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

Burkpx: A Multiplex Serodiagnostic Bead Assay To Monitor Burkholderia Pseudomallei Exposures In Non-human Primates

Kimberly Celona Northern Arizona University **Austin Shannon** Northern Arizona University **Derek Sonderegger** Northern Arizona University **Jinhee Yi** Northern Arizona University **Erik Settles** Northern Arizona University **Paul Keim** Northern Arizona University **Mary Barnes** Tulane University **Elizabeth Didier** Tulane University **Kathrine Phillippi-Falkenstein** Tulane University **Daniel Sanford** Battelle Memorial Institute

Burkholderia pseudomallei, a tier 1 select agent in the United States, is the causative agent of melioidosis in humans and non-human primates (NHP). Endemic to Southeast Asia and Northern Australia, infections with this tropical soil-dwelling bacteria can cause upwards of 40% mortality without proper treatment. Diagnosis of a B. pseudomallei infection can be difficult due to its strikingly similar symptomology with other infectious agents. The standard and most used diagnostic assay for B. pseudomallei exposure in humans and NHP animal models is determined by the indirect hemagglutination assay (IHA). This assay is based upon the cross reactivity of serum antibodies to B. pseudomallei whole-cell lysate antigens from several strains bound to red blood cells. Interpreting results from IHA titers can be difficult due to background seropositivity and the type of population assessed. In humans, the IHA has a reported sensitivity of 79% and specificity of 72%. However, in NHP populations, the IHA has not been evaluated and standardized. Thus, a true diagnostic cut-off has not been established lending to unknown sensitivity and specificity metrics. This is a critical gap in diagnostics given that NHPs are predominately used in clinical trials to determine the efficacy of treatments and development of vaccines prior to moving to human clinical trials, as well as biodefense research exploring routes of infection (i.e., aerosolization).To that end, we have developed a multiplexed serological assay, called BurkPx, which uses purified B. pseudomallei proteins and carbohydrates bound to 21 fluorescently-distinct MAGPIX magnetic beads in an effort to surmount the sensitivity and specificity issues of the IHA and to standardize a serodiagnostic assay for NHP. We screened B. pseudomallei-challenged (n=115) and non-challenged (n=126) rhesus macaques to explore time-dependent antibody responses and to train two multivariate models, LASSO and Ridge Regression, in differentiating exposure from non-exposure. These multivariate models produce a probability score (p-hat) indicating the likelihood of a B. pseudomallei infection. When cross validating the model using independent data, 63.5% sensitivity and 100% specificity were observed. However, removal of samples collected prior to day seven after challenge increased the sensitivity to 95%. Following this assessment, we tested our assay with sera collected from rhesus macaques housed at the Tulane National Primate Research Center (TNPRC) in Covington, Louisiana, during an accidental release of B. pseudomallei in the fall of 2014. This collection included B. pseudomallei culture-confirmed (n=2) and IHA-positive animals (n=38), suggesting B. pseudomallei exposure. The represented IHA positive animals with low IHA titers could represent B. pseudomallei false positive animals since the IHA assay has not been validated for macaques. Using the BurkPx assay, we identified 100% of the culture-positive animals, as well as additional culture-negative macaques that might have been exposed. These data suggest that the BurkPx assay can be a valuable monitoring tool for B. pseudomallei exposure and baseline background seropositivity in NHP for research and diagnostic applications.

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