

## Point-of-Care Detection of of Multi-Drug Resistant Mycobacterium tuberculosis

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Linear-After-The-Exponential (LATE)-PCR, invented in our laboratory, is an advanced form of asymmetric PCR that uses two primers of unequal concentration and differing melting temperatures to generate double-stranded DNA amplicons exponentially, followed by linear amplification of one strand of each amplicon. One of the two primers can also be used to synthesize cDNA from RNA prior to the start of amplification. Such assays can detect as little as a single DNA or cDNA molecule. The resulting single-stranded DNA can be quantified at end-point by use of multiple sequence-specific probes, or mis-match tolerant probes, or via our convenient Dilute-'N'-Go sequencing protocol. PrimeSafe™, also invented in our laboratory, is a PCR additive that suppresses mis-priming throughout all thermal cycles. PrimeSafe™, therefore, enhances construction of multiplexed LATE-PCR assays allowing simultaneous detection and analysis of several target sequences from the same bacterial or viral genome in a single tube. Thus, these new platform technologies are more informative than conventional PCR and are ready for widespread use in many fields.

We are using these technologies to construct an assay for rapid detection and analysis of MDR TB in the laboratory or in the field. Point-of-care testing will be carried out in the BioSeeq, a novel portable device developed by Smiths Detection, Inc. Because the assay is based on LATE-PCR not symmetric PCR, it does not require the use of nested primer. It is highly specific and sensitive down to at least 10 target molecules, even in the presence of 10,000 fold excess human genomic DNA. The BioSeeq system includes a sample preparation device which isolates, purifies, and amplifies genomic DNA in a hands-off manner requiring a minimum of user training. The BioSeeq also analyzes amplified products in four colors over a wide temperature space. The final assay will be able to detect any sequence variation over relatively long stretches of DNA using innovative probes of the same color fluorophore. Use of several colors will enable simultaneous scans of several regions of the TB genome for genetic changes indicative of drug resistance. We anticipate that all of these events will be accomplished in under two hours from the time that a sample is taken to the time that an initial diagnosis is provided. The very same sample amplified in the field can then be returned to the laboratory for even more detailed epidemiological analysis using our efficient "Dilute-'N'-Go" protocol that permits sequencing of several amplicons from the same multiplex reaction.