Thesis Title Improvement of HIV-1 DNA Detection by One-tube Seminested PCR Followed by Solution Hybridization Enzyme Immunoassay

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Abstract

PCR is a useful tool for study of HIV-1. To apply the PCR in routine clinical laboratory, more efficient PCR and detection method should be modified. In this study, a seminested PCR for detection of HIV-1 was optimized and evaluated. The seminested PCR used three primers, one of them was labeled with biotin. The reaction was carried out in a single tube to avoid carryover of amplified products which usually occur in transferring step of conventional two reactions nested PCR. The detection limit of seminested PCR was 10 copies of HIV-1 genome on ethidium bromide stained gel which is 10 fold less than the conventional nested PCR. To simplify the detection procedure, the solution hybridization enzyme immunoassay (SHEIA) for the specific biotinylated PCR products was developed and evaluated. The biotinylated amplified products were

immobilized on a streptavidin plate and denatured by NaOH. The immobilized amplified products formed hybrids with digoxigenin probe and were detected by anti-digoxigenin labeled with horse radish peroxidase. The coefficient of variation of SHEIA was 7.71-22.07%, and its cutoff optical density was 0.211. The result of SHEIA agreed with the result from gel electrophoresis in all 19 samples tested, 11 seropositive individuals (OD value 1.823-2.643) and 8 seronegative individuals (OD value 0.056-0123). The author conclude that seminested PCR coupled with SHEIA was practical, sensitive, specific, rapid and should be suitable for mass screening of samples.