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

A Simple And Rapid Lateral Flow Assay For Accurate Diagnosis Of Acute Human Brucella Melitensis Infection

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Brucella melitensis is a biological threat agent with potential for significant mortality and morbidity in both military personnel deployed on the front lines in combat zones, and in civilian populations. Early accurate diagnosis of infection is imperative for effective management. Polymerase chain reaction (PCR)-based assays detection of pathogen-specific genes in blood/serum of infected patients, the mainstay of early diagnosis of human Brucellosis, are reportedly of suboptimal clinical sensitivity and not useful in combat zones or resource-constrained environments; hence, new highly sensitive assays that are simple to perform anywhere and rapidly output easily interpretable results are urgently needed. In this regard, pathogen-derived molecules, including proteins shed in body fluids / blood in sufficient amounts during the actual process of infection (i.e., components of the pathogen circulatory proteome / CP) have excellent potential for development of assays capable of detecting B. melitensis infection on the same day or a few days of onset of clinical symptoms. Here, work toward development of a simple and rapid in vitro diagnostic lateral flow assay (IVD) prototype, based on pathogen proteins "shed" in circulation during acute infection (i.e., the CP), is described. To define the pathogen CP, we first generated acute-infection-phase sera (sera) by experimentally infecting cohorts (n = 8 per cohort) of mice via subcutaneous and intranasal routes (to mimic portals of entry during human infection) with 107 C.F.U. of virulent B. melitensis strain 16M, and then collected sera on day 3, day 7, and day 14, following infection. Sera from one uninfected cohort, collected on day 0, served as the pre-infection control. We then employed a proprietary proteomics-based platform proteome mining tool called Proteomics-based Expression Library Screening (PELS) to define CPs by “mining” pooled sera from individual cohorts across each time point and route of infection. The PELS principle exploits the exquisite specificity of a receptor and its cognate ligand, followed by identification of interacting proteins via tandem mass spectrometry and interrogation of relevant public non-redundant databases with the output mass spectral data. PELS resulted in the identification of 297 B. melitensis "shed" proteins as components of the pathogen CP, across both routes of infection and all time points. Application of stringent bioinformatics-based reductive selection criteria prioritized a subset of 42 “shed” proteins, from which 22 candidate diagnostic domains (i.e., protein domains unique to Brucella melitensis / Brucella species), were targeted for generation of monoclonal antibodies (MAbs). MAbs demonstrating exemplary specificity for and strong reactivity against cognate peptide immunogen in an EIA are currently under evaluation for development of a prototype sandwich / competitive lateral flow-based IVD in collaboration with NanoComposix, a commercial IVD manufacturer, which is ongoing. Successful assay / device development will enhance diagnostic capabilities of clinics in war zones and medical centers serving the military (thus positively impacting DTRA’s overall mission), as well as those of institutions dispensing healthcare to civilians. The assay will also contribute to the armamentarium of tools for surveillance for human Brucellosis globally.

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