# In Search for a Better Tuberculosis Diagnostic Test

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Tuberculosis (TB) remains one of the most common deadly infectious diseases on the planet and the diagnosis, treatment and prevention of this disease present serious global public health challenges.[1]

The TB treatment regimen takes too long to cure and is complicated to administer, and anti-TB drugs can be toxic.[2] There is no efficacious vaccine for TB.[3]

Rates of new infections are still rising in many endemic areas where TB co-infects those with HIV/AIDS.[4]

Treating this co-infection is further complicated by the surge of multi-drug resistant strains of *Mycobacterium tuberculosis (Mtb)*.[5] TB diagnosis remains antiquated and inadequate in most parts of the world.[6,7]

Given that one-third of the world's population is assumed to have been exposed to *Mtb* and to have developed latent infection, diagnostic assays based on the host immune response (whether cellular or humoral) often fail to distinguish active TB from latent cases.[8,9]

Unfortunately, identifying individuals with active pulmonary disease (the source of disease transmission) and distinguishing them from those with latent (non-transmissible) infection is critical to provide prompt and appropriate therapy to minimize the spread of TB. Active pulmonary TB is typically diagnosed by finding *Mtb* in sputum by acid-fast smear, a labor-intensive process requiring trained personnel and with widely varied sensitivity in different settings (20-60%).

Moreover, sputum smear has limited utility in patients with paucibacillary TB (e.g. HIV co-infected patients) and those unable to produce sputum samples (e.g. children).[10] In young children, testing often requires repeated gastric lavage, an invasive and unpleasant method of sample collection.[11]

In more developed regions of the world, sputum smear is complemented by culture (a process that takes up to eight weeks) and, in some laboratories, by the Cepheid Xpert *MTB*/RIF automated nucleic acid amplification test.[12] In view of these worldwide diagnostic limitations, the World Health Organization (WHO) and several other governmental and non-governmental institutions have recently emphasized the urgent need for better TB diagnostics.[13]

One study concluded that a rapid and widely available diagnostic test for TB, with ≥85% sensitivity for smear-positive and smear-negative cases and 97% specificity, could save ~400,000-600,000 lives annually.[14]

To overcome these obstacles and advance the TB diagnostics field, we are working on the development of a diagnostic test based on the presence of *Mtb* protein antigens in human bodily fluids.[15-17] While our focus in the current work is on the detection of *Mtb* antigens in urine, it is possible to interrogate other specimen types like blood, spinal fluid, pleural fluid, saliva, and feces (latter primarily for children) to allow diagnosis of individuals with pulmonary as well as a range of other clinical manifestations of active TB. Our antigen detection test will distinguish between those with active and latent disease and thus will represent an important new strategy for control of this global disease.

The test will diagnose infection with both drug- sensitive and drug-resistant strains of *Mtb*. It is important to note that we expect the test will also be a powerful tool to monitor the efficacy of TB treatment, a need made much greater by the alarming increase in rates of multi-drug resistant TB (MDR-TB) and, more recently, extensively drug resistant TB (XDR-TB).[18]

Currently, standard methods for monitoring TB treatment efficacy are based on culture, causing extensive diagnostic delays.

***Brief synopsis of preliminary data***

Our laboratory has used a state-of-the-art and powerful strategy to identify *Mtb* proteins that were produced *in vivo* in patients with active pulmonary TB and were subsequently excreted in the urine. Using a mass spectroscopy-based technique to analyze the urine of patients with active pulmonary TB (collected in Brazil, Colombia, Peru, and Zimbabwe), we identified several *Mtb* proteins, of which five were initially selected as potential pathogen- derived biomarkers for assay development and clinical validation (Table 1).

Table 1. Selected *Mtb* Biomarkers Identified in the Urine of Patients with Pulmonary TB

| **Putative identification** | **Rv Annotation** |
|---|---|
| MoaA-related protein | Rv1681 |
| Ornithine carbamoyltransferase | Rv1656 |
| Homoserine O-acetyltransferase | Rv3341 |
| Putative S-adenosyl-L-methionine- dependent methyltransferase | Rv1729c |
| Hypothetical protein | Unassigned |

Recombinant proteins were produced, purified and used for antibody production in rabbits. Antibodies were purified and used to assemble capture ELISAs specific for each selected biomarker. A detection limit of approximately 10-100pg/ml was achieved for each recombinant biomarker.

***Key points of validation attained to date***

Following the assay's optimization, we began the clinical validation of the capture ELISA for the Rv1681 biomarker using banked urine specimens assembled through collaborative studies sited in Texas/Mexico, Boston, and Brazil.[17] The Rv1681 assay detected the protein in unconcentrated urine specimens from 11/25 (44%) culture-confirmed pulmonary TB patients and 1/21 (4.8%) subjects in whom TB was initially clinically suspected but then ruled out by conventional methods. Rv1681 protein was not detected in urine specimens from non-TB patients with *E. coli*-positive urine cultures, subjects with confirmed non-TB tropical diseases (schistosomiasis, Chagas' disease, and cutaneous leishmaniasis), or healthy subjects.

These results strongly support the potential diagnostic utility of the biomarker Rv1681, and point towards development of a test that should be highly specific for the diagnosis of active TB. Subsequent to these initial promising findings, we evaluated the performance of ELISAs assembled to detect the other biomarkers using a new panel of urines collected from patients with and without active pulmonary TB. For these experiments, we have benefited greatly from access to banked urines from TB patients and controls. While none of the individual assays showed sufficient sensitivity to support development of a single biomarker detection test for accurate diagnosis of TB, the sensitivity of detection of multiple biomarkers in combination could substantially increase the sensitivity of the test. We are currently working on the optimization of multiplexed detection assays for these biomarkers.

We expect that an assay that includes 3-4 biomarkers will have sensitivity and specificity levels which will significantly increase the diagnostic limitation of the current available tests.

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