

Thesis Title Identification of Specific Antigen of *Pseudomonas pseudomallei* and Monoclonal Antibody Production for the Diagnosis of Melioidosis.

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ABSTRACT

The currently used serodiagnostic methods for acute septicemic melioidosis are not yet reliable and satisfactory because of the unavailability of specific antigen. Most reports depend largely on the use of crude unfractionated bacterial extract for the detection of serum antibodies. In this study, fractionated 19.5-kDa antigen was prepared from unfractionated whole cell antigen of *P. pseudomallei* and evaluated for its potential usefulness in the diagnosis of acute septicemic melioidosis. The antigen was used for detecting antibody in sera from patients with acute septicemic melioidosis by indirect ELISA and the results were compared with those from patients with other bacterial infections, using normal human sera from either endemic or nonendemic areas of infection as a baseline. The results were considered positive if the O.D. value of a 1:250 serum dilution were higher than mean +2SD. The fractionated 19.5-kDa antigen exhibited

satisfactory results with 92% sensitivity, 91% specificity, 81% positive predictive value and 96% negative predictive value based on a background obtained with normal sera from the nonendemic area. These value were 82% sensitivity, 96% specificity, 94% positive predictive value and 87% negative predictive value based on normal sera from the endemic area.

Monoclonal antibodies were produced by immunizing BALB/c mice using unfractionated whole cell antigens with a purpose of developing specific reagent for antigen detection. Several specific clones were established and the 5F8 was selected for detailed study because it was highly specific for *P. pseudomallei* and could detect all 56 isolates currently available. It was IgM agglutinating antibody and could bind to the surface envelope of *P. pseudomallei* (detected by immunofluorescence test). This MoAb could be used successfully for detecting antigen based on B-SA ELISA method at the antigen concentration as low as 12.5 ng/ml. The preliminary data using an artificial mixture of antigen with urine or serum from normal individuals showed it to be promising for detecting antigen in the urine. However, whether or not this method will be useful in the diagnosis of acute melioidosis depends on further investigation when clinical specimens are available for analysis.