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RESEARCH ARTICLE

QUANTITATIVE DETECTION OF HIV-1 RNA - CLINICAL USEFULNESS IN DISEASE MANAGEMENT

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ABSTRACT

The use of PCR for virus detection and quantification offers the advantages of high sensitivity and reproducibility combined with an extremely broad dynamic range. HIV-1 RNA levels in plasma of a patient is a significant marker for the successful monitoring of immunocompromised patients, present study includes the quantification of HIV-1 RNA levels in patients infected with HIV. The study signifies about the co-relation of HIV-1 RNA levels with respect to patient's viremia.

Keywords: Immunocompromised patients, Retroviral therapy, TaqMan RT PCR Probes, Threshold Value.

INTRODUCTION:

HIV, an etiologic agent of AIDS, where HIV infection can be transmitted by sexual contact, exposure to infected blood or blood products, or by an infected mother to the fetus. Most untreated people infected with HIV-1 eventually develop AIDS (1). These individuals mostly die from opportunistic infections or malignancies associated with the progressive failure of the immune system. With the advancements of amplification technologies for HIV, the monitoring of the disease is becoming very easy and clinically effective (2). Viral load in the peripheral blood can be quantitated by measurement of the HIVp24 antigen in serum, done serologically, or by direct measurement of titer viral RNA in plasma using nucleic acid amplification or signal amplification technologies. p24 antigen is the principal core protein of HIV and is found in serum either free or bound by anti-p24 antibody free p24 antigen can be measured with commercially available enzyme immunoassays, although the usefulness of p24 antigen as a marker of viral load is limited since the antigen is detectable in only 20% of asymptomatic patients and 40-50% of symptomatic patients. Advances in Medical Biotechnology have provided molecular diagnostic tools that are essential for the exploration of AIDS pathogenesis and the optimization of treatment of HIV type 1 (HIV-1) infection. HIV RNA in plasma can be quantitated by nucleic acid amplification technologies, such as the PCR. Recent and most advanced technologies includes from Abbott, Genprobe, Roche etc. HIV-1 Test, v2.0 for use with the High Pure System uses PCR technology to achieve maximum sensitivity and dynamic range for quantitative detection of HIV -1 RNA in EDTA anti-coagulated plasma (3-5). Present study was done to evaluate the diagnostic efficacy of the the usage of Roche RT PCR for the quantification of HIV-1 RNA in serum specimens.

MATERIALS AND METHODS:

Fourteen blood specimens were collected for the proposed study from the different departments of SMI Hospital, Dehradun in EDTA vials. Further serum was separated from these specimens and subjected for processing. All the specimens were further processed by Roche HIV RNA Extraction system with required precautions as provided by WHO and CDC. In addition to all the specimens, negative, low positive and high positive controls were also used for the proper validation of the assay. Roche HIV-1Test, version 2.0 for use with the High Pure System is an in-vitro nucleic acid amplification test for the quantitation of HIV-1 RNA in human plasma, using the High Pure System Viral Nucleic Acid Kit for manual specimen preparation & COBAS Analyzer for automated amplification & detection. The test can quantitative HIV-1 RNA over range of 57 to 10,000,000 copies/ml. One copy of HIV-1 RNA is equivalent to 1.7+0.1 International Units based on the WHO 1st International Standard for HIV-1 RNA (6,7).

Specimen Preparation;

HIV-1 Test, v2.0 processes EDTA plasma specimens and isolates HIV-1RNA through a manual specimen preparation based on nucleic acid binding to glass fibres. The procedure process 500

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microlitres of plasma as the started specimen. The HIV-1 virus particles are lysed by the incubation at an elevated temperature with a protease and chaotropic lysis/binding buffer that releases nucleic acid and protects the released HIV-1 RNA RNAases in plasma. Protease and a known number of HIV-1 QS Armored RNA molecules are introduced into each specimen along with lysis reagent. Further, isopropanol is added to the lysis mixture, centrifuged through a column with a glass fiber insert (8,9). Unbound substances, such as salts, proteins and other cellular impurities are removed by centrifugation. The absorbed nucleic acids are eluted with an aqueous solution.

Molecular target:

With the high genetic variability of the virus, two regions of the HIV-1 genome are concurrently targeted for amplimer generation & further detection by Roche TaqMan HIV-1Testv2.0. Two target-specific and one QS-specific dual labeled oligonucleotide probes permit independent identification of the HIV-1 amiplicon and of the HIV-1 QS, v2.0 amplicon. Accordingly, the appropriate selection of the primers and dual labeled oligonucleotide probes is critical to the ability of the test to amplify and detect diverse HIV-1 isolates; also uses reverse transcription and PCR amplification primers that define sequences within highly conserved region of the HIV-1 gag gene and of HIV-1 LTR region (10-12).

Detection of PCR products in the COBAS Taqman HIV-1 Testv2.0 for Use with The High Pure System

The use of dual labeled fluorescent probes provides accumulation of the fluorescence, by monitoring of the emission intensity of fluorescent reporter dyes released during the amplification process. The probes consist of HIV-1&HIV-1QS specific oligonucleotide probes with a reporter dye &quencher dye.

RESULTS:

Out of fourteen specimens processed for the HIV-1 RNA quantification, five cases amplifies only quantitation standard, whereas target was not detected. Nine patient's plasma level showed HIV-1 RNA titre in between 5.22 X 10^2 IU/ml-1.3 X 10^5 10/ml. Every time all the standards, including negative control, low positive control and high positive control were in their range.

Table 1: Titre values in IU/ml for HIV-1 RNA in the human serum samples and controls.

Sr. No.	Analyte for HIV-1 RNA quantification	Amplification status for Target and QS	Cycle Threshold (Ct values) w.r.t. cycle no.		Titer values of HIV-1 RNA in IU/ml
			QS	Target	
1	Case 1	Detected & quantified	29.2	30.1	**4.1 X 10^3**
2	Case 2	Detected & quantified	28.2	33.4	2.3 X 10^3
3	Case 3	Detected & quantified	28.8	32.6	5.22 x 10^2
4	Case 4	Target not detected but QS amplified	29.6	-	-
5	Case 5	Detected & quantified	28.6	32.2	3.23 x 10^2
6	Case 6	Detected & quantified	28.8	29.2	4.61x10^4
7	Case 7	Detected & quantified	29.2	30.1	1.3 X10^5
8	Case 8	Detected & quantified	28.6	31.2	2.7X10^2
9	Case 9	Target not detected but QS amplified	29.6	-	-
10	Case 10	Target not detected but QS amplified	27.4	-	-
11	Case 11	Detected and quantified	26.9	32.2	3.9X10^3
12	Case 12	Detected & quantified	29.6	29.2	6.4X10^4
13	Case 13	Target not detected but QS amplified	29.4	-	-
14	Case 14	Detected and quantified	29.6	32.2	7.9X10^2
15	Negative Control	Target not detected but QS amplified	28.6		
16	Low Positive Control	Detected & quantified	28.6	32.2	3.23×10^2
17	High Positive Control	Detected & quantified	28.1	29.8.	2.23 X 10^5

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DISCUSSION AND CONCLUSION:

There are different levels of HIV classification. Group O appears to be restricted to west-central Africa and group N - a strain discovered in 1998 in Cameroon - is extremely rare. In 2009 a new strain closely relating to gorilla simian immunodeficiency virus was discovered in a Cameroonian woman (13). It was designated HIV-1 group P. More than 90 percent of HIV-1 infections belong to HIV-1 group, M. Within group M there are known to be at least nine genetically distinct subtypes (or clades) of HIV-1. These are subtypes A, B, C, D, F, G, H, J and K. Occasionally, two viruses of different subtypes can meet in the cell of an infected person and mix together their genetic material to create a new hybrid virus (a process similar to sexual reproduction, and sometimes called "viral sex"). Many of these new strains do not survive for long, but those that infect more than one person are known as "circulating recombinant forms" or CRFs. For example, the CRF A/B is a mixture of subtypes A and B. The classification of HIV strains into subtypes and CRFs is a complex issue and the definitions are subject to change as new discoveries are made. Some scientists talk about subtypes A1, A2, A3, F1 and F2 instead of A and F, though others regard the former as sub-subtypes. The assay can quantitate HIV -1 RNA over the range of 4.7-10,000,000 IU/ml. Clinical Applications of HIV-1 viral load is of very significance for Disease Progression and HIV-1 transmission (14-16). The current study or the use of HIV-1 RNA quantification is intended for use in conjunction with clinical presentation & other laboratory markers of disease progress for clinical management of HIV-1 infected patients and can be used to assess patient prognosis by measuring the baseline HIV-1 RNA level or to monitor the effects of antiviral therapy by measuring changes in EDTA plasma HIV-1 RNA levels during the course of antiretroviral treatment. HIV-1 Test, v2.0 for use with the High Pure System is not intended to the used as a screening test for the presence of HIV-1 in blood or blood products or as a diagnostic test to confirm the presence of HIV-1 infection.

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Conflict of Interest: None

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