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# Short Report: Rapid Detection of *Burkholderia pseudomallei* in Blood Cultures Using a Monoclonal Antibody-Based Immunofluorescent Assay

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*Abstract.* Melioidosis is a severe bacterial infection caused by *Burkholderia pseudomallei*. Rapid antimicrobial therapy is necessary to improve patient outcome, which is aided by direct detection of *B. pseudomallei* in clinical samples. A drawback for all antigen assays is that the number of *B. pseudomallei* in blood usually falls below the achievable level of detection. We performed a prospective cohort study of 461 patients with 541 blood cultures to evaluate the utility of a pre-incubation step prior to detection of *B. pseudomallei* using a monoclonal antibody-based immunofluorescent assay (Mab-IFA). The Mab-IFA was positive in 74 of 76 patients with melioidosis (sensitivity = 97.4%), and negative in 385 patients who did not have blood cultures containing *B. pseudomallei* (specificity = 100%). The Mab-IFA could be a valuable supplementary tool for rapid detection. We recommend the use of the Mab-IFA to test blood cultures that flag positive in regions where melioidosis is endemic.

Melioidosis is a severe bacterial infection caused by the Gram-negative bacterium *Burkholderia pseudomallei*. Most documented cases occur in Thailand and northern Australia, although cases are increasingly reported from other parts of the world.[1] In northeast Thailand, the annual incidence of melioidosis is 21.3 per 100,000 people, and the crude mortality rate is 42.6%.[2] Rapid instigation of appropriate parenteral antimicrobial therapy (ceftazidime or a carbapenem drug) is critical to efforts to improve outcome from melioidosis, which is aided by direct detection of *B. pseudomallei* in clinical samples before the more lengthy process of culture and confirmatory identification. Methods that achieve this detection include polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), and antibody-based immunofluorescence (IFA), which can be applied to a range of sample types, including sputum, pus, urine, respiratory secretion, and body fluids.[3–6] Around one-half of patients with melioidosis have positive blood cultures,[7] and an important drawback for all antigen assays is that the median *B. pseudomallei* count in blood is 1 cfu/mL,[8] which is far below the achievable level of detection by the IFA. Pre-incubation of blood cultures would be predicted to increase the sensitivity of IFA, and despite this extra step would still reduce the time to diagnosis compared with conventional culture and biochemical identification. This is particularly important for patients with a positive blood culture and no other positive samples. We undertook a clinical evaluation of this idea using a monoclonal antibody-based (Mab) IFA.

A prospective cohort study was performed at Sappasithiprasong Hospital, Ubon Ratchathani, northeast Thailand between May and September of 2012. Ethical approval was obtained from the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University. Patients were included if they had at least one blood culture taken by hospital staff. Routine practice is to take 10 mL of blood and inoculate this into a 30-mL BACTEC Plus Aerobic/F bottle (BD, Sparks, MD), which is incubated in an automated BD BACTEC FX (BD, Sparks, MD) instrument for 7 days. Bottles that flagged positive were cultured, and bacterial isolates were identified using standard laboratory methodology.[9] All positive culture bottles were tested using the Mab-IFA within 2 hours of flagging positive. The monoclonal antibody used is specific to exopolysaccharide of *B. pseudomallei*.[6] The Mab-IFA reagent consisting of primary detection Mab 4B11 and Alexa Fluor 488-conjugated goat anti-mouse immunoglobulin G (IgG) secondary antibody (Molecular Probes, Carlsbad, CA) was prepared as described previously.[6] Ten microliters of working IFA reagent was mixed with an equal volume of blood culture on a glass slide, a coverslip applied; and left at room temperature for 5 minutes. Bacteria were observed using a fluorescent microscope (Olympus, Tokyo, Japan) at 1,000× magnification.

In total, 461 patients with 541 blood cultures were recruited. Of these patients, 76 patients had 91 samples that were culture-positive for *B. pseudomallei*, and 385 patients had 450 samples that were culture-negative for *B. pseudomallei*. The time interval between taking the blood culture and the sample flagging as positive and being examined by the IFA ranged from 1 to 3 days (median = 1 day, interquartile range = 1–2 days). The IFA was positive in 74 of 76 patients with melioidosis (diagnostic sensitivity = 97.4%), and negative in 385 patients who did not have blood cultures containing *B. pseudomallei* (diagnostic specificity = 100%). The positive predictive value was 100% (74 of 74 patients), and the negative predictive value was 99.5% (385 of 387 patients); 34 of 76 melioidosis patients (44.73%) had no other samples that were positive for *B. pseudomallei*, and the Mab-IFA was positive in all 34 of these samples (100%). The two false-negative IFA results occurred in patients who had blood cultures taken on admission that flagged as positive 2 days later. The first patient responded to ceftazidime treatment and was discharged alive, and the second patient died before the culture flagged as positive.

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In total, 450 blood culture samples from non-melioidosis cases were culture-positive for one or more of the following organisms: 215 Gram-positive bacteria (*Bacillus* spp. [10], *Enterococcus* spp. [7], coagulase-negative staphylococci [112], *Staphylococcus aureus* [56], *Streptococcus* group A [7], *Streptococcus* group B [4], *Streptococcus* group D [11], other *Streptococcus* spp. [5], *S. pneumoniae* [2], and *S. viridans* [1]); 207 Gram-negative bacteria (*Acinetobacter* spp. [41], *B. cepacia* [1], *Chromobacterium violaceum* [1], *Citrobacter* spp. [1], *C. diversus* [2], Diptheroids [22], *Enterobacter* spp. [1], *E. aerogenes* [3], *E. cloacae* [9], *Escherichia coli* [57], *Haemophilus influenzae* [2], *Klebsiella pneumoniae* [21], *Proteus mirabilis* [1], *Pseudomonas* spp. [27], *P. aeruginosa* [8], *Salmonella* serogroup B [2], *Salmonella* serogroup C [4], and *Salmonella* serogroup D [4]); and 44 fungi (*Candida* spp. [8], *C. albicans* [7], *Cryptococcus neoformans* [13], and *Penicillium marneffei* [16]).

Detection of *B. pseudomallei* in blood cultures only after they have flagged as positive in an automated system is less optimal than direct detection in the clinical sample, but it provides a presumptive diagnosis 24–48 hours earlier than would be achieved using conventional culture and bacterial identification. We recommend the use of the Mab-IFA to test blood cultures that flag positive in those regions of the world where melioidosis is endemic. This use will increase reagent and labor costs, but one strategy to minimize costs is to limit testing to those samples that are positive for Gram-negative rods based on conventional Gram stain. The Mab-IFA could be a valuable supplementary tool for the rapid detection of *B. pseudomallei* infections, allowing earlier initiation of ceftazidime or imipenem therapy in patients with melioidosis.

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