A novel molecular method for HIV-1 proviral DNA detection: non-radioactively-reversed probe hybridization and nested PCR

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Abstract

A novel molecular method for HIV-1 proviral DNA detection comprising two main techniques: nested PCR, amplifying a target sequence of the ENV-gene of HIV-1, and nonradioactively-reversed probe hybridization for the detection of the amplified target sequence. The dual amplification of inserted HIV-1 proviral DNA in each DNA sample to be tested was performed by nested PCR in two steps: firstly with two outer primers covering the target sequence of the ENV-gene of HIV-1; secondly with two 5'-biotinylated primers specific to the target sequence. The biotinylated PCR product could be visualized as a single band of 141bps in length on agarose gel stained with ethidium bromide. For the confirmation of the primary result, a method of reversed probe hybridization, using a nylon membrane immobilized with the oligonucleotide probe specific to the target sequence, was established. The oligonucleotide probe was given a homopolymer tail with terminal deoxynucleotidyl-transferase; the tail was spotted onto a nylon membrane and bound covalently by UV irradiation. Owing to its length, the tail bound to the nylon, leaving the oligonucleotide probe free to hybridize. Hybridization of the amplified target sequence to the immobilized probe was accomplished by a simple colorimetric reaction involving the enzymatic oxidation of a colorless chromogen that yielded a purple color wherever hybridization occurred.

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