



PHLN

Public Health Laboratory Network

# Melioidosis (*Burkholderia pseudomallei*)

## Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *Burkholderia pseudomallei*.

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### Nomenclature:

Melioidosis is sometimes referred to as Whitmore's disease or the 'Vietnam timebomb'.

*Burkholderia pseudomallei* was previously called *Pseudomonas pseudomallei*. Before that the species was known in reverse chronological order as *Pfieferella whitmori*, *Bacillus whitmori* and *Bacillus pseudomallei*.

## 1. Introduction

The genus *Burkholderia* contains a number of distinct species; however, only three members of the genus are regarded as pathogenic to humans, namely *Burkholderia mallei*, *Burkholderia pseudomallei* and the *Burkholderia cepacia* genomocomplex. This case definition specifically deals with melioidosis, the infection caused by *B. pseudomallei*. The other two species will not be discussed further.

*B. pseudomallei* has come to attention over the last decade as an emerging pathogen of tropical and subtropical regions, with diagnosis of cases in an increasing list of countries between 20° north and 20° south of the Equator. *B. pseudomallei* is a risk mainly to people who have significant outdoor soil or water exposure and a history of underlying chronic disease (particularly diabetes, chronic renal failure or alcohol dependency). Given the ubiquitous distribution of this organism in soil and surface water in endemic areas and the lack of a human vaccine, targeted education of these risk groups has been used as a preventive measure. The recognition that potable water supplies can become a potential source of *B. pseudomallei* infection, diagnosis of acute cases in more temperate parts of Australia, and the recent listing by CDC of *B. pseudomallei* as a possible biological weapon have made

melioidosis a possible risk to a much larger proportion of the Australian population than suggested by the location and number of acute cases diagnosed annually.

*B. pseudomallei* infection takes a variety of forms, none of which are easily identified from clinical features alone. These include acute septicaemia with or without pneumonia, and localised infection in almost any soft tissue location. Of particular note is occult *B. pseudomallei* prostatitis, which may prove difficult to diagnose. Infection can relapse after apparently adequate initial therapy and does so in around 25% cases. Infection may also present with a delayed onset up to three decades after the original exposure. The differential diagnosis is wide and in the relevant clinical setting includes Gram negative septicaemia, tuberculosis and community-acquired *Acinetobacter* pneumonia. Person-to-person transmission has been recognised only very rarely. There have been several well documented instances of laboratory acquired infection, resulting from exposure to aerosols generated during unprotected procedures. Infection is believed to occur mainly due to dermal inoculation of contaminated soil or surface water. However, inhalation is likely to be an alternative route of infection, from experience of melioidosis during the Vietnam conflict, and ingestion has not been completely ruled out.

## 2. Laboratory diagnosis/tests

### 2.1 Culture for *B. pseudomallei*

*B. pseudomallei* is a non-fastidious species, able to grow on minimal media supplemented with any of a wide range of carbon and nitrogen sources. Non-selective diagnostic laboratory media such as blood agar are sufficient for isolation of *B. pseudomallei* from blood and other sterile fluids. However, selective media such as Ashdown's selective agar (ASA) are required to isolate *B. pseudomallei* from non-sterile sites.

#### 2.1.1 Media

*B. pseudomallei* can be isolated from blood, tissue specimens and exudate from non-sterile sites by inoculation of clinical specimen material directly onto blood agar or by subcultures from blood culture bottles. ASA (Ashdown's) agar should be used for culture of clinical specimens from non-sterile sites. The numbers of *B. pseudomallei* colony-forming units are often very low in tissue and exudate specimens. *B. pseudomallei* is thus easily lost among heavy growth of commensal bacterial flora. In addition, when a potential environmental source has been identified it may be necessary to attempt culture of soil or surface water specimens. ASA inhibits the growth of some *B. pseudomallei* strains from environmental specimens and should be incubated for at least 24 hours longer than for clinical specimens. *B. cepacia* selective agar (Oxoid CM0995) is used in some centres as an alternative selective media for *B. pseudomallei*, though it will obviously not inhibit strains of *B. cepacia* that may be difficult to distinguish from *B. pseudomallei*. A novel selective medium (BPSA) has been specifically designed to recover *B. pseudomallei* and distinguish it from environmental Gram negative species such as *B. cepacia* and *Pseudomonas aeruginosa*. BPSA does not inhibit mucoid variant strains of *B. pseudomallei* to the same extent as ASA but the results of clinical evaluation studies have yet to be published. 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.

#### 2.1.2 Suitable specimens

Blood

Blood samples should be inoculated immediately into blood culture bottles. These may be stored at room temperature during transport to the laboratory and subsequently incubated at 37°C.

Abscess/inflammatory exudate

Swabs should be placed in an appropriate transport medium and transported to the laboratory ASAP.

Sputum

Sputum should be collected into an appropriate sterile container, transported to the laboratory ASAP (if longer than 2 hours, keep cool but do not refrigerate). Avoid prolonged cold storage.

Autopsy specimens

Specimens collected during post mortem examination are liable to be contaminated by resident commensal flora. These should be conveyed to the laboratory ASAP and inoculated promptly to avoid overgrowth of contaminants.

### 2.1.3 Test sensitivity

No quantitative data available.

Depends on the quality and type of the specimen, the type of media chosen, and whether enrichment is used prior to direct plating. Prior antibiotic treatment or cold storage of specimens after collection may inhibit the growth of *B. pseudomallei*.

### 2.1.4 Test specificity

Up to 20% *B. pseudomallei* strains can be incorrectly identified as another bacterial species when identification relies on biochemical (substrate utilisation) means. The isolation of a *B. pseudomallei*-like organism, even from a normally sterile site, should be confirmed by supplementary tests such as nucleic acid amplification or agglutination of specific antisera. The isolation of *B. pseudomallei* is notifiable in all parts of tropical Australia.

### 2.1.5 Predictive values

A negative culture does not exclude the diagnosis of melioidosis.

### 2.1.6 Suitable acceptance criteria

On blood agar after several days incubation, bacterial colonies often take on a wrinkled, honeycomb appearance. A proportion of strains do not exhibit this property. *Pseudomonas stutzeri* and occasional *B. cepacia* strains have a similar wrinkling appearance on agar. Colonies produce a positive oxidase test and are almost universally resistant to Gentamicin and Colistin.

### 2.1.7 Suitable internal controls

Properly documented, relevant, internal quality control program for each type and batch of selective medium (ASA) used.

### 2.1.8 Suitable test validation criteria

Culture-based isolation of *B. pseudomallei*, when its identity has been confirmed by a genotypic or similarly discriminating method is the best available standard. No single method is sufficiently reliable to be regarded as a 'gold standard'.

## 2.1.9 Suitable external QC programme

*B. pseudomallei* culture does not form part of the RCPA quality control programme.

## 2.1.10 Special considerations

It is important that laboratories correctly identify the species present in a specimen to avoid false positive and false negative aetiological diagnoses. In some locations up to 98% clinical isolates can be identified easily using standard clinical laboratory methods. In other locations, strain diversity and particularly the presence of mucoid variants may cause a lower recovery rate via selective media (ASA) and erroneous results when relying on substrate utilisation panels for definitive identification. *B. pseudomallei* can be differentiated from *B. cepacia* by supplementary test such as nucleic acid amplification or gas-liquid chromatography for bacterial cell wall fatty acid methyl esters. Both these methods are known to generate equivocal results that do not distinguish some clinical *B. pseudomallei* strains from *B. cepacia*.

## 2.2 Identification of *B. pseudomallei*

There are two levels of action in the identification of *B. pseudomallei*:

A. Diagnostic laboratories – Most diagnostic laboratories should be capable of presumptive identification of *B. pseudomallei*.

B. Reference laboratories – Isolates, particularly where a cluster of cases is involved, should be forwarded to a State reference laboratory or clinical laboratory with special interest in *Burkholderia* spp. if confirmation by supplementary tests, molecular sub-typing or comparison with related environmental isolates is required.

### 2.2.1 Conventional biochemical tests

Colony appearance on solid media, Gram stain appearance, oxidase reaction and antibiotic susceptibility are the key to preliminary recognition of *B. pseudomallei*. Commercially available substrate utilisation panels are prone to variable, subjective end-points and often rely on data from a small and possibly unrepresentative library of reference *B. pseudomallei* strains. In some centres up to 98% isolates of *B. pseudomallei* can be successfully identified by substrate utilisation panels. Cultures inoculated into API and Vitek systems should be at least 24 hours old for optimal results.

#### 2.2.1.1 Predictive values

A negative result in one substrate utilisation test does not completely exclude *B. pseudomallei*. Further testing or more prolonged incubation of the test may be necessary to confirm the identification.

#### 2.2.1.2 Suitable test criteria

An isolate that exhibits biochemical characteristics consistent with documented reactions for *B. pseudomallei*. Expected results for *B. pseudomallei* are Gram negative bacilli with polar staining, 'safety pin' appearance, oxidase, gelatinase and ADH positive, Gentamicin and Colistin resistant but Amoxicillin-Clavulanic acid sensitive. The wrinkled appearance of colonies growing on solid media is not universal, and heavy growth produces a characteristic sheen when growing on blood agar.

### 2.2.1.3 Suitable internal controls

Each batch of biochemical substrate tested with positive and negative control strains. Results of all testing recorded and the records maintained.

### 2.2.1.4 Suitable validation criteria

Correct biochemical reactions exhibited by a standard *B. pseudomallei* strain.

### 2.2.1.5 External QC program

A suitable programme does not exist for safety reasons.

## 2.2.2 Kits/automated systems for biochemical identification

Various substrate utilisation kits exist for the identification of *B. pseudomallei*. Of the numerous kits available in Australia, the API 20E and 20NE Listeria and the Microbact 24 are commonly used.

MicroScan (Baxter, Baxter Healthcare Corporation, West Sacramento, USA), Vitek (BioMerieux, Marcy- l'Etoile, France), MicroStation (Biolog, Heyward, USA) and Cobas (Becton Dickinson Instrument Systems, Sparks, USA) are the automated systems in use. All automated systems may be subject to the same *B. pseudomallei* identification problems that apply to manual substrate utilisation panels. API and Vitek inoculations should be made with cultures that are at least 24 hours old for optimal results.

### 2.2.2.1 Special considerations

For accurate results it is imperative to follow the manufacturer's recommended procedure exactly.

## 2.2.3 Molecular identification

Probes have been developed for the detection of *B. pseudomallei*, particularly for detection in clinical samples by nucleic acid amplification. Molecular probes are only in use in some reference laboratories and have not been made commercially available for use by clinical laboratories. They do not offer a significant improvement in sensitivity over culture-based systems. Nucleic acid amplification is used in many reference centres to confirm identification of *Burkholderia* species and can be used for *B. pseudomallei*. Some clinical strains cannot be easily distinguished from *B. cepacia* by NAA tests.

## 2.3 Molecular subtyping

Subtyping for epidemiological purposes is available in only a small number of reference laboratories that can perform DNA macrorestriction analysis (by pulsed-field gel electrophoresis, or PFGE). Rapid genotyping methods such as automated ribotyping have been successfully applied to *B. pseudomallei* and approach the level of discrimination achieved with PFGE.

## 2.4 Serodiagnosis

A variety of serodiagnostic methods are available. These include indirect haemagglutination and ELISA. Diagnostic criteria, action thresholds and interpretation of results depend on the local epidemiology of melioidosis. In endemic areas, there may be a high incidence of seropositivity in an

asymptomatic population. Reagents may also be subject to batch-to-batch variation. A positive serology result meeting local interpretive thresholds for recent infection only indicates a presumptive diagnosis.

#### 2.4.1.1 Predictive values

A negative result in a single antibody test does not exclude a diagnosis of melioidosis. Repeat testing at a later date may be required to detect a late onset seroconversion. A positive result does not necessarily confirm a diagnosis of acute *B. pseudomallei* infection and may reflect prior exposure at an unspecified date.

#### 2.2.1.2 Suitable test criteria

A serum specimen that exhibits a positive reaction by haemagglutination or ELISA.

#### 2.2.1.3 Suitable internal controls

Each batch of tests should be tested with a suitable positive and negative control. Results of all testing recorded and the records maintained.

#### 2.2.1.4 Suitable validation criteria

Correct serological reactions exhibited by a standard positive serum strain.

#### 2.2.1.5 External QC program

RCPA Quality Assurance Programs Pty. Ltd.

## 3. References

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