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Development of PCR based assay for the detection of Mycobacterium tuberculosis

Tuberculosis is an infections disease caused by the *M .tuberculosis* complex and is a major source of morbidity and mortality in humans. Though many of the currently used PCR assays are exquisitely sensitive, they lack the required specificity. Hence a PCR assay based on a previously cloned DNA sequence flanking the insertion element Is 1081 was developed for detection of *M.tuberculosis* in this study.

Among the primer combinations used, the primer pair MTBFb and MTBRb was selected for optimization of the assay. The assay was optimized in relation to the concentration of deoxyribonucleotides (dNTP), primers, $MgCl_2$, *Taq* Polynerase and the cycle number and annealing temperature.

The assay was found to be sensitive and capable of detecting ~ 10pg of *M. tuberculosis* DNA. It was also found to be specific for the *M.tuberculosis* complex.

The assay was compared with the PCR assay (using primers Pt90 currently used in Sri Lanka. Two samples, which were negative using the primer pair Pt8 & Pt(, were found to be positive when PCR amplified using the assay developed by us. This is probably due to the lack of the insertion element IS 6110 (the target of r1XPt8 & Pt9) in these

isolates. This indicates the utility of the assay developed by us. A multiplex (diplex) PCR assay containing both sets of primers is currently being evaluated for the detection of *M. tuberculosis* in clinical samples in Sri Lanka.