Creation of a Clinical Assay for Detection of Mycobacterium Tuberculosis Infection as Well as Rifampin Resistance Using Linear-After-The-Exponential PCR (LATE-PCR)

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Background: The World Health Organization reports that in 2006 alone, there were approximately 9.2 million new cases and 1.7 million deaths from tuberculosis (TB). Approximately 98% of these tuberculosis related deaths occur in developing countries (WHO 9). Tuberculosis has beset the human population since recorded history and remains the infectious disease with the highest morbidity and mortality among human civilization (Schlossberg 1). Numerous TB control programs have been instituted with great success such as directly observed treatment short course (DOTS). Nevertheless, TB remains a burden to the population where primary care services are inadequate and resources are scarce.

The increased prevalence of multidrug-resistant tuberculosis (MDR-TB) further complicates the heavy burden that lies on primitive healthcare systems of developing countries. Strategies to deal with MDR-TB are still in the early stages of implementation and therefore nowhere near the appropriate level of control necessary to slow down disease transmission. Patients with MDR-TB would ideally be diagnosed at their first encounter with a healthcare professional and thereby isolated and treated appropriately so that transmission of this strain would be limited. Isoniazid (INH) and rifampin are currently the most widely used first line drugs for TB infection. Musser showed that approximately 95% of rifampin resistant strains have a mutation which occur in an 81 base pair core region of the bacterial RNA polymerase gene (1995). Additionally, any mutation that occurs within this region will lead to rifampin resistance (Varma-Basil et al, 2004). Telenti et al reports that 90% of rifampin resistant TB strains are also Isoniazid (INH) resistant (1993) therefore rapid diagnosis of rifampin resistant TB infection could lower the transmission of MDR-TB significantly.

Linear-After-the-Exponential PCR (LATE-PCR) is a form of asymmetric polymerase chain reaction that uses primers at different concentrations in order to generate double stranded amplicons exponentially followed by linear amplification of a single strand of the amplicon. The gene sequence can then be easily identified using fluorescent probes or direct sequencing of the single stranded amplicon. PrimeSafe is a new reagent developed in our laboratory that prevents all forms of mispriming while dramatically reducing the occurrence of non-specific product and improving assay efficiency. LATE-PCR along with PrimeSafe makes it possible to use lower temperature detection with fluorescent probes since the limiting primer is exhausted and subsequently does not compete with the probe. Lower temperature probes have lower background fluorescence, are easier to design and, most importantly, are more allele discriminating.
Objective: The aim of this project is to create a rapid, clinical assay able to detect TB infection as well as rifampin resistance in human samples such as, but not limited to, sputum, blood, urine, and feces. The assay will be sensitive to the single mycobacterium level and sample preparation will be conducted in a single closed container ensuring easy handling for healthcare professionals. This clinical assay is for implementation in developing countries where TB is major burden on their healthcare system. Ultimately, the assay will directly detect resistance to other commonly used TB antibiotics such as Isoniazid via multiplexing and direct sequencing.

Methods: Project is currently patent pending and therefore methods can not be addressed due to non-disclosure agreement.

Results: Project is currently patent pending and therefore results can not be addressed due to non-disclosure agreement.

Conclusion: Project is currently patent pending and therefore conclusions can not be addressed due to non-disclosure agreement.

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References:


