A-12 Development of PCR based assay for *Mycobacterium tuberculosis*

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Using a specific DNA sequence for *M.tuberculosis* obtained from the 3' region of recombinant clone pLZM 2, 3 sets of amplification primers were designed for the development of the PCR assay. The PCR assay was carried out using FP3/Rp3 (primer set) as it gives a high intense band compared to the other 2 sets of primers. The oligonucleotides were customed synthesized from Genosis, Biotechnologies (Europe) Ltd., UK.

PCR amplification of *M.tuberculosis* DNA using the forward primer, FP3 : A A C T G T G C G A C A A C A A T C A T C and reverse primer, RP3 : T G A C C A A G C C C T A T T C G T C G resulted in the amplification of a 134 bp fragment.

The final composition of the PCR mix was 10mM tris HCl (pH 8.3), 50mM NaCl, 1.5mM $MgCl_2$, 0.01% (wt/vol) gelatin, 0.2 mM (each) deoxynucleoside triphosphate (dATP, dTTP, dGTP, dCTP), 0.1mM (each) primers. Prior to PCR amplification reaction mix (50 µl) incubated at 94°C for 5 min and followed by the addition of 2.5U of Taq polymerase. *M.tuberculosis* DNA was amplified using the following parameters, denaturation at 94°C for 2 min, annealing at 65°C for 2 min and primer extension at 72°C for 3 min for 30 cycles. At the end of the 30 cycles the samples were allowed to remain at 72°C for 15 min.

The sensitivity of the PCR assay with the primers FP3 and RP3 was determined by using decreasing amounts of *M.tuberculosis* DNA. Approximately 3 fg of *M.tuberculosis* DNA could be amplified sufficiently and reproducibly to give a clearly detectable band on agarose gels.

Specificity of the PCR assay was determined by amplifying human DNA, *Mycobacterium smegmatis* DNA and DNA belonging to microorganisms commonly found in human respiratory tract instead of *M.tuberculosis* DNA. The primer set did not amplify any of the above DNA indicating it to be specific for *M.tuberculosis*.

Several positive *M.tuberculosis* samples (identified previously using the PCR assay based on the insertion element IS 986) were assayed using the PCR assay developed by us. All samples were accurately identified and the PCR assay developed by us appears to be equally sensitive.