Thesis Title Detection and Monitoring of Mycobacterium tuberculosis in clinical samples using DNA amplification

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## ABSTRACT

Tuberculosis is still a global health problem. The confirmed diagnosis of the disease depends on the results of acid-fast staining and culture for *Mycobacterium tuberculosis.* The low and variable sensitivity of acid-fast staining and the lengthy time required for culture are still the problematic. In this study, the organism could be detected as DNA products at cell concentrations as low as 20 cells in clinical specimens by using the polymerase chain reaction (PCR) with the MPB64 protein coding gene as the primer and with confirmation by a digoxigenin incorporated PCR probe. A total of 201 patients including 95 CSF patients, 8 sputum patients, 44 induced sputum patients, 45 broncho-alveolar lavage (BAL) patients, 8 urine patients and 1 pleural fluid patient were attending the general medical clinic and pulmonary disaese clinic at Ramathibodi Hospital during the interval 1991-1992. For CSF specimens, sensitivity, specificity, positive and negative predictive values and accuracy compare with culture, were 100, 94.3, 83.3, 100 and 95.6% respectively. For pulmonary

tuberculosis the respective from induced specimens values were 100, 100, 100, 100 and 100% and from BAL specimens were 100, 100, 100, 100 and 100%. DNA detection results did not differ for induced sputum and BAL specimens whether fresh or kept at room temperature for two weeks. For monitoring TB cases during therapy for tuberculous meningitis, PCR products of TB-DNA could be detected many weeks and 8 weeks in the case of pulmonary disease. In the future, detection of TB might be focused on RNA instead of DNA in order to detect only living bacterial cells and thus more suitable correlate for assessments of chemotherapy.