Highly Multiplexed Detection of Antibodies in Whole Blood during Tuberculosis Infection

1Charles Greef, 1Greg Husar, 2Christen Gray, 3David Danks, 2Pamela Nabeta, 2Mark Perkins, 1Michael J. Lochhead*
1MBio Diagnostics Inc., Boulder, CO, USA; 2Foundation for Innovative New Diagnostics (FIND), Geneva, Switzerland; 3Carnegie Mellon University, Pittsburgh, PA, USA

OVERALL PROJECT GOAL:
Identity a set of TB antigen targets to provide a triage (rule-out) test with high sensitivity (and moderate specificity) to refer TB suspects for reference testing, or b) a rule-in test with high specificity (and moderate sensitivity) that has the potential to replace or augment an existing microscopy assay in a format that meets a point-of-care target product profile.

BACKGROUND:
A simple blood test for active TB disease remains a critical, unmet need worldwide. In 2011, the World Health Organization recommended against the use of commercially available serological tests for TB, due to poor performance [1]. Most serological tests to date have been based on a single or small number of antigens and have not provided adequate diagnostic accuracy. The hypothesis of the research described here is that a multipanel of antigen-antibody interactions could provide a signature indicative of active disease. FIND has led a Mycobacterium tuberculosis (MBio) project to identify a set of 57 potentially discriminatory antigens [2]. To further down-select to a diagnostic antigen set, it was determined that testing fresh specimens in endemic countries was critical.

The portable MBio multiplex array platform was selected as uniquely suited for this task and a multi-phase clinical research program was initiated. This poster presents results from the Project Phase I designed to establish the operational functionality of the MBio system.

METHODOLOGY
MBio Cartridge
- 57 MBio proteins were sourced from FIND partners and were supplied to MBio as purified proteins in individual aliquots.
- MBio Diagnostics developed a disposable cartridge-based assay that incorporates the 57 MBio proteins and 31 control spot features in a fluidic channel protein array.
- The MBio TB assay can be run with diluted whole blood, serum, or plasma and is completed in less than 60 minutes.
- The MBio SnapPrep® Reagent was used for all studies.

Reference Testing
- The same 57 MBio proteins on the MBio cartridge were also configured in a Luminex bead array assay at NMI, Tuubingen, Germany under the direction of Dr. Thomas Joss.

Clinical & Laboratory Workflow

RESULTS

MBio-FIND TB SEROLOGY SYSTEM DESCRIPTION

The MBio Array system incorporates a protein microarray in a disposable fluidic cartridge, enabling spatially multiplexed immunoassays [3]. A proprietary planar waveguide illumination approach enables fluorescence-based assays with a unique combination of robustness and simplicity [3].

MBio-FIND TB Array
- The MBio FIND TB array comprises an 88 spot array
- 57 separate MB antigen spots
- 3 control and normalization spots
- 88 simultaneous, semi-quantitative antigen-antibody measurements using 10 microliters of whole blood or 5 microliters of serum or plasma.

MBio-FIND TB System Implementation at Clinical Sites

Phase I Endpoint Goals

Phases I through III

Phase I Endpoint Goals

Clinical Study Overview

Consortium

Phase I: 600 TB suspects

Phase II: 1200 TB suspects

Phase III: 5700 TB suspects

Phase I: Peru & Vietnam

Phase II: Peru & Vietnam

Phase III: Peru, Vietnam, China

Phase I Platform Analytical Endpoints

Concordance with MBio and Luminex reference

Operational Endpoints

Indeterminate Rate

Reliability

Ease of Use

Phase I: Peru & Vietnam

Phase II: Peru & Vietnam

Phase III: Peru, Vietnam, China

References