitable test validation criteria

Identification methods used for *B. pseudomallei* should undergo verification or validation using a well-characterised, geographically diverse collection of isolates including near neighbour species.

3.1.8. Suitable internal controls

There should be a properly documented, relevant internal quality control program for selective media used for *B. pseudomallei* culture. Adjunctive identification methods such as antisera and nucleic acid amplification should also undergo quality control.

3.1.9. Suitable external quality assurance program (proficiency testing)

The RCPA Biosecurity external quality assurance program includes *B. pseudomallei*.

3.1.10. Laboratory safety

In Australia *B. pseudomallei* is classified as a risk group 2 organism with a recommendation to handle the organism in a biosafety cabinet. However, this is not practical and not done in many endemic areas, including in some laboratories in northern Australia. Available evidence suggests the risk of laboratory-acquired infection is very low^{29,30}.

Evaluation of laboratory exposure to *B. pseudomallei* should consider the nature of the exposure including high risk features such as aerosolisation or percutaneous inoculation, and any staff comorbidities that increase melioidosis risk. There is limited evidence to guide management and follow-up following laboratory exposure to *B. pseudomallei*³¹. Post-exposure prophylaxis with trimethoprim-sulfamethoxazole, doxycycline, or amoxicillin-clavulanate may be offered. Exposed staff members may have baseline and follow up serology testing and should be evaluated clinically for development of melioidosis. Expert advice is recommended.

3.1.11. Outbreak investigation and molecular epidemiology

Whole genome sequencing is the standard method for characterising the molecular epidemiology of *B. pseudomallei*, and can be used for *in silico* multilocus sequence typing (MLST), core genome single nucleotide polymorphism calling and phylogenetic analysis, and core-genome MLST^{32,33}. There is a well curated online MLST database which includes many Australian and international isolates (<https://pubmlst.org/organisms/burkholderia-pseudomallei>). *B. pseudomallei* sequence types (STs) are geographically restricted at the continental level^{34,35}, with the exception of rare instances of homoplasy (where isolates from

the same ST do not share recent ancestry)³⁶, and rare instances of long-range transmission³⁷. Genomics can be used to predict geographic origin at the continental level³⁸.

While the vast majority of melioidosis cases are sporadic, outbreaks occasionally occur^{12,14}. Typing and genomic comparison of clinical and environmental isolates can enable pinpointing of the source³⁹. The diversity of isolates associated with clusters related to shared environmental exposure can be high, however. International consensus guidelines have been developed for environmental sampling and testing²².

3.2. Nucleic Acid Testing

Nucleic acid amplification testing directly on clinical specimens may provide timely melioidosis diagnosis. However, culture remains the mainstay because it enables antimicrobial susceptibility testing and genomic sequencing for epidemiological investigations. It is not uncommon for patients to remain culture positive for *B. pseudomallei* for days to weeks after commencing melioidosis treatment, particularly where there is a high organism burden, and the benefit of molecular testing after antimicrobial administration is potentially less than for other microorganisms. The sensitivity of nucleic acid amplification tests is less than that of culture, particularly on blood specimens. The most widely used test is an in-house assay targeting the type III secretion system TTS1-orf2 locus⁴⁰. Nucleic acid amplification may also be used by diagnostic and reference laboratories to confirm identification of *B. pseudomallei* and differentiate it from other *Burkholderia* species²⁷.

The BioThreat panel run on the BioFire FilmArray can detect *Burkholderia mallei/pseudomallei* but this test is for use on liquid, powder and surface swabs, and is not validated for use on clinical samples. Importantly, the BioThreat panel comes with a caveat that it may be cross-reactive with other *Burkholderia* species. Further testing would therefore be needed to determine the significance of a positive result.

3.2.1. Suitable specimen types

Suitable clinical specimens depend on the clinical presentation and infective foci, and may include sputum, urine, pus, tissue, and body fluids.

3.2.2. Specimen collection and handling

Specimens should be kept cool and transported to the laboratory as soon as possible after collection.

3.2.3. Test sensitivity

Sensitivity varies by the specimen type and the molecular targets chosen. The TTS1-orf2 assay has high sensitivity on pus and sputum, and lower sensitivity on blood specimens^{41,42}.

3.2.4. Test specificity

Specificity varies by the molecular targets chosen, but has been reported to be 100% on the TTS1-orf2 assay⁴⁰.

3.2.5. Predictive values

A negative nucleic acid amplification test does not preclude melioidosis.

3.2.6. Suitable test acceptance criteria

Detection of in-house targets should be accompanied by acceptable performance of all negative and positive controls included in the assay. Commercial assays should be interpreted according to manufacturer's instructions.

3.2.7. Suitable test validation criteria

Nucleic acid amplification methods used for *B. pseudomallei* should undergo verification or validation using a well characterised, geographically diverse collection of isolates including near neighbour species as well as known positive clinical samples.

3.2.8. Suitable internal controls

Testing on direct clinical samples should ideally include an extraction and an amplification control to rule out inadequate DNA concentration or inhibition.

3.2.9. Suitable external quality assurance program and proficiency testing

The RCPA Biosecurity external quality assurance program includes *B. pseudomallei*.

3.3. Serology

Serology has limited utility in the diagnosis of acute melioidosis due to poor performance characteristics. Its main role is as an adjunct to diagnosis in patients with infection at a site difficult to sample, such as neurologic melioidosis where there is meningoencephalitis or brain abscess. The indirect haemagglutination assay (IHA) is the most widely used assay. This test is not well standardised between laboratories due to reliance on sheep red cells sensitised to local *B. pseudomallei* strains, and has poor sensitivity early in infection, and

poor specificity in endemic areas to high levels of background seropositivity⁴³. Newer enzyme-linked immunoassays for detection of antibodies against haemolysin coregulated protein 1 (Hcp1) and O polysaccharide have demonstrated better performance but are not widely available⁴⁴. Diagnostic criteria, action thresholds and interpretation of results depend on the local epidemiology of melioidosis.

3.3.1. Suitable specimen types

Serum

3.3.2. Specimen collection and handling

Specimens should be kept cool and transported to the laboratory as soon as possible after collection.

3.3.3. Test sensitivity

Sensitivity varies by the assay used.

3.3.4. Test specificity

Specificity varies by the assay used.

3.3.5. Predictive values

Serology has limited utility in the diagnosis of acute melioidosis, particularly in endemic areas due to high background antibody positivity. False negative results are common in early disease.

3.3.6. Suitable test acceptance criteria

There should be acceptable performance of all negative and positive controls included in the assay.

3.3.7. Suitable test validation criteria

In house methods used for *B. pseudomallei* serology should undergo verification or validation using known positive clinical samples.

3.3.8. Suitable internal controls

Positive and negative controls should be included.

3.3.9. Suitable external quality assurance program and proficiency testing

Serology for melioidosis is conducted by few laboratories, and a specimen exchange arrangement may be needed for proficiency testing.

4 Laboratory nomenclature for national data dictionary

SNOMED CT code	Concept name	Description
428111003	Melioidosis	Disease
116399000	B. pseudomallei	bacteria

5 References

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6 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

RFLP – Restriction fragment length polymorphism

RNA – Ribonucleic acid

RT – Reverse transcriptase

RT-PCR – Reverse transcription polymerase chain reaction

SBT – Sequence based typing

Serotype – Pathogens of the same species that are antigenically different

SNT – Serum neutralisation

SSBA – Security sensitive biological agent

STI – Sexually transmitted infection

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

TGA – Therapeutic Goods Administration

UTM – Universal transport medium

VTM – Viral transport media

WGS – Whole genome sequencing

WHO – World Health Organization

WHO CC – WHO Collaborating Centre

XDR – Extensively drug resistant