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Evaluation of a New, Highly Sensitive and Specific Primer Set for Reverse-transcriptase PCR Detection of HIV-1 Infected Patients: Comparison with Standard Primers

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Abstract: In the present study, a primer pairs in the HIV-1 POL gene were evaluated by performing RT-nested PCR and PCR and results were compared with universal GAG primers. In this study 80 HIV-1 seropositive patients from the patient cohort of the infectious disease division at Imam Khomeini Hospital in Tehran and 40 HIV-1 seronegative blood donors were evaluated by performing PCR and RT-PCR with the widely used SK primers (gag) and new designed primer set. Determination of PCR and RT-PCR sensitivity (copy number) was performed. The newly designed POL primer pairs was shown to be highly sensitive (100%) and specific (100%) for detection of HIV-1 RNA in Iranian patients. New primer designed detected HIV-1 RNA and DNA in all 80 plasma samples and 69 PBMC samples. SK38/39 could only detect HIV-1 RNA and DNA in 54 plasma samples and 37 PBMC samples, respectively. The optimum PCR profile for a specific primer pairs was defined as that which detected one copy of proviral plasmid DNA and 500 copies of viral RNA. Present results demonstrated that the use of primers designed for highly conserved regions of viral genome along with appropriate optimization of the test, leads to results with higher sensitivity. The high sensitivity of the developed RT-PCR allows its use as a qualitative screening test in patients receiving antiretroviral therapy in Iran.

Key words: New sensitive primer, HIV-1, Reverse-transcriptase PCR

INTRODUCTION

The standard diagnostic techniques for diagnosis of HIV-1 and assessment of blood and blood products are Enzyme immunoassays (EIAs) and Western Blot (WB). However, in both cases, due to a long incubation period before seroconversion, unidentified cases may occur (Newel, 2006; Sethoe *et al*., 1995). During the past several years, Nucleic Acid Testing (NAT) such as PCR has been used as a strong detection system in laboratories and blood bank organizations. PCR is recommended for diagnosis of HIV-1 proviral DNA in infants of infected mothers, monitoring HIV-1 infection prior to appearance of antiviral antibodies and in patients with indeterminate serological results. In addition, PCR can be used in laboratories without appropriate equipments and/or conditions for culturing the virus (Chamberland *et al*., 2001; Yilmaz, 2001). The use of reverse transcription PCR on viral isolates can also help in detection of various types of viruses circulating in a society (Steegen *et al*., 2006; Izopet *et al*., 1996).

One of the unique characteristics of HIV-1 is its high mutation rate that is not distributed equally around the world. This genomic heterogeneity has increased doubts with regard to the use of classical serological assays as the main techniques for diagnosis of HIV-1 (Van Binsbergen, 1996).

A key factor in determining sensitivity and specificity of PCR is selection of appropriate primers for amplification of the target sequence. It has been shown that inappropriate primers yield false negative results, which is misleading for the clinical team responsible for patient care (Engelbrecht, 1996). One of the primer sets suggested by international organizations and is commonly used in many laboratory settings is SK38/39 that is specifically designed to amplify the GAG fragment of HIV-1 (Vandamme *et al*., 1995). However, by widespread use of these primers, it has been shown in several studies that they can not be considered as optimal primers for diagnosis of predominant circulating HIV-1 in various countries, causing false negative results in a group of subjects studied (Owens *et al*., 1996).

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