## PALADINS: PROTECTIVE APPROACHES LEVERAGING AD-APTIVE AND IN-NATE SYSTEMS

# Optimization Of Serological Assays For The Analysis Of Antigen-specific Antibody Responses In Melioidosis Patients

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Melioidosis, caused by Burkholderia pseudomallei, is a severe tropical infectious disease predominantly endemic in Southeast Asia and Northern Australia. Delays in diagnosis and treatment of the disease can result in high mortality rates and no vaccines currently exist for protection against this Tier 1 select agent. Detection of antibodies against B. pseudomallei antigens may aid in the early diagnosis of melioidosis. This study was aimed at optimizing serological assays for detection of antibody responses against different Burkholderia antigens in melioidosis patients. Using pooled plasma samples obtained from melioidosis patients and healthy donors, we optimized enzyme-linked immunosorbent assay (ELISA) conditions for the analysis of IgG, IgM, IgA and IgG subclasses against five purified Burkholderia antigens. The polysaccharide and protein targets that were selected have previously been identified as serodiagnostic targets and/or potential vaccine candidates and included O-polysaccharide (OPS), capsular polysaccharide (CPS), hemolysin co-regulated protein 1 (Hcp1), alkyl hydroperoxide reductase C (AhpC) and the deubiquitinase TssM. Following optimization of the antigen concentrations and antibody dilutions, the ELISAs were evaluated using plasma samples obtained from culture-confirmed melioidosis patients (n=210) and healthy donors (n=210) from northeast Thailand. Our results showed that higher levels of antigen-specific IgG, IgM, IgA, IgG1, IgG2 and IgG3 were present in melioidosis patients compared to healthy donors. In addition, we observed that antibody responses against the protein antigens were predominantly IgG1 and IgA whereas antibody responses against the polysaccharide antigens were primarily IgG2 and IgA. Collectively, our results indicate that we have developed robust ELISAs that will be useful for characterizing humoral immune responses to Burkholderia antigens in melioidosis patients. We anticipate that these optimized assays will be useful for improving serodiagnosis of melioidosis in clinical settings. Furthermore, these assays will be useful for assessing exposure of individuals to B. pseudomallei in both endemic and non-endemic regions as well as for evaluating immune responses against melioidosis vaccine candidates currently being developed to protect the warfighter.

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