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Rapid Antigen Detection Assay for Identification of *Burkholderia* (*Pseudomonas*) *pseudomallei* Infection

A. ANUNTAGOOL,[1] P. INTACHOTE,[1] P. NAIGOWIT,[2] AND S. SIRISINHA[1,3]*

Laboratory of Immunology, Chulabhorn Research Institute,[1] and Department of Microbiology, Faculty of Science, Mahidol University,[3] Bangkok, and Department of Medical Science, National Institute of Health, Ministry of Public Health, Nonthaburi,[2] Thailand

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A simple antigen detection test was developed for rapid diagnosis of melioidosis caused by *Burkholderia* (*Pseudomonas*) *pseudomallei* infection. The method was based on the use of a specific monoclonal antibody in a sandwich enzyme-linked immunosorbent assay to capture antigen in clinical specimens culture positive for *B. pseudomallei*. The results showed the sensitivity and specificity of the test to be 75 and 98%, respectively.

Melioidosis is an important bacterial disease endemic in Southeast Asian countries and northern Australia. Clinical diagnosis remains a problem, as the spectrum varies widely from acute fatal septicemia to mild localized infections (3). Moreover, subclinical infection, as manifested by positive seroconversion, is relatively common in people in the area where the infection is endemic, a finding that complicates the evaluation of serological tests for antibody in these patients (6). Identification of the causative agent, *Burkholderia* (*Pseudomonas*) *pseudomallei*, by culture requires at least 2 to 5 days (11). More recently, methods for the detection of *B. pseudomallei* antigens have been described and evaluated (4, 10). However, these methods require either expensive equipment or reagents, thus making them difficult to set up in poorly equipped peripheral laboratories in the areas where the infection is endemic. Results of a more simple latex agglutination method (9) to detect *B. pseudomallei* antigens were not satisfactory with regard to sensitivity (18%). Therefore, in this communication we describe a reliable, simple, and rapid antigen detection method that can be readily carried out in any diagnostic laboratory in areas where the infection is endemic.

The method described herein was based on the use of a monoclonal antibody (MAb) specific for *B. pseudomallei* antigen produced and characterized earlier by our group (5). The supernatant fluid from a hybrid producing immunoglobulin M (IgM) antibody (5F8) was concentrated by ammonium sulfate precipitation, and the antibody was purified by gel filtration chromatography using Sephadex G-200. The MAb-based enzyme-linked immunosorbent assay (ELISA) developed for *B. pseudomallei* antigen detection was essentially the same as the one described for other systems (7). In the present protocol, the capture antibody was MAb 5F8 and the detection antibody was biotinylated polyclonal rabbit IgG anti-*B. pseudomallei*. The antibody-coated microtiter plate was blocked with skim milk instead of bovine serum albumin, and 3,3′,5,5′-tetramethyl benzidine was used as the chromogen for the detection of the streptavidin-horseradish peroxidase reaction. The enzymatic reaction was determined from an optical density value measured at 450 nm. All incubation conditions and reagent concentrations were predetermined for optimal results by checkerboard titration. Crude bacterial extracts and affinity-purified *B. pseudomallei* antigen used as a reference were prepared as described previously (5). The latter was prepared by using a MAb-conjugated Sepharose 4B column, and the adsorbed antigen was eluted with 3 M sodium thiocyanate. The assay method could detect an affinity-purified antigen at a concentration of 8 ng of carbohydrate per ml. As few as 420 CFU of *B. pseudomallei* in a 50-μl volume of hemoculture broth (1.8×10^4 CFU/ml) could be detected. Results presented in Table 1 clearly show that the method is highly specific for *B. pseudomallei* antigen.

The method was then evaluated for its diagnostic value by using clinical specimens from patients culture positive or negative for *B. pseudomallei*. Pus and sputum samples were immediately put in a transport medium from which one aliquot was cultured, and the organisms were identified bacteriologically from that aliquot by a diagnostic laboratory. The other aliquot was kept frozen at −20°C until the time of antigen assay. Table 2 shows that 44 of the 59 specimens culture positive for *B. pseudomallei* were also antigen positive. This was strikingly different from the results in Table 3 showing that only 1 of the 54 specimens which gave no growth for *B. pseudomallei* (but positive growth for other gram-negative bacteria) was positive. The one apparent false positive could have been due to the presence of a small number of *B. pseudomallei* CFU that were missed because of overgrowth of other gram-negative bacteria in the specimen. It should be mentioned that

TABLE 1. Immunoreactivity of a MAb-based streptavidin-biotin sandwich ELISA against crude extracts of gram-negative bacteria

Source of extracts[a]	Optical density (450 nm)
B. pseudomallei	>2.000
Burkholderia cepacia	0.057
Pseudomonas aeruginosa	0.030
Pseudomonas putida	0.053
Xanthomonas maltophilia	0.030
Proteus mirabilis	0.027
Klebsiella pneumoniae	0.033
Enterobacter cloacae	0.041
Escherichia coli	0.037
Salmonella typhi	0.038
Salmonella enteritidis	0.039
Salmonella krefeld	0.026

[a] The bacteria on blood agar plates were scraped off, suspended in phosphate-buffered saline, and boiled for 3 min, after which the protein content was adjusted to 10 μg/ml. A 50-μl volume was used for analysis.

* Corresponding author. Mailing address: Department of Microbiology, Faculty of Science, Mahidol University, Rama 6 Rd., Bangkok 10400, Thailand. Fax: (662) 644 5411 or (662) 247 1222.

TABLE 2. Sensitivity of a MAb-based ELISA for antigen detection in clinical specimens culture positive for *B. pseudomallei*

Specimen	No. tested	No. antigen positive[a]	Sensitivity (%)
Sputum	13	11	85
Pus	35	24	69
Pleural fluid	3	2	67
Urine	8	7	88
Total	59	44	75

[a] Antigen-positive specimens were those with an optical density reading (450 nm) at least three times the background value.

TABLE 3. Results of antigen assay in clinical specimens[a] culture negative for *B. pseudomallei*

Specimen	No. tested	No. antigen assay positive	Specificity (%)
Sputum	5	0	100
Pus	26	1[b]	96
Urine	23	0	100
Total	54	1	98

[a] Taken from patients culture positive for other gram-negative bacteria.
[b] The organisms found in this one antigen-positive specimen were identified by a diagnostic laboratory as *Pseudomonas* species.

although cross-reactivity with gram-negative bacteria was not encountered during the laboratory evaluation (Table 1), neither *Burkholderia mallei* nor *Legionella pneumophila* was available for testing. In a separate experiment, we examined 49 additional sputum specimens from nonmelioidosis patients, and all were found to be negative by this assay system.

Currently the definitive diagnosis of melioidosis is by the culture method, which is still used as the "gold standard." It is, however, time-consuming and can be falsely negative, particularly when specimens contain only small numbers of organisms and are heavily contaminated by normal flora. The more widely used serological methods for antibody detection are difficult to interpret, particularly in areas where the infection is endemic, where the background antibody levels may be elevated. This drawback still persists even when more-purified antigens are used for detection (2, 5). The quantitation of specific IgM antibody, as carried out in several laboratories (6), is not completely satisfactory because false positives still occur. We recently reevaluated this problem and found that the IgM antibody detection method was not superior to the IgG antibody detection method (1). Using the fluorescein isothiocyanate–anti-fluorescein isothiocyanate system to amplify a sandwich ELISA for the detection of antigen in urine (dilution, 1:10), Desakorn and her associates found the method to be 81% sensitive and 96% specific (4). However, the assay method required the use of MAb to fluorescein isothiocyanate, which is expensive and not readily available, thus making it impractical for remote health centers. Walsh and associates (10) reported the sensitivity of a direct immunofluorescent-antibody test to be 73%. However, this method required the use of a fluorescent microscope, which is available only in some large diagnostic laboratories. The assay method described in the present report had sensitivity comparable to that of the direct immunofluorescent-antibody test but required simpler laboratory equipment and could be readily adapted for use in peripheral laboratories. By this method, it is possible to have a specific result within a few hours for three of four patients with melioidosis.

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