REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Capillary Blood Is An Effective Clinical Matrix For Rapid Diagnosis Of Acute Plague In Field Forward Settings

Dawn Birdsell Pathogen and Microbiome Institute **Voahangy Andrianaivoarimanana** Institut Pasteur de Madagascar **Dave Trudil** New Horizon Diagnostics **Larry Loomis** New Horizon Diagnostics **Caroline Balagot** Naval Health Research Center **Vipin Rastogi** US Army CCDC **Vladimir Motin** University of Texas UTMB **Stephen Francesconi** Defense Threat Reduction Agency **Minoarisoa Rajerison** Institut Pasteur de Madagascar **David Wagner** Pathogen and Microbiome Institute **Lovasoa N. Randriantseheno** Institut Pasteur de Madagascar **Jason Sahl** Pathogen and Microbiome Institute **Carina Phillips** Pathogen and Microbiome Institute **Jessica Marshall** Pathogen and Microbiome Institute **Jessica Tartal** New Horizon Diagnostics **Aidan Mucci** New Horizon Diagnostics **Shruti Rastogi** New Horizon Diagnostics **Greg Siragusa** New Horizon Diagnostics

Background information: Capillary blood is an attractive clinical matrix for blood-borne pathogens given the limited training required and ease of collection, making it ideal for field-forward settings. Plague, caused by the blood-borne pathogen Yersinia pestis, is a biological threat agent with a high mortality rate if untreated but without a current licensed vaccine. Thus, efforts to protect warfighters need to be focused on rapid diagnosis at a very early acute disease phase followed by appropriate antimicrobial treatment. Plague progresses rapidly and presents in two predominant forms: bubonic and pneumonic. Bubonic plague is the predominant form of naturally occurring human plague, but pneumonic plague is the likely form in a biowarfare scenario.

Because of its rapidity, immunodiagnostic detection of plague must be antigen based. In Madagascar, which reports >75% of annual global plague cases, diagnosis of human plague in rural foci is conducted using a rapid diagnostic test that targets the F1 antigen of Y. pestis, which is specific to Y. pestis, and bubo aspirates and sputa as the clinical matrices for bubonic and pneumonic plague, respectively. However, these matrices can be difficult to collect and, in the case of bubo aspirates, highly invasive and painful. In addition, it has been demonstrated that F1-negative Y. pestis strains can still be infectious via an aerosol route.

Purpose, objective, rationale of the research: To evaluate the utility of a lateral flow immunoassay (LFI) targeting both the F1 and lcrV antigens of Y. pestis to accurately diagnose acute plague using capillary blood from a finger prick with the goal of FDA submission.

Methods: We evaluated the two-target LFI and capillary blood as a clinical matrix by comparing them to existing approaches used for diagnosing plague in Madagascar. We recruited patients suspected to have either bubonic or pneumonic plague. Due to the rapidity and lethality of plague, antimicrobials were typically administered as soon as symptoms were identified, often before sample collection. But this was followed as soon as possible (between 0 to 5 days, with >75% within 1 day) by collection of traditional samples (buboes or sputa) for medical workup and blood for this study. Primary confirmation testing (qPCR and culture) was performed on traditional matrices (buboes or sputa).
Results: Out of 101 participants, 64 were confirmed positive, three as probable, and 34 as suspect based on existing diagnostic tests. Of the 64 confirmed, 43 were identified as positive by the two-target LFI using capillary blood, as were 16 of the 37 probable and suspect cases. Statistical testing indicated that there exists fair agreement between the two test schemes – LFI with capillary blood vs. traditional reference tests (qPCR and culture) using bubo aspirates or sputa.

Preliminary conclusions, and impact to the DTRA mission and warfighter: Prior to this study, the presence of Y. pestis antigen in capillary blood was unknown. The two-target LFI developed in this study coupled with capillary blood can diagnose acute plague making it possible for warfighters to be rapidly and accurately diagnosed in field-forward settings using minimally invasive sampling.