

# Recent Developments in the Laboratory Diagnosis of Melioidosis

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## Abstract

Melioidosis, an infection caused by *Burkholderia pseudomallei*, is endemic in Southeast Asia and Northern Australia. Septicemic melioidosis is a leading cause of death among community-acquired septicemia in Northeastern part of Thailand. More than half of these patients die within the first 2 days after hospital admission, before the bacteria can be identified. A major factor that contributes to the high mortality is a delay in the isolation and identification of the causative organism. Laboratory investigations are important to provide a definite diagnosis of melioidosis because the clinical manifestations of melioidosis can not be differentiated from septicemia caused by other organisms. Recent developments in various aspects of laboratory diagnosis of melioidosis, including bacterial isolation by culture, detection of bacterial antigens, detection of antibodies to the bacteria, and detection of bacterial genetic materials, have exhibited potentials for a significant improvement that may lead to rapid diagnosis of melioidosis, especially for septicemic melioidosis patients whom the early diagnosis is critical. The comparison of the performance of these new tests and their critical evaluation in clinical situation will provide the proper selection of more accurate and rapid diagnostic methods for this disease in the near future. (*J Infect Dis Antimicrob Agents* 1995;13:77-80.)

## INTRODUCTION

Melioidosis, an infection caused by *Burkholderia pseudomallei*, remains to be an important health problem in Southeast Asia both in terms of diagnosis and treatment. Northeastern part of Thailand is an endemic area of the disease with an estimated number of cases of 2,000-5,000 patients per year. The clinical spectra of the disease vary from acute sepsis to chronic localized infection. The mortality of septicemic melioidosis is very high, ranges from 40-80 percent. The bacteria are resistant to most commonly used antibiotics. Early diagnosis and appropriate antibiotics treatment are very critical for reducing the mortality. Laboratory investigations are important to provide a definite diagnosis of melioidosis because the clinical manifestations of melioidosis can not be differentiated from septicemia caused by other organisms. The scope of this article is to review recent developments on the laboratory diagnosis of melioidosis. Laboratory diagnosis of melioidosis can

be categorized into 4 groups, (i) bacterial isolation by culture, (ii) detection of bacterial antigens, (iii) detection of antibodies to the bacteria, and (iv) detection of bacterial genetic materials.

## Bacterial isolation

Isolation of *B. pseudomallei* by culture is the main method for identification of the bacteria. This method is reliable, sensitive and economical. However, it generally takes 3-5 days to obtain the results. Approximately one-third of the septicemic melioidosis patients expire before the bacterial culture result becomes available. Two developments that provide significant improvement in culture methods include the development of selective culture media and the use of automated culture systems. *B. pseudomallei* grows easily in common bacterial culture media but tend to be overgrown by other organisms. Ashdown described the use of selective agar to reduce the growth of other organisms. The

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traditional Ashdown media for isolation of *B. pseudomallei* is composed of trypticase soy broth incorporating glycerol, crystal violet, neutral red and gentamicin (1). Preculture in broth such as modified Ashdown's selective media incorporating colistin into trypticase soy broth with glycerol and crystal violet or threonine-basal salt solution containing colistin prior to plating onto Ashdown agar further increased the isolation efficiency of this organism from clinical specimens (2,3). This method is useful for isolating the bacteria in specimens contaminated with other organisms such as sputum, urine and soil, but is not necessary for blood culture.

Several methods for reducing the culture time have also been introduced. Early subculture onto plate after 24-48 hours of incubation, even before the culture media are turbid, reduces the culture time. This method is however labor-intensive, and is therefore not cost-effective in the area that melioidosis are not hyperendemic. Automation in bacterial culture work has been introduced recently and a few bacteriology laboratories in the endemic area of melioidosis have been using this system of isolation of *B. pseudomallei* as well. An automated culture system is based on the colorimetric detection of carbon dioxide concentration by means of a sensor internally attached to the bottom of the blood culture bottles. The sensor is monitored regularly every 10 minutes by a reflectometer (4). Positive cultures are recognized by a computer-driven algorithm that monitor initial and increased carbon dioxide concentration as an indicator of bacterial growth before the culture is turbid as seen by naked eyes. Preliminary studies at Khonkaen Regional Hospital in Northeastern Thailand showed that the growth of *B. pseudomallei* in blood culture specimens using an automated BacT/Alert culture system can be detected within less than 48 hours, of which more than half are detectable within the first 24 hours (Tiangpitayakorn, personal communications). The culture can then be proceeded for bacterial identification, biochemical characterization and antibiotics sensitivity tests which require at least another 24 hours before the species of the bacteria can be identified.

### Detection of bacterial antigens

Several methods of bacterial antigen detection have been developed. These include the detection of secreted bacterial antigens in blood and urine, as well as the detection of the whole bacteria in clinical specimens. The former includes the detection of *B. pseudomallei*

exotoxin (5), detection of *B. pseudomallei* antigens by polyclonal antibody (6), and more recently, the detection of *B. pseudomallei* antigen in urine (7). An enzyme-linked immunosorbent assay (ELISA) based on a specific monoclonal antibody to *B. pseudomallei* exotoxin was described. This assay was capable of detecting at least 16 ng/ml of exotoxin present in bacterial culture supernatant fluid (5). However, its application for detecting the exotoxin in clinical specimens was not reported. An avidin-biotin ELISA based on polyclonal antibody to crude *B. pseudomallei* antigens that was able to detect 3.9 ng/ml of the antigens was described, although the evaluation of its application in patients has also not been reported (6). The level of bacterial antigens in clinical specimens may be too low to be detectable by the above assays. An assay that was able to detect *B. pseudomallei* antigen in urine of melioidosis patients was recently described (7). This ELISA system was based on the use of FITC-conjugated polyclonal rabbit antisera to *B. pseudomallei* and amplified by peroxidase conjugated anti-FITC monoclonal antibody. The bacterial antigens recognized by this assay were mainly lipopolysaccharide (LPS) and probably included other heat stable antigens. The detection limit of this assay was 12.2 ng/ml of purified LPS. This system was able to detect the antigens in unconcentrated urine of 96 and 80 percent of septicemic and localized melioidosis cases, respectively. False positive rate was low and was found mainly in patients with significant gram-negative bacteriuria. Comparison of this test with the latex agglutination indicated that it was much better for detecting *B. pseudomallei* antigen in urine (8). The performance of this test appears to be superior to other antigen detection systems tested to date. Further evaluation of this test in large scale clinical settings should be carried out.

In addition, the presence of *B. pseudomallei* whole bacteria in clinical specimens can be detected either by immunofluorescence using polyclonal antibodies to *B. pseudomallei* (9), or by agglutination with latex beads coated with anti-*B. pseudomallei* antibody (10). These assays enabled rapid identification of the bacteria from pus and sputum without culture. They may also be used in combination with bacterial culture systems to rapidly identify the bacteria after short term culture.

### Detection of specific antibody

Indirect hemagglutination assay (IHA) is the first serodiagnostic method of melioidosis developed and

is still widely used. This semi-quantitative assay measures the levels of antibody that can agglutinate *B. pseudomallei* crude antigens coated onto the surface of erythrocytes, and is relatively specific for melioidosis. *B. pseudomallei* LPS was shown to be the major antigenic composition in the crude antigen preparation in IHA test. The most important drawback of this assay is its limited use in clinical situations in endemic areas due to the presence of background antibody in a large percentage of healthy population. The increase in cut-off titre level to avoid false-positive results resulted in the decrease in the sensitivity of the test (11). In addition, it was found that several patients with fatal septicemic melioidosis had the IHA antibody titre below cut-off level (12).

Several more recent ELISA systems for detection of specific IgG antibody have been developed using various preparations of purified antigenic components of the bacteria as antigens. These antigens include a purified bacterial endotoxin (13), a 19.5 kDa antigen (14), and an antigen prepared by affinity purification using *B. pseudomallei* specific monoclonal antibody (14,15). Antibody detection systems using these antigen preparations were shown to be highly specific and sensitive in comparison to IHA test. Using limited numbers of specimens, the specificity and sensitivity of these assays are generally above 90 percent. However, the comparisons of the performance among these assays and their critical evaluations in clinical setting in endemic area are still lacking. In addition, the detection of specific IgM antibody have been developed including an IgM immunogold and ELISA methods using crude bacterial antigens (16,17). The assays were shown to be specific and sensitive. However, none of these assays are currently in use in diagnostic laboratories. More recently, an ELISA system for detecting IgM antibody to immunoaffinity-purified antigen (Dharakul, unpublished data) was also developed. The clinical usefulness of these IgM assays has yet to be evaluated.

### Detection of bacterial genetic materials

Recent studies showed that specific *B. pseudomallei* genetic material could be detected, either by nucleic acid hybridization or by amplification of specific fragments of the bacterial genome. Specific DNA probe for *B. pseudomallei* was developed and used to hybridize bacterial DNA in artificially inoculated clinical specimens. The sensitivity of the hybridization method was relatively poor, and cannot be used for detecting bacterial DNA

in clinical specimens (18). Recent publications on PCR amplification of *B. pseudomallei* showed more promising results. The PCR primers were designed from the regions encoding 23S ribosomal RNA (19), 16S rRNA (20), and the junction between 16S-23S rRNA genes (21). These assays could differentiate *B. pseudomallei* from other pathogens, with an exception of *B. mallei* which is genetically identical to *B. pseudomallei* at the region used for amplification. Amplified DNA can be detected by hybridization and enzymatic reaction or by staining with ethidium bromide. Preliminary data showed that a nested PCR system may be needed for detecting the bacterial DNA directly from patient blood. The nested PCR assay for amplifying 16S rRNA gene could detect bacterial genetic material equivalent to 2 bacteria/specimen tested, and was able to detect the bacteria in blood from the majority of septicemic melioidosis patients, as sensitive as conventional blood culture method (20).

In summary, recent developments in various aspects of laboratory diagnosis of melioidosis have exhibited potentials for significant improvement that lead to the rapid diagnosis of melioidosis, especially for septicemic melioidosis patients, where the diagnosis is most needed. The comparison of the performance of these tests and their critical evaluation in real clinical situation will provide better diagnostic methods for this disease in the near future.

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