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Rapid Immunofluorescence Microscopy for Diagnosis of Melioidosis

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An immunofluorescent (IF) method that detects *Burkholderia pseudomallei* in clinical specimens within 10 min was devised. The results of this rapid method and those of an existing IF method were prospectively compared with the culture results for 776 specimens from patients with suspected melioidosis. The sensitivities of both IF tests were 66%, and the specificities were 99.5 and 99.4%, respectively.

Melioidosis, the disease caused by *Burkholderia pseudomallei*, is endemic in Southeast Asia. Melioidosis accounts for approximately one-fifth of all community-acquired cases of septicemia in areas of endemicity, such as northeast Thailand, where it is associated with a mortality rate of about 50% (1, 5). Rapid diagnosis in rural Thailand is important, since the antibiotics empirically prescribed for the treatment of patients presenting with sepsis of unknown cause may not include the relatively expensive antibiotics, ceftazidime or a carbapenem, that are required for the treatment of melioidosis. Culture of *B. pseudomallei* from any specimen is diagnostic and represents the "gold standard," but a delay of 24 to 48 h or more between the time of specimen plating and bacterial growth plus presumptive identification often occurs. A simple, rapid test performed directly with clinical samples may influence the time taken to begin effective treatment and the subsequent outcome. We have previously described a direct immunofluorescent (IF) technique for the detection of *B. pseudomallei* in clinical samples (3) and have used this technique in our diagnostic and research laboratory in a provincial hospital for more than a decade. It is relatively labor intensive and takes more than 2 h to complete; here, we report on the results of a simplified method that can be completed in 10 min.

A prospective study was conducted between June 2002 and October 2004 by a study team at Sappasithiprasong Hospital, Ubon Ratchathani, northeast Thailand. Patients with suspected melioidosis were actively sought during twice-daily rounds of the medical and intensive care wards. Specimens for microbiological examination were taken from all patients and were cultured by standard procedures (4).

The methodology for preparation and storage of the IF conjugate was performed as described previously (2), except that bacteria were killed with formalin rather than heat. Two direct IF methods were applied in parallel to clinical samples of respiratory secretions, pus from sterile sites, urine, and blood culture fluid to detect *B. pseudomallei*. The standard direct IF technique was performed as described previously (3), except that 5% skim milk in phosphate-buffered saline was used as the blocking agent. The rapid method was a one-step technique in which 1 drop (10 μl) of specimen was mixed on a clean glass microscopic slide with an equal volume of conjugate and a coverslip applied. The white blood cells present in pus were lysed prior to examination by the addition of an equal volume of distilled water, and respiratory secretions were mixed with an equal volume of sterile distilled water before examination. Conjugate was used at a 1:200 dilution in blocking buffer. The slides were examined with a fluorescent microscope with a ×100 oil-immersion lens. A positive result by either test was recorded when the periphery of the bacilli showed a strong apple-green fluorescence. A slide known to be positive (for a clinical isolate of *B. pseudomallei*) was prepared and examined in each test run.

Testing was performed directly on 776 specimens (respiratory secretions, urine, or pus from sterile sites) from 646 patients with suspected melioidosis. *B. pseudomallei* was cultured from 154 samples taken from 120 patients. Of these, 108 samples (70%) from 84 patients were positive by either the standard or the rapid IF method (Table 1); 96 samples were positive by both IF methods, 6 were positive by the standard IF method only, and 6 were positive by the rapid IF method only. Of the 46 specimens that were not positive by either IF method, 9 (20%) grew viable colonies only after enrichment in a selective broth, compared to 2 of the 108 specimens positive by either method ($P < 0.001$). The sensitivity of the standard IF method was 66%, and that of the rapid IF method was also 66%.

Four of the 622 specimens (from 526 patients) that were culture negative for *B. pseudomallei* were positive by either IF method. Three were false positive by both methods, and an additional sample was false positive only by the standard IF method. Two of these were urine samples that grew *Pseudo-*

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TABLE 1. Standard and rapid IF techniques versus conventional microbial culture for the detection of *B. pseudomallei* in 772 clinical specimens from patients with suspected melioidosis

Specimen type	No. (%) of samples			
	Total	Culture positive for *B. pseudomallei*	Positive by standard IF	Positive by rapid IF
Pus	88	25	12 (48)	12 (48)
Respiratory secretion	304	87	56 (64)	56 (64)
Urine	384	42	34 (81)	34 (81)
Total	776	154	102 (66)	102 (66)

monas aeruginosa and *Acinetobacter* spp., respectively, on Ashdown's medium; and two were respiratory secretions that were negative on Ashdown's medium but that grew mixed respiratory flora on blood agar. The specificity of the standard IF method was 99.4%, and that of the rapid method was 99.5%.

The IF techniques were also evaluated for their abilities to presumptively identify *B. pseudomallei* from specimens in blood culture bottles. A total of 241 blood cultures were taken from 227 patients with suspected melioidosis. Five milliliters of blood was inoculated into aerobic BacT/Alert FA bottles (BioMérieux, Durham, N.C.), which were incubated for 7 days at 37°C. The bottles were inspected daily, and the contents subcultured onto blood agar if the indicator changed color, and routinely on days 1, 2, and 7. *B. pseudomallei* was isolated from 42 of 241 blood culture specimens. The other organisms isolated included *Escherichia coli* ($n = 34$), *Pseudomonas* spp. ($n = 31$), *Enterobacter* spp. ($n = 14$), *Acinetobacter* spp. ($n = 11$), *Klebsiella* spp. ($n = 11$), *Salmonella* spp. ($n = 9$), other gram-negative rods ($n = 6$), *Staphylococcus aureus* ($n = 22$), coagulase-negative staphylococci ($n = 24$), *Streptococcus* spp. ($n = 10$), other gram-positive cocci ($n = 2$), gram-positive rods ($n = 6$), and fungal organisms ($n = 19$). Positive bottles were simultaneously examined by the standard and rapid IF methods. All samples culture positive for *B. pseudomallei* were positive by both the standard and the rapid IF methods; there were no false-positive test results. Thus, the sensitivity and specificity of both the standard and the rapid IF methods were 100%.

There were a small number of discordant results between the two IF methods, but the use of both methods in parallel increased the sensitivity from only 66 to 70%. The overall sensitivity of the direct IF method reported previously (3) was 73%. The lower result here may reflect the effect of earlier empirical treatment for melioidosis or presentation for care earlier in the course of the illness, both of which result in lower bacterial loads and diagnostic yields. The small number of false-positive results may reflect the presence of nonviable organisms affected by prior antibiotic administration. We conclude that the rapid IF method has a sensitivity and a specificity equivalent to those of the previously reported standard IF method.

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