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PRAPAPUN LEKNGAM : AN INNOVATIVE DEVELOPMENT OF PCR DIAGNOSTICS FOR DETECTING HIV-1 PROVIRAL DNA IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS. THESIS ADVISOR : WATCHAREE H. ATTATIPPAHOLKUL, Ph.D., 114 p. ISBN 974-665-162-5

We have validated a newly designed PCR diagnostic using our improved method of reverse dot-blotting hybridization and ELISA based-detection system for early detection of HIV-1 proviral DNA extracted from lysed PBMCs of 100 HIV-1 seropositive and 60 seronegative individuals.

In this study, DNA was extracted from peripheral blood mononuclear cells (PBMCs) with the M&W method which was efficient, simple, rapid, and inexpensive. PBMCs were lysed directly in a lysis buffer containing quanidineisothiocyanate under very high salt conditions which resulted in total nucleic acids binding to silicon dioxide particles, which act as solid phase. The DNA was amplified in a nested PCR, first with outer primers and then with inner primers nested within the first primers. Nested PCR was performed using biotin-labeled amplification primer which resulted in biotinylated PCR products. Several methodological parameters of nested PCR were evaluated, including annealing temperature and time, $MgCl_2$ concentration, amount of outer PCR product, amount of Taq DNA polymerase and cycle number of PCR reaction. Biotinylated PCR products were analyzed preliminarily by agarose gel electrophoresis and ethidium bromide staining. The final confirmation was done by reversed dot blot hybridization using membrane immobilized with its corresponding oligonucleotide probe and color detection via alkaline phosphatase conjugated streptavidin. We were able to detect HIV-1 proviral DNA in all 100 samples from 100 HIV-1 seropositive individuals of which 89 seropositive samples were detected by agarose gel electrophoresis and 11 HIV-1 seropositive sample showed negative results by gel electrophoresis but these 11 samples could be detected by the reversed hybridization. All 60 samples of 60 HIV-1 seronegative individuals gave consistently negative results. Our data indicated that our a newly designed PCR diagnostic including nested PCR using two biotinylated inner primers and reverse dot blot hybridization is practical, sensitive, and specific. It is practical, sensitive, and specific for the detection of HIV-1 proviral DNA in clinical samples especially blood donors during the window period before seroconversion, newborns of seropositive mothers which can not be determined by any available serological diagnostic assays and seronegative people at high risk for the acquired immunodeficiency syndrome.

These techniques may constitute a good alternative to other diagnostic assays described to date. Moreover, thay are readily applicable and appropriate for using in routine diagnostic laboratory.