**Thesis Title** Detection of HIV-1 Proviral DNA in Peripheral Blood Mononuclear Cells by Nested PCR and Biotinylated Oligonucleotide Probe Hybridization.

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## ABSTRACT

Nested-PCR technique for detection of Human Immunodeficiency virus Type 1 (HIV-1) have been developed in the present study. The three important steps; DNA extraction, amplification and detection of amplified products were investigated. DNA was extracted from peripheral blood mononuclear cells with crude lysate salting out procedure which was more advantageous than the other 3 procedures; UCLA, salting out and crude lysate especially concerning its feasibility and efficiency.

DNA was amplified in a two-step PCR, one with outer primers and the other with inner primers

nested within the outer primers. Two parameters of PCR procedure including ingradients of reaction mixture especially $MaCl_2$ concentration, and step cycle profile had been optimized for each primer pair in order to obtain highest efficiency. The PCR product was visualized by agarose gel electrophoresis and ethidium bromide staining, then identified by dot blot hybridization using biotinylated oligonucleotide probe specific to amplified sequences. All sample were analysed with 2 sets of nested primers complementary to conserved region of HIV-1 within gag and env sequences. Among 100 seropositive samples, 88 were positive after ethidium bromide staining of PCR products, 100 were positive after hybridization. Samples from 100 seronegative blood donors were absolutely negative in PCR with both detection method using isotopic and non-isotopic probes, thus demonstrated the specificity of amplification and detection. So the developed technique including nested PCR and biotinylated oligonucleotide probe hybridization will be a useful technique to detect HIV-1 proviral DNA in human blood samples especially blood donors during the window period before seroconversion and newborns of seropositive mothers which can not be determined by any available serological diagnostic assays.