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Evaluation &validation of a highly multiplexed LATE-PCR single-tube assay for M(X)DR-TB

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Background: In 2006, the World Health Organization (WHO) and the Stop TB partnership called for strengthening of diagnostic services and highlighted the need for the development of rapid diagnostics to fight the tuberculosis pandemic. In 2011, WHO estimated that approximately 630,000 (5.3%) of the 12 million TB cases had multiple drug resistant (MDR)-TB, while more than 80 countries have reported cases of extremely drug-resistant (XDR)-TB. Only a small fraction of reported cases (<20%) were correctly diagnosed and even fewer were treated according to WHO standards. In response the WHO endorsed the Genotype® MTBDR*plus* (version 1.0) line probe assay (LPA) in 2008 and the Xpert®MTB/RIF assay in 2010. But these tests only provide evidence for resistance to isoniazid and/or rifampicin. There continues to be a critical need for a more comprehensive convenient diagnostic technology. The highly multiplexed LATE-PCR assay for M(X)DR-TB described here was designed to meet that need.\

Our goal was to firmly establish that a highly multiplexed Linear-After-the-Exponential (LATE) PCR single closed-tube assay can simultaneously detect and distinguish multiple mutations in multiple gene targets that are known to confer resistance to isoniazid, rifampicin, ethambutol, ofloxacin, amikacin, kanamycin and capreomycin.

Methods & Materials: In this initial study, DNA from clinical isolates with different *rpoB, katG, embB, inhA promoter, gyrB, gyrA and rrs* genotypes were selected from a DNA bank housed at Stellenbosch University. Each DNA samples was amplified and the singled-stranded DNA products were scanned for mutations at end-point using the same mixture of Lights-On/Lights-Off Probes. The resulting fluorescent signatures were compared to that of H37Rv, a pan-susceptible "wildtype" strain.

Results: Each clinical isolate harbouring a unique mutation had its own, highly reproducible fluorescent signature distinct from that of H37Rv, as well as all other isolates with different mutations.

Conclusion: This study achieved the intended transfer of the Brandeis University technology to Stellenbosch University. This study also demonstrates that this single tube multiplexed assay can simultaneously distinguish the different mutations that confer resistance to rifampicin, isoniazid, ethambutol, fluoroquinolones, aminoglycosides and ethionamide in less than three hours.

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Classification of tuberculous meningitis using Marais Criteria

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Background: Tuberculous meningitis (TBM) diagnosis is difficult, being the worst prognostic form of extrapulmonary tuberculosis. Diagnostic delays contribute significantly to mortality and neurologic sequelae.

Methods & Materials: We performed an observational, descriptive and transversal study applying new consensus criteria for the definition of Meningeal Tuberculosis diagnosis and British Medical Research Council stage prognosis. In the study period, 68 patients were identified for tuberculous meningitis, 40 were excluded, and the final sample consisted of 28 cases

Results: The diagnostic group "Possible" was the most common with 35.7%, followed by Definite with 28.6%, Probable 21.4%, and 14.3% were excluded as Non-tuberculous meningitis in which an alternative diagnosis was established or considered dual disease. 60.71% were less than 5 years of age. 78.6% were male. 80.77% patients had symptoms for more than 5 days. 88.46% patients had focal neurological deficits. 64% patients showed alteration of consciousness. 64.3% underwent neuroimaging, which 50% demonstrated hydrocephalus. 75% of patients were in Stage III of the prognostic British Medical Research Council classification. 7.1% died.

Conclusion: Classification of tuberculous meningitis allows early diagnosis and treatment. Tuberculous meningitis manifestations vary and TBM is usually diagnosed when brain damage has already occurred.

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Detection of toxigenic *Clostridium difficile* by Loop-Mediated Isothermal Amplification (LAMP)

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Background: *Clostridium difficile*-associated disease (CDAD) is a leading cause of nosocomial diarrhea in adults. Therefore, rapid and