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NISSARA SRISAIMANEE : DETECTION AND IDENTIFICATION OF *MYCOBACTERIUM* SPECIES BY PCR-ELISA METHOD. THESIS ADVISORS: ANGKANA CHAIPRASERT, Dr.rer.nat., MONGKOL KUNAKORN, M.D.
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Recently, there is an increase of tuberculosis and mycobacterial diseases caused by nontuberculous mycobacteria. Infections with more than one mycobacterial species are increasingly reported in immunosuppressed patients. The rapid and correct diagnosis of mycobacterial infections is important for an effective patient treatment. The seminested PCR-ELISA method was developed to detect mycobacterial DNA coding for 16S rRNA. The seminested PCR using Fl-16SOL, 16SOR and 16SNSR with optimized conditions could detect amplified products of about 499 bp from all mycobacterial DNA. Cross amplification was observed in *Nocardia asteroides* and *Rhodococcus equi*. The assay was sensitive enough to detect as little as 100 fg of mycobacterial DNA except for the group of rapid growing mycobacteria, the detection limits of which were ranged from 1 ng to 10 pg. The seminested PCR combined with ELISA could detect as little as 10 fg of slow growing mycobacterial DNA equivalent to 2-3 mycobacterial genomes.

The specificities of the capture probes were assessed by analysis with 96 mycobacterial strains (22 species) and 33 nonmycobacterial strains (30 species). The specificity of pAll1, pTbc1, pMar1 were 94%, 93% and 82% respectively and that of pAvi1, pInt1, pChe1, pFor1 were 100% species-specific. The oligonucleotide probes Tbc1 and Avi1 were tested with DNAs from 108 CSF samples, the sensitivity and specificity of the detection method were 56% and 84% respectively corresponding to the culture and patient's history. The assay should be used for rapid detection and concurrent identification of slow growing mycobacteria without conventional culture. For the rapid growing mycobacteria further study is required to improve the sensitivity of detection method.