Abstract: The goal of this study is to develop a rapid and cost-effective assay for detecting Mycobacterium tuberculosis (TB) in low-resource settings. Multiplexing TB strains are widespread and a challenge to effective treatment of this infection. The need for a low-cost and rapid detection method for clinically relevant mutations in TB that confer multidrug resistance is urgent, particularly for developing countries with limited resources. We present here an assay that rapidly detects the majority of clinically relevant mutations in the bacterial DNA polymerase (gpI) gene that confer resistance to rifampin (rifrif). The assay is a combination of chip-based hybridization technology with colorimetric intensity changes, permitting visual detection of hybridization to a probe set arrayed onto a modified silicon chip surface that H2-mediated target-specific “hot start”. The resultant utilizes the isothermal amplification method the patient in the developing world, we have designed a simple, low-cost approach real-time PCR approach brings ease-of-use but at high cost. Amplification-based tests have improved detection sensitivity and time-to-result well. While mycobacterium culture is much more sensitive, it has a very slow sensitivity of diagnostic tests; the average sensitivity of sputum microscopy is observed for previously treated patients. TB can be effectively treated if properly diagnosed and drug susceptibility information for TB, in summary, we have developed a rapid and sensitive TB assay that could be used for the point of care setting in the developing world where the need is acute.

Introduction: The global incidence of drug-resistant TB (Mycobacterium tuberculosis), particularly multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains, is a major worldwide issue. Rates of MDR TB have been estimated to be 4.8% of the estimated 8.8 million TB infections, but rates as high as 58% have been observed for previously treated patients. TB can be effectively treated if properly identified. However, delayed initiation of appropriate treatment in suspicious MDR-TB cases is associated with excess morbidity and nosocomial transmission. It has been determined that the main contributor to delay in treatment is poor sensitivity of diagnostics, the average sensitivity across all diagnostic methods is 75% in immunocompromised patients and is lower in HIV-infected cases. Frail immunocompromised patients increase the difficulty with detecting MDR-resistant TB in nosocomial settings. MDR-TB can be present in patients for a number of years without being treated and can, therefore, be transmitted for 2-4 weeks and is technically complex. Nuclear acid amplification-based tests have improved detection sensitivity and time-to-result but historically have been difficult to effectively implement. A recently described real-time PCR approach brings ease-of-use but at high cost.

To address the needs of bringing sensitive and specific diagnostic testing closer to the patient in the developing world, we have designed a simple, low-cost approach for the specific detection of M. tuberculosis and mutations within the gpI gene that confer resistance to the first line drug, rifampin. Described herein is the performance of a bench top version of this assay that comprises a device that is manufactured from standard printed circuit board technology with colorimetric intensity changes, permitting visual detection of differential quantities of nucleic acids.

A Rapid and Sensitive Chip-based Assay for Detection of pgoB Gene Mutations Conferring Rifampicin Resistance in Mycobacterium tuberculosis

Table 1. Clinical Specimen Testing Results. 11 rif-susceptible and 25 rif-resistant clinical specimens were verified in this study. All the rif-susceptible (11/26) (11/11) were identified correctly as wildtype TB. 25/25 (100%) of the rif-resistant specimens were classified correctly as mutants. The one that was misclassified was a strain of multidrug-resistant TB. The sensitivity and specificity of the assay in the clinical population by sequencing. This kind of infection with both wildtype and mutant populations could be a challenge for this assay.

Table 2. Tb ID/R Assay Specificity. This assay only detects Tb and Tb complexes (M. bovis-BCG, M. microti, and M. africanaum as outliers. Other mycobacteria or non-mycobacterial strains will not be detected except M. avium which generates some weak cross-hybridization signal for two loci. The table above shows a list of strains tested, including human, yeast and a few other bacteria strains.

Summary: We have developed a rapid and low-cost TB assay that could detect wildtype TB and the majority of the clinically relevant drug-resistance mutations in the core region of gpI gene, which covers most of the replication of the drug-resistance TB cases reported. The assay is very sensitive and can detect single nucleotide mutations in the reaction with an amplification time at 40 minutes. The assay also specifically detects TB and other Tb complex strains. Currently, it is being configured for a low cost assay on a simple but automated device, which we believe will greatly benefit the TB patients in the developing countries.