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Evaluation of an IS 6110 – based PCR assay for laboratory detection of *M. tuberculosis* complex DNA in clinical samples

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Due to the slow growth rate of the causative agent, the diagnosis of Tuberculosis (TB) takes considerable time period leading to the complication and spread of the disease. Towards this end, use of Polymerase Chain Reaction (PCR) technology, has revolutionized diagnosis of TB by reducing the diagnostic time. The aim of the present study was to compare two primer pairs and DNA extraction methods for the PCR based detection of *M. tuberculosis* complex (MTB) DNA in clinical samples for the routine laboratory diagnosis of TB. Two DNA extraction methods (Modified Boom's method and Roche commercial kit) and two IS 6110-based primer pairs were compared with respect to the sensitivity, time and quality/quantity of DNA. Extra pulmonary and pulmonary specimens from 45 TB suspected patients referred to the Molecular Medicine Unit, University of Kelaniya from February 2007 to April 2008 were analyzed. Results indicated 50 % and 70 % of the samples extracted from modified Boom's method and commercial kit, respectively, had high quality DNA, while 17 % and 67 % of the specimens extracted by the Boom's method and commercial kit, respectively, had over 200 µg/ml DNA. Both primer pairs exhibited similar level of sensitivity (200 fg of MTB DNA). In comparison to the time consuming culture, which takes 4 to 6 weeks, the modified Boom's method and commercial kit combined with PCR takes only 48 and 24 hrs, respectively. Of the 19 positives (42.22%) 11 were males while 17 and 02 were extra-pulmonary and pulmonary TB, respectively. The commonest clinical indication for sending samples was suspected disseminated TB. Presence or absence of fever or presence or absence of very high ESR (>100 mm) did not have a significant positive or negative predictive value for PCR. Moderately high ESR (>50 mm) had a negative predictive value of 0.8 and Mantoux test had a positive predictive value of 0.8. According to the time required for completion, labour, quality/quantity of DNA (statically significant at p=0.05) and reproducibility the commercial kit proved to be an efficient DNA extraction procedure. Both sets of primers elicited similar discriminating power. There was not a single clinical indicator with satisfactory predictive values, which is useful in clinical decision making regarding the need for PCR diagnosis in individual patients. We report a simple, rapid and reproducible PCR assay for routine laboratory diagnosis of MTB DNA from both pulmonary and extra-pulmonary specimens.

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