Highly Multiplexed Detection of Antibodies in Whole Blood during Tuberculosis Infection

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OVERALL PROJECT GOAL:

Identify a set of TB antigens to create a) 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 adjunct smear microscopy, 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 multiplexed panel of antigen-antibody interactions could provide a signature indicative of active disease.**
- FIND has led a *Mycobacterium tuberculosis* (Mtb) proteome effort and identified 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 multiplexed 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

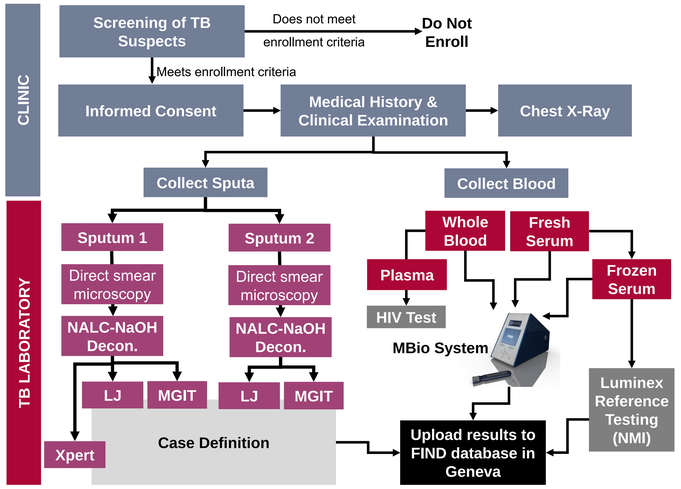
MBio Cartridge

- 57 Mtb 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 Mtb 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 SnapEsi® Reader was used for all studies

Reference Testing

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

Clinical & Laboratory Workflow



Clinical Study Overview

Study Phase	Enrollment	Sites	Purpose	Schedule
Phase I	200 TB Suspects	Peru & Vietnam	Validate functionality of the MBio-TB Platform	Complete
Phase II	600 TB Suspects	Peru & Vietnam	Select a potential diagnostic antigen set	Enrollment Complete Data Q2 2014
Phase III	1200 TB Suspects	3 Sites, to include a HIV-prevalence site	Validate the selected antigen set	Start Summer 2014, pending PhII results

Phase I results are presented here

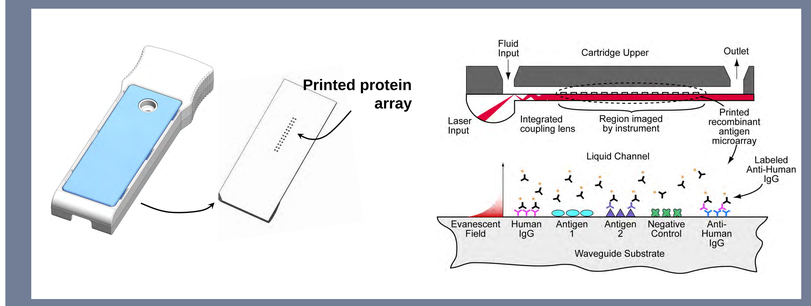
Phase I Endpoint Goals

Platform Analytical Endpoint	Goal
Concordance between MBio field platform and Luminex reference	Demonstrate direction concordance of the MBio and Luminex platforms around their respective mean fluorescence readings for each protein
Operational Endpoints	**Goal**
Indeterminate Rate	< 10% indeterminate rate, inclusive of failed cartridges, and unreadable or lost data
Reliability	Incoming quality control (IQC) passed and documented Robustness of instrumentation; documented breakdowns in equipment, software, data output
Ease of Use	User error rates <5% (part of overall indeterminate rate) Successful proficiency testing after one day's training of all operators (microscopist-level) at each site

MBio-FIND TB SEROLOGY SYSTEM DESCRIPTION

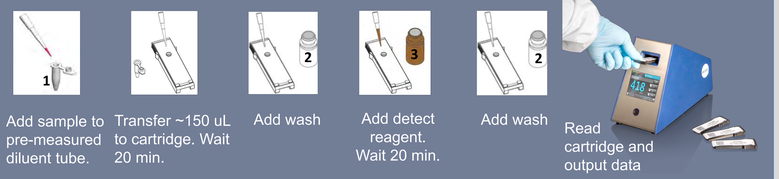
The MBio Array system incorporates a protein microarray in a disposable fluidic cartridge, enabling spatially multiplexed immunoassays [3].

A proprietary planar waveguide illumination approach enables fluorescence-based assays with a unique combination of robustness and simplicity [3].



Assay Protocol

- Samples: 10 uL whole blood or 5 uL serum
- Total assay time < 1 hour
- Batch compatible - multiple cartridges run in parallel
- On-instrument read time < 1 minute

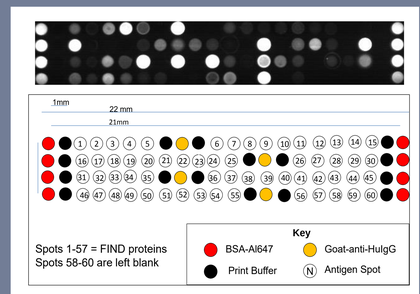


Add sample to pre-measured diluent tube. | Transfer ~150 uL to cartridge. Wait 20 min. | Add wash | Add detect reagent. Wait 20 min. | Add wash | Read cartridge and output data

MBio-FIND TB Array

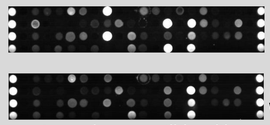
The MBio-FIND TB array comprises an 88 spot array

- 57 separate Mtb antigen spots
- 31 control and normalization spots
- 88 simultaneous, semi-quantitative antigen-antibody measurements using 10 microliters of whole blood or 5 microliters of serum or plasma.



UNDILUTED WHOLE BLOOD DEMONSTRATION

Diluted whole blood (6%) was the basis of this study. In a point-of-care application it will be desirable to use undiluted whole blood. To demonstrate that the MBio-FIND TB Assay is compatible with undiluted whole blood, the following demonstration was performed. A lyophilized reagent formulation was dried into the inlet port of the cartridge. 150 uL of whole blood was added directly to the cartridge, and then the remaining parts of the assay were performed (see workflow at left).

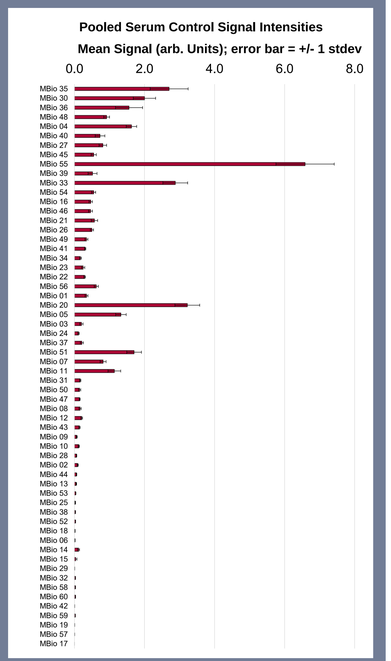


Assay is compatible with undiluted whole blood

RESULT

- Undiluted whole blood shows qualitatively similar intensity pattern as 6%.
- Higher signal on 6% suggests detect reagent depletion
- Assay optimization for whole blood expected to further improve correlation

Representative Sample Output:
Every sample run on an MBio cartridge generates 57 separate antigen-antibody reactivities. The plot below is a representative, semi-quantitative output for a positive control sample [4] consisting of a serum pool from a set of blood donors.



RESULTS

MBio-FIND TB SYSTEM IMPLEMENTATION AT CLINICAL SITES

VIETNAM
TB Reference Laboratory and Microbiology Department
Pham Ngoc Thach Tuberculosis and Lung Disease Hospital (PNTH)
Ho Chi Minh City, Vietnam
Principal Investigator: Ngoc Lan Nguyen Thi





PERU
Instituto de Medicina Tropical "Alexander von Humboldt"
Universidad Peruana Cayetano Heredia (UPCH)
Lima, Peru
Principal Investigator: Eduardo Gotuzzo

Implementation Results

- System installation at each site in < 1 hour
- Training completed in less than one day for multiple operators at each site
- Incoming QC (IQC) assay successfully performed for all kits shipped during Phase I
- Observed testing on Day 2 using stored blood samples demonstrated achieved proficiency



Operator training at PNTH, Ho Chi Minh City, Vietnam

Phase I Platform Analytical Endpoints (Concordance with Luminex)

Z-Score Method:

1. For frozen serum samples, calculate mean antigen signal intensity and standard deviation for all samples
2. Define three categories and classify each antigen on MBio & Luminex:
   - *NEGATIVE*: more than one standard deviation below the mean
   - *NEUTRAL*: within one standard deviation of mean
   - *POSITIVE*: more than one standard deviation above mean
3. Calculate percentage agreement between platforms for each datapoint (e.g., positive-positive, neutral-neutral, and negative-negative constitute agreement; any other pairing discordant)

Results:

- **All antigens showed concordance across the dataset of > 75%**
- **The majority of antigens showed >90% concordance**
- **Performance metric met**
- **MBio shows good concordance with Luminex reference**

Indeterminate Rate	Reliability	Ease of Use
• Initially 7.4% indeterminate. After adjustment of instrument controls algorithm, indeterminate rate decreased to 0.77% • Full serology profiles generated for 99.2% of cartridges used for serology testing in Phase I (644 of 649 cartridges) • GOAL ACHIEVED	• All three production lots shipped during Phase I passed IQC at each site • No instrument or software failures over the 673 cartridges tested • GOAL ACHIEVED	• Only two user errors out of 673 cartridges tested with successful repetition. (The 673 cartridge total includes proficiency & IQC cartridges) • Proficiency criteria met by all operators after one demonstration and one run. • GOAL ACHIEVED

CONCLUSIONS / NEXT STEPS

The MBio platform met all Phase I analytical and operational endpoints, and the project moved to Phase II

- Concordance with Luminex reference
- Complete serological profiles on 99.3% of samples run
- Ease-of-use demonstrated
- Reliable instrument and cartridge performance
- Simple training

Phase II patient enrollment in Peru and Vietnam (a total of 600 TB suspects) was completed in February 2014. Approximately 2400 cartridges were run (whole blood, fresh serum, fresh plasma). Data analysis expected to be complete in Q2 2014.

ACKNOWLEDGEMENTS

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References

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