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Highly sensitive plasma RNA quantification by real-time PCR in HIV-2 group A and group B infection

Séverine Delarue[a], Emmanuelle Didier[a], Florence Damond[b], Diane Ponscarme[c], Karen Brengle-Pesce[d], Matthieu Resche-Rigon[e], Muriel Vray[f], Marie Gueudin[g], François Simon[a,*]

[a] Service de virologie, CHU Saint Louis, Faculté de Médecine Paris – Diderot, INSERM U 941 Paris, France
[b] Service de virologie, CHU Bichat, Faculté de Médecine Paris – Diderot, Paris, France
[c] Service des Maladies Infectieuses, CHU Saint Louis, Faculté de Médecine Paris – Diderot, Paris, France
[d] Département Recherche et Développement, bioMérieux, Grenoble, France
[e] Département de Biostatistique et Informatique Médicale, CHU Saint Louis, Faculté de Médecine Paris – Diderot, Paris, France
[f] Unité d'Epidémiologie des maladies émergentes, INSERM/Institut Pasteur, Paris, France
[g] Laboratoire de virologie, CHU de Rouen, Faculté de Médecine Pharmacie GRAM EA 2646, Rouen, France

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ABSTRACT

Plasma HIV-2 viral load has been reported as predictive of AIDS in HIV-2 infected patient but the lack of sensitivity of the current HIV2 viral load assay is a limitation for the monitoring of the HIV-2-infected patients.
Objective: To validate a new quantification assay based on a synthetic HIV-2 RNA transcript and real-time PCR with primers and probes selected in the LTR region, together with high-performance reagents and a protective RNA carrier.
Study design: We quantified 23 HIV-2 group A and B supernatants and 58 plasma samples with our TAQMAN-PCR assay and compared the results to those of our previously published in a real time reference PCR performed onto Light Cycler technology, the LC-PCR with a detection of 2.0 $\log_{10}$ copies/ml.
Results: The performance of TAQMAN-PCR was significantly improved, yielding a detection limit of 17 RNA copies/ml. There was a major difference (1–5 $\log_{10}$ copies/ml) between LC-PCR and TAQMAN-PCR values for HIV-2 group B supernatants. Twenty-six of 27 plasma samples with levels higher than 2.0 $\log_{10}$ copies/ml in LC-PCR were positive by TAQMAN-PCR. Ten of the 31 plasma samples below the LC-PCR detection limit were quantifiable with the TAQMAN-PCR.
Conclusions: The new primers and probe in the LTR region can circumvent HIV-2 diversity, making our method suitable for use in HIV-2 group B-infected patients. Use of a high-performance RT enzyme and RNA carrier protection contributed to improving the detection limit. This study confirms that plasma viral load is lower than 17 copies/ml in a large number of HIV-2-infected patients.

1. Background

In France, 2.1% of newly diagnosed cases of human immunodeficiency virus (HIV) infection are due to HIV-2, and 0.4% are due to HIV-1/HIV-2 co-infection [1]. Studies conducted mainly in West Africa, Portugal and France have led to a better understanding of the natural history of HIV-2 infection [2–5]. By comparison with HIV-1 infection, one of the main characteristics of HIV-2 infection is a lower viral replication rate, resulting in low or undetectable plasma RNA viral load, lower transmissibility, and a longer symptom-free period [6–9].

In the ANRS CO5 HIV-2 cohort, 36% of patients have undetectable plasma HIV-2 RNA (<100 copies/ml) and only 18% have levels higher than 3.0 $\log_{10}$ copies/ml [4]. The plasma HIV-2 RNA level correlates with the degree of immunodeficiency, and treatment is recommended when plasma RNA becomes detectable [10,11].

Several teams, including ours, have developed plasma HIV-2 RNA or DNA amplification techniques based on the polymerase chain reaction (PCR) [7,12,13] nucleic acid sequence-based amplification (NASBA) [14] or viral reverse transcriptase assay [15]. A recent international quality control exercise highlighted the difficulties of standardizing HIV-2 viral load assays and the pitfalls of basing therapeutic intervention on this parameter [16,17].

* Corresponding author at: Service de virologie, CHU Saint Louis, 75475 Paris cedex 10, France. Tel.: +33 01 42 49 43 07; fax: +33 01 42 49 92 00.
E-mail addresses: francois.simon@sls.aphp.fr, fsimon2010@gmail.com (F. Simon).

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The high genetic diversity of HIV-2, with eight groups (A to H) so far reported, may complicate the detection of viral nucleic acids. Group A is generally found in patients originating from countries bordering the Atlantic ocean (Cape Verde, Guinea, Guinea-Bissau and Senegal) and group B in patients from countries bordering the Bight of Benin (Ghana, Ivory Coast, Liberia, Sierra Leone) or located in the hinterland (Mali, Burkina Faso) [12]. The other groups have been defined on the basis of only one (G, H) or two full-length sequences (F), or partial sequences (C, D, E), in patients from Ivory Coast (G, H), Liberia (C, D) and Sierra Leone (E, F) [18–22].

We have previously developed a quantitative real-time PCR method for plasma HIV-2 RNA assay [12] using Roche Light Cycler technology (Roche diagnostics, Branchburg, NJ, USA), and subsequently improved its performance (detection limit 2.0 $\log_{10}$ copies/ml, 100 copies) [23]. However, the need to improve sensitivity and to overcome HIV-2 genetic diversity required a new approach.

2. Objectives

To evaluate, on *in vitro* culture supernatants and plasma clinical samples, the sensitivity of a new quantitative real-time HIV-2 PCR method using an RNA transcript as standard, together with high-performance amplification reagents and a RNA-protective carrier.

3. Study design

The new PCR assay is referred to below as "TAQMAN-PCR" and the reference assay based on Light cycler technology [23] as "LC-PCR".

3.1. Primers and probe

The primers and probe were selected in the highly conserved LTR region (nucleotide positions 200–380) in HIV-2 groups A to H, using the HIV database (http://www.hiv.lanl.gov/content). These primers and probe have been successfully used for HIV-2 proviral DNA amplification [23]. Fig. 1 summarizes the selected sequences.

3.2. HIV-2 RNA standard

An RNA transcript was prepared from a previously synthesized HIV-2 DNA LTR plasmid based on the prototype ROD HIV-2 group A sequence [23]. The transcript sequence is shown in Fig. 1 B. The plasmid was linearized by BamH1 digestion for 2 h at 37 °C prior to transcription with the T7 RiboMAX™ Express Large Scale RNA Production System (PROMEGA, 69260 Charbonnieres, France) following the manufacturer's recommendations. The Qiagen RNA Easy mini-kit was used for purification, as instructed by the manufacturer. The concentration of RNA transcripts was 7.65×10^{12} copies/μL by UV spectrophotometry (Nanodrop ND-1000, NanoDrop Technologies Inc) and purity was checked by using RNA Nano Chip and RNA 6000 Nano reagents on an Agilent Bioanalyzer.

3.3. Samples

We tested 23 diluted culture supernatants of PBMC collected from HIV-2-infected patients living in France. The supernatants, stored at −80°C, were quantified using the reference LC-PCR method, a real-time PCR assays. This is a one-step Q-RT-PCR method based on gag region amplification in a Light Cycler apparatus. HIV-2 strain NIH-Z (Group A) virions counted by electron microscopy were used to prepare HIV-2 standards for LC-PCR [12]. Gag and/or LTR sequencing indicated that 12 isolates belonged to HIV-2 group A and 11 to group B (data not shown).

Fifty-eight plasma samples were collected from 48 patients having given their written consent to participate in the ANRS HIV-2 cohort. Plasma viral load was quantified with our reference LC-PCR as previously reported [12,23]. Thirty-one samples were below the detection limit of 2.0 $\log_{10}$ copies/ml.

3.4. Nucleic acid extraction

RNA was extracted with a NucliSENS® EasyMAG™ automat (bioMérieux, Marcy l'Etoile, France) based on Boom technology [24]. Extracts were aliquoted and stored at −80°C. Sample volume was 1 ml for culture supernatants and between 1 ml and 400 μL for human samples, depending on the amount available. Copy numbers were adjusted for the dilution factors and expressed in $\log_{10}$ RNA equivalent copies/ml.

3.5. RNA-protective carrier adjunction

In order to protect the small amount of RNA from a potential endonuclease excess, we added 3 μg of RNA carrier used in the Q-PCR mix [25]. The lyophilized RNA carrier (1350 μg, QIAGEN, Hilden, Germany) was reconstituted with 1350 μL of sterile water to obtain a concentration of 1 μg/μL, and was then aliquoted and stored.

A) Primers and Probe

Primer LTR2 U17 : 5' TAG TCG CCG CCT GGT CA 3'

Primer LTR2 L17 : 5' TTC CTG CCG CCC TTA CT 3'

Probe HIV-2 : FAM 5' ACCCTGGTCTGTTAG 3' MGB

B) RNA Transcript

GCCTCAATAAAGCTTGCCTTGAGTGCTCAAAGTAGTGTGTGCCCGTCTGTtGTGTGACTCTGGTaACTAGAGATCC
CTCAGACCCtTTTAGTCAGTGTGGaAAATCTCTAGCAGTGGCGCCGAATTCCGAAtTCTAGTCGCCgCCTGGTCAtT
CGGTGtTCACCTGAGTAACaAGACCCTGGTCTGTTAGGACCCTTCTTGCTTTGGGAAACCgAGGCAGGAAAGTTC
CTAGCAGGTTGGCGCCCGAACAGGGACTTGAAGAAGACTGAGAAGTCTTGGAACACGGCTGAGTGAAGGCAGT
AAGGGCGGCAGGAA

Fig. 1. (A) HIV-2 LTR primer and probe sequences. (B) HIV-2 LTR RNA transcript sequence based onto HIV-2 ROD group A strain; primers are highlighted in grey and the probe is underlined.