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Use of Protein-Specific Monoclonal Antibody-Based Latex Agglutination for Rapid Diagnosis of *Burkholderia pseudomallei* Infection in Patients with Community-Acquired Septicemia[▽]

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A latex agglutination test employing monoclonal antibody specific to a 30-kDa protein of *Burkholderia pseudomallei* was used to detect the organisms in blood culture specimens from 1,139 patients with community-acquired septicemia. The sensitivity, specificity, and positive and negative predictive values of the test were 96.75%, 99.61%, 96.75%, and 99.61%, respectively.

Melioidosis is an infection caused by *Burkholderia pseudomallei*, a gram-negative organism. Southeast Asian countries and the northern part of Australia appear to be areas where this disease is endemic (4). In the northeastern part of Thailand, the disease is an important source of community-acquired septicemia, and the prevalence is around 30% (2). The clinical manifestations of this disease cannot be differentiated from septicemia caused by other organisms. Fifty percent of patients with acute septicemic melioidosis die within 48 h after admission (14). To reduce the mortality rate, early diagnosis and appropriate antibiotic treatment are required (19). Current laboratory diagnosis of melioidosis depends on bacterial culture (3, 18), although molecular techniques for this purpose have been reported (6, 9, 10). However, bacterial culture is a time-consuming method. To shorten the diagnostic time, we previously developed a monoclonal antibody-based latex agglutination (LA) test for the identification of *B. pseudomallei* and evaluated it by testing blood culture fluid taken from an automated culture system in a major hospital in the northeastern region of Thailand (8). The monoclonal antibody used was specific for a 30-kDa protein antigen of *B. pseudomallei*, which in our previous study was found only in Ara$^-$ clinical isolates but not in other gram-negative bacteria, including *Burkholderia thailandensis*, which is often found in soil and water. In this study, we applied the test in the nonselected situation by testing a total of 1,139 blood culture specimens collected from patients clinically suspected of having community-acquired septicemia. These patients had been admitted to 11 hospitals, at regional and provincial levels, located throughout the area in the northeastern region of Thailand where melioidosis is endemic, during the rainy season, when melioidosis usually occurs (12). The blood sample was cultured by either a manual or automated (BacT/Alert) culture system. The blood culture fluid was taken when it was turbid or when the machine alerted by alarm, and some samples were subjected directly to the LA test. The sample was Gram stained, and an aliquot of 0.5 ml was subcultured in 2 ml of brain heart infusion broth (BHIB) and incubated at 37°C for 3 h. After incubation, it was subjected to antibiotic sensitivity tests, biochemical tests for species identification, and the LA test. Blood culture samples that showed no bacterial or fungal growth after incubation for 7 days were subjected directly to the LA test, subcultured in BHIB, and then subjected to the LA test.

Of 1,139 specimens tested, 123 were positive for *B. pseudomallei*, 849 were positive for other bacteria as identified by conventional biochemical tests, and the rest were negative. The LA test gave positive results for 119/123 specimens with *B. pseudomallei* and 4/849 specimens with positive results for other bacteria, including group A streptococci, *Enterobacter cloacae*, *Staphylococcus aureus*, and mixed growth of *Staphylococcus epidermidis* and *Streptococcus faecalis*. The false-positive results might have been due to the cross-reaction of the monoclonal antibody with some gram-positive bacteria. A similar situation was reported as being due to the cross-reaction of the monoclonal antibody with group A streptococci and *Bacillus* spp. (8). The false-negative results for four specimens that were positive for *B. pseudomallei* may have been because the LA test is not sensitive enough to detect the organisms in these specimens. The LA test showed negative results for all of the 167 specimens with no growth of bacteria. Thus, by using hemoculture and biochemical tests as the "gold standard," the sensitivity, specificity, and positive and negative predictive values of the LA test were 96.75%, 99.61%, 96.75%, and 99.61%, respectively. The test performance observed in this study confirmed the value of the test in our previous report.

We also used the LA test to identify the organisms directly in 309 blood culture specimens, which were obtained from two regional hospitals. Positive results with the LA test were found for 20/21 blood culture specimens positive for *B. pseudomallei*, 1/273 of those positive for other organisms, and 0/15 specimens with no bacterial growth. The one specimen with a false-positive LA test, compared to conventional biochemical tests,

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yielded growth of group A streptococci. The LA test used directly with blood culture specimens showed 95.24% sensitivity, 99.65% specificity, a 95.24% positive predictive value, and a 99.65% negative predictive value, which were similar to those obtained from testing BHIB subcultures. Our previous study showed that the LA test gave higher specificity with BHIB subculture than when used with hemoculture directly (96.49% versus 85.96%). The discrepancy between the previous and the present study results could be due to the use of fresh specimens both blood culture and BHIB specimens, while frozen specimens were used in the previous study. In the frozen blood culture specimens which gave false-positive results, there were nonspecific materials, which could have been eradicated by centrifugation at low speed (8).

Another report of a large-scale study described the performance of a monoclonal antibody LA test employing monoclonal antibody specific to a 200-kDa protein antigen of *B. pseudomallei* (1). The test showed results that were similar to ours. The other methods that have been reported to be useful for the diagnosis of melioidosis, as they can distinguish *B. pseudomallei* from other species of *Burkholderia*, are those with a molecular approach based upon DNA hybridization and PCR (5, 7, 11, 13, 15, 16, 17, 20). However, such a test requires special equipment and well-trained personnel. Moreover, it has been noted that the results of PCR for the diagnosis of melioidosis in clinical settings have been rather disappointing compared with the classical culture method currently used as the gold standard (6).

In conclusion, the LA test employing monoclonal antibody specific for a 30-kDa protein antigen of *B. pseudomallei* was shown to work well in diagnostic laboratories in rural areas where there is minimal equipment and less-experienced laboratory personnel, with sensitivity, specificity, and positive and negative predictive values all approaching 100% for the diagnosis of community-acquired septicemic melioidosis. The test was able to distinguish *B. pseudomallei* from its nonvirulent counterpart and was able to detect the organisms early in the course of the disease with a rapid turnaround time. Thus, it appears to be very useful for the diagnosis of melioidosis, which is a potentially fatal disease and needs early diagnosis for appropriate antibiotic treatment (19).

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