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Field evaluation of an open and polyvalent universal HIV-1/SIVcpz/SIVgor quantitative RT-PCR assay for HIV-1 viral load monitoring in comparison to Abbott RealTime HIV-1 in Cameroon

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Abstract

With the increasing demand of HIV viral load (VL) tests in resource-limited countries (RLCs) there is a need for assays at affordable cost and able to quantify all known HIV-1 variants. VLs obtained with a recently developed open and polyvalent universal HIV-1/SIVcpz/SIVgor RT-qPCR were compared to Abbott RealTime HIV-1 assay in Cameroon. On 474 plasma samples, characterized by a wide range of VLs and a broad HIV-1 group M genetic diversity, 97.5% concordance was observed when using the lower detection limit of each assay. When using the threshold of 3.00 log₁₀ copies/mL, according to WHO guidelines to define virological failure (VF) in RLCs, the concordance was 94.7%, 360/474 versus 339/474 patients were identified with VF with the new assay and Abbott RealTime HIV-1, respectively. Higher VLs were measured with the new assay, +0.47 log₁₀ copies/mL (95% CI: 0.42-0.52) as shown with Bland-Altman analysis. Eleven samples from patients on VF with drug resistance were not detected by Abbott RealTime HIV-1 versus two only with the new assay. Overall, our study showed that the new assay can be easily implemented in a laboratory in RLCs with VL experience and showed good performance on a wide diversity of HIV-1 group M variants.

Keywords

HIV-1; Viral load; Genetic diversity; Subtype; Africa

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1. Introduction

Reducing the burden of HIV/AIDS remains a key public health challenge. The major objective of the Joint United Nations AIDS Program (UNAIDS) and the World Health Organization (WHO) is to end the HIV/AIDS epidemic as a public health threat by 2030 (UNAIDS, 2013). Therefore HIV transmission has to be reduced significantly and by 2020, 90% of people living with HIV should know their HIV status, 90% of them should receive antiretroviral treatment (ART) with 90% of them achieving viral suppression (UNAIDS, 2013). It is estimated that at least 17 million of the more than 37 million people living with HIV are receiving ART in 2015 (UNAIDS, 2016), but to reach the 90-90-90 UNAIDS objectives all individuals diagnosed with HIV should now receive ART. The initial scale-up of ART in resource-limited countries (RLC) was possible because they adopted the WHO public health approach which is based on standardized first and second-line regimens and clinical monitoring when biological monitoring was not possible (Gilks et al., 2006). However, with this approach ART failure is not detected in a timely period and as a consequence high rates of virological failure (VF) and drug resistance with accumulation of numerous mutations are observed in various settings (Aghokeng et al., 2014; Boyd et al., 2015; Konou et al., 2015; Stadelin and Richman, 2013). Therefore, WHO recommends since 2013 viral load (VL) testing to monitor patients on ART (WHO, 2013). Although access to VL tests has increased, it remains largely insufficient in RLCs, especially in sub-Saharan Africa (Haas et al., 2015). The rapid expansion in ART coverage, necessary to reach the UNAIDS objective, creates also an urgent need for scale-up and strengthening of laboratory capacity for timely monitoring of HIV treatment. The low coverage of VL tests is due to cost and complexity of VL detection equipments, cost of reagents, need for human resource training and laboratory infrastructure as well as operational challenges in sample collection, transport and storage (Roberts et al., 2016; Rouet and Rouzioux, 2007).

Today, different viral load assays using various molecular detection and quantification techniques such as real-time RT-PCR (Reverse Transcription Polymerase Chain Reaction), NASBA (Nucleic Acid Sequence Based Amplification) or bDNA (branched-chain DNA signal amplification) are commercially available (Fiscus et al., 2006; Rouet et al., 2011). Moreover, 'in-house' and generic real-time RT-PCR assays using open polyvalent platforms have also been developed (Aitken et al., 2013; Li et al., 2010; Preiser et al., 2006; Rouet et al., 2008). These latter tests enable reduction of reagent costs and optimize the range chart of existing equipment to allow their use also with 'in-house' or generic assays for other pathogens. Another challenge for VL tests is the genetic diversity of HIV. Most commercial VL assays were primarily designed for HIV-1 group M subtype B, which is the most prevalent subtype in high-resource countries. However, HIV-1 M non-B variants (subtypes and circulating (CRF) or unique (URF) recombinant forms) are the most prevalent in RLCs, and they represent 90% of all circulating HIV-1 strains worldwide (Hemelaar et al., 2011). Therefore efforts have been done to improve the capacity of VL assays to detect and quantify a wide diversity of HIV-1 strains. The number of commercially available assays that can detect and quantify to some extent HIV-1 group O increased also and assays to monitor HIV-2 VL have recently been developed (Rouet et al., 2011; Ruelle et al., 2014; Avettand-Fenoel et al., 2014). Nevertheless, quantification discrepancies leading to clinically

significant differences still exist, because not all tests perform equally on the different HIV-1 variants and no VL assay is perfect (Mourez et al., 2015).

With the increasing demand of HIV viral load tests in RLCs, where a wide diversity of HIV-1 variants co-circulate, there is a need for assays at affordable cost and able to quantify all known HIV-1 variants. We recently developed and described an 'in house' quantitative RT-PCR assay, called HIV-1/SIVcpz/SIVgor RT-qPCR assay, that detects and quantifies a wide diversity of HIV-1 group M variants, the divergent HIV-1 group O, as well as the simian precursors of HIV-1: SIVcpz P_{tt} , SIVcpz P_{ts} and SIVgor from chimpanzees and gorillas (Etienne et al., 2013). The test was initially compared with a previously validated Generic HIV Viral Load assay (Biocentric, Bandol, France) (Rouet et al., 2007, 2010) on a panel of plasma samples known to represent a large diversity of HIV-1 group M subtypes and CRFs. The VLs were highly correlated ($r=0.95$) and the analytical specificity and sensitivity were 100% and 97.4%, respectively. Moreover, HIV-1 group O plasma samples were better quantified compared to the Abbott RealTime HIV-1 (Abbott Molecular, IL, USA) (Etienne et al., 2013). In the present study, we further evaluated the performance of this recently developed assay for its capacity to detect and quantify HIV-1 variants with the aim to implement the assay use in RLCs. Therefore, the assessment was conducted in Cameroon where all HIV-1 groups, numerous subtypes and CRFs co-circulate. Results were compared with VL obtained with an FDA-approved assay, Abbott RealTime HIV-1. This assay is widely used and known to detect and quantify a wide diversity of HIV-1 group M and O strains (Mourez et al., 2015; Ndiaye et al., 2015; Tang et al., 2007).

2. Material and methods

2.1. Study population, sample collection and processing

The study was conducted in Cameroon in the CREMER Virology Laboratory in Yaoundé, Cameroon, a national reference laboratory and accredited by WHO for HIV antiretroviral resistance surveillance. HIV-1 RNA VL results were compared for 474 samples from HIV-1 positive patients on first-line antiretroviral treatment (ART). Patients were attending HIV services of the Central and Military Hospitals of Yaoundé in Cameroon for the screening visit prior to inclusion in the ANRS12169/2-LADY Trial, designed to evaluate the efficiency of different second-line ART regimens (Ciaffi et al., 2015). Whole blood was collected on EDTA tubes and sent within six hours to the laboratory of CREMER. After centrifugation, at least three plasma aliquots of 1 mL were stored at -80°C when sufficient material was available. One aliquot was used for routine VL testing with the Abbott RealTime HIV-1 (Abbott molecular, IL, USA), the second one was used for VL testing with the recently developed HIV-1/SIVcpz/SIVgor RT-qPCR assay and the last one was kept as backup for further analyses (i.e viral load control test, genotypic drug resistance testing). The freeze/thaw cycles until use were the same for each assay.

2.2. HIV-1 RNA viral load quantification

For the universal HIV-1/SIVcpz/SIVgor RT-qPCR assay, HIV-1 RNA was extracted from 140 μL of plasma with the QIAamp viral RNA minikit (Qiagen, Courtaboeuf, France) according to the manufacturer's protocol on the Qiacube instrument (Qiagen, Courtaboeuf,

France) and eluted in 60 μ L. Standards covering a range of HIV RNA concentrations and positive and negative controls of the Generic HIV-1 viral load test (Biocentric, Bandol, France) were used as previously described and were extracted with the same protocol (Etienne et al., 2013). HIV-1 RNA quantification was done in a 20 μ L reaction volume containing 10 μ L of RNA extract, primers and the probe at 500 nM, 1 $\times$ of TaqMan Fast Virus 1-step Master Mix (Applied Biosystems, CA, USA) and RNase-free water to the final volume. Cycling conditions were as follows: reverse transcription at 50 $^{\circ}$ C for 5 min, RT inactivation and initial denaturation at 95 $^{\circ}$ C for 20 s, and amplification with 50 cycles at 95 $^{\circ}$ C for 3 s and 58 $^{\circ}$ C for 30 s (Etienne et al., 2013). The 7300 Real-Time PCR system (Applied Biosystems, CA, USA) was used for cycling and data acquisition. The lower detection limit (LDL) was estimated $\sim 2.70 \log_{10}$ copies/mL with a plasma input of 140 μ L.

The Abbott RealTime HIV-1 assay (Abbott Molecular, IL, USA) was done according to the manufacturer's protocol. HIV-1 RNA was extracted manually from 600 μ L of plasma using the mSample preparation system (Abbott Molecular, IL, USA). Cycling and data acquisition were performed with the m2000rt Molecular Diagnostic Instrument (Abbott Molecular, IL, USA). The LDL of the Abbott RealTime HIV-1 assay was 1.60 $\log_{10}$ copies/mL.

2.3. Genotyping and classification of HIV-1 variants

All samples were tested with a previously developed 'in-house' enzyme-linked immunosorbent assay (ELISA) using V3-loop peptides from the gp120 envelope region to identify infections with HIV-1 group N, O and P (Vergne et al., 2003; Villabona-Arenas et al., 2015). All samples with VL $\geq 3.00 \log_{10}$ copies/mL in at least one of the two VL assays were sequenced in the *pol* gene. Nucleic acids were manually extracted from 200 μ L of plasma using QIAamp viral RNA minikit (Qiagen, Courtaboeuf, France) according to the manufacturer's protocol. Protease and RT were amplified as a single fragment (1281 bp) in the first round with previously described primers (Monleau et al., 2014). The forward primer was PR1 (HXB2 position 1958–1984, 5'-THAARTGTTTCAACTGTGGCAARGAAG-3'). Briefly, a one-step RT-PCR was performed with forward primer PR1 and reverse primer TR2as. Nested PCR was then performed using PR2 and TR3as. The amplification conditions and the enzymes used are the same as previously described (Monleau et al., 2014). PCR products were directly sequenced using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, CA, USA). Identification of relevant HIV-1 drug resistance mutation (DRMs) and prediction of antiretroviral drug resistance were performed using the ANRS-v25 algorithm (<http://www.hivfrenchresistance.org/2015/Algo2015-hiv1.pdf>, accessed 06 January 2016). Phylogenetic tree analysis using the Maximum-likelihood method with PhyML (Guindon et al., 2010) and recombination analysis using similarity and bootscanning plots (Simplot software package version 3.5.1.0; 300-bp window size and 20 bp increment) were done to identify HIV-1 M subtypes and circulating (CRF) and unique (URF) recombinant forms (Lole et al., 1999).

2.4. Statistical analysis

All VL data were analyzed as $\log_{10}$ -transformed values. To determine the linear relationship between the assays, the Pearson's correlation coefficient (r) was calculated. Bland-Altman analysis was used to assess the agreement between the two methods of viral load

measurement (Bland and Altman, 1999). Correlation and Bland-Altman analyses were performed using pairs of quantifiable VL values.

3. Results

3.1. HIV-1 genetic diversity of the samples used in the comparative analysis

Among the 474 samples used for the VL comparisons, none reacted with peptides of HIV-1 group N, O and P when tested with the V3-loop peptide ELISA. A total of 362 samples had VL 3.00 log₁₀ copies/mL in at least one of the two VL assays. Genotyping was successful for 323 (89%) of them. For the remaining samples genotypes were not available due to insufficient material or PCR tests that failed. Phylogenetic and recombination analyses identified the following HIV-1 variants in decreasing order of importance: 180 (55.7%) CRF02_AG, 23 (7.1%) subtype G, 17 (5.3%) CRF22_01A1, 15 (4.6%) CRF11_cpx, 12 (3.7%) subtype F(1 F1 and 11 F2), 11 (3.4%) subtype D, 11 (3.4%) subtype A (including 3 A3), 7 (2.2%) CRF13_cpx, 6 (1.9%) CRF09_cpx, 4 (1.2%) CRF18_cpx, 4 (1.2%) CRF37_cpx, 4 (1.2%) CRF01_AE, 2 (0.6%) subtype H, 1 (0.3%) CRF06_cpx and 26 (8.1%) URFs.

3.2. Comparison of the VL results between Abbott RealTime HIV-1 and the universal HIV-1/SIVcpz/SIVgor RT-qPCR

We first compared the number of samples detected above the lower detection limit (LDL) of each assay. A total of 367/474 (77.4%) plasma samples had VL above the LDL of 1.60 log₁₀ copies/mL with the Abbott RealTime HIV-1 assay, and 361/474 (76.2%) samples were above the LDL of 2.70 log₁₀ copies/mL with the new assay. Of these, 358 (75.5%) had detectable VL loads in both assays. Despite the different lower detection limit, there was a concordance between detectable and undetectable HIV-1 RNA values for 462 (97.5%) of the 474 samples. Overall, we observed thus 12 discordant samples, i.e. nine that were not identified with the new assay and three not detected with the Abbott RealTime HIV-1 assay. Among the nine samples not detected with the new assay, seven had VL measured below its lower detection limit of 2.70 log₁₀ copies/mL, i.e. between 1.65 and 2.66 log₁₀ copies/mL with Abbott RealTime HIV-1. For the two other samples, VL values with Abbott RealTime HIV-1 were 3.67 and 4.65 log₁₀ copies/mL. However, when repeating VL test on new extracts, with the new assay on these two samples: the first sample had a VL of 3.34 log₁₀ copies/mL (CRF13_cpx) but the second sample remained undetectable (CRF22_01AE). Finally, for the 3 samples not detected with the Abbott RealTime HIV-1 assay (1 CRF02_AG and 2 not amplified in *pol*), VLs were between 3.26 and 3.38 log₁₀ copies/mL with the new assay.

Fig. 1 shows the correlation between the results of both assays (Pearson correlation coefficient $r = 0.84$; $P < 0.001$). The Bland-Altman difference plot (Fig. 2) shows a mean difference of +0.47 log₁₀ copies/mL (95% confidence interval (CI); 0.42-0.52) ($r = 0.028$ $p = 0.60$) corresponding to higher VLs measured with the new assay as compared to Abbott RealTime HIV-1. In total, 16 (4.5%) of the 356 samples that could be quantified in both assays were outside the limits of agreement (average difference ± 1.96 standard deviation of the difference); 10 samples had lower VL and for 6 samples it was the opposite. The samples

that were outside the limits of agreement are from diverse HIV-1 variants and represented the general genetic diversity of the study samples; 8 CRF02_AG, 3 from different subtypes, and 3 from other CRFs and 2 not amplified in *pol*, as shown in Fig. 2.

3.3. Comparison of VL at the threshold of 3.00 log₁₀ copies/mL and implications for patients in resource-limited countries

We also compared the VL of the 474 HIV-1 plasma samples obtained with the two assays at the threshold of 3.00 log₁₀ copies/mL which is recommended by WHO to define virological failure in resource-limited countries (RLCs) (WHO, 2016). At this threshold, the new assay detected 360 samples versus 339 for the Abbott RealTime HIV-1 assay. Overall, 449/474 (94.7%) VL results above or below this value were concordant; 337 with VL ≥ 3.00 log₁₀ copies/mL and 112 with VL < 3.00 log₁₀ copies/mL in both assays. Among the 337 samples, 303 were previously genotyped and 99% of them had at least one mutation associated with drug resistance (Guichet et al., 2016). We identified thus 25 discordant samples, 23 of which had VL > 3.00 log₁₀ copies/mL with the new assay (VL range; 3.23-4.17 copies/mL) but ranged between undetectable to 2.96 log₁₀ copies/mL with the Abbott RealTime HIV-1 assay (Table 1). The VL differences between the discordant samples ranged between 0.39-1.72 log₁₀ copies/mL, and were > 0.5 log₁₀ copies/mL or > 1.0 log₁₀ copies/mL for 95% and 70% of them, respectively. The two samples below the threshold of 3.00 log₁₀ copies/mL with the new assay, are the same as described above. Genotyping was successful for 20 (80%) of 25 discordant samples. Phylogenetic analysis identified a wide diversity of HIV-1 variants and reflect the overall subtype/CRF distribution of the entire study population: CRF02_AG (n = 12, 60%), F2 (n = 1), G (n = 2), CRF09_cpx (n = 1), CRF11_cpx (n = 1), CRF13_cpx (n = 1), CRF22_01A1 (n = 1) and CRF37_cpx (n = 1). Among the 20 samples genotyped, 13 strains carried HIV-1 drug resistance mutations (DRMs) conferring resistance to one (n = 2), two (n = 9) or three (n = 2) drugs of the first-line antiretroviral treatment (ART), and in remaining seven samples no drug resistance was predicted and no DRMs identified. Overall, this means that the new VL assay detected 11 additional patients for whom VF was related to drug resistance but failed to detect two patients with DRMs (Table 1). On the other hand, the Abbott RealTime HIV-1 assay did not detect 11 patients with DRMs.

4. Discussion

The overall aim of the study was to evaluate the performance of a recently developed open and polyvalent universal HIV-1/SIVcpz/SIVgor RT-qPCR HIV-1 viral load assay in a laboratory setting in sub-Saharan Africa, where many HIV-1 variants co-circulate and to compare results to an FDA-approved assay, Abbott RealTime HIV-1. The tests were performed on 474 plasma samples characterized by a wide range of VL (undetectable to > 7.00 log₁₀ copies/mL) and a broad HIV-1 group M genetic diversity predominated by CRF02_AG (56%) in addition to 5 different subtypes (A, D, F, G, H) (18%), 8 different CRFs (18%) and 26 URFs (8%).

Using the lower detection limit of each assay 97.5% concordance was observed between the two assays, and only two samples detected with Abbott RealTime HIV-1 were not identified

with the new assay. Repeat testing confirmed that a single sample remained negative with the new assay, this difference could be due to PCR inhibition or a technical error. Important to note is that the other discordant samples had low VL and were below the lower detection limit of the new assay. The different lower detection limits for the two tests are most likely due to different input volumes of plasma used in each assay, 600 μL for Abbott RealTime HIV-1 versus 140 μL for the new assay. Despite these different thresholds, the good concordance is most likely due to the fact that VLs are in general higher (mean difference: $+0.47 \log_{10}$ copies/ml) with the new assay. This difference can be related to the different target regions of the HIV-1 genome between the two tests, which is the long terminal repeat (LTR) for the new assay and integrase for the Abbott RealTime HIV-1 as well as other reagents used. A recent review on VL assays on dried blood spots, reported that VL assays targeting the LTR region seem more sensitive (Parkin, 2014) and the Cobas TAqMan HIV-1 test v.2.0 added a LTR target to improve quantification (Sizmann et al., 2010). A positive mean difference ($+0.24 \log_{10}$ copies/mL, $n = 112$) was also observed in studies comparing the Generic HIV Viral Load assay (Biocentric) assay, which targets the LTR, to the Abbott RealTime HIV-1 in Gabon (Mouinga-Ondémé et al., 2014). Our previous study showed that our new assay and the Generic HIV Viral Load assay (Biocentric) performed equally on HIV-1 group M variants, $0.02 \log_{10}$ copies/mL difference only (Etienne et al., 2013). However, the generic HIV-1 viral load test (Biocentric) detects and quantifies only HIV-1 group M in contrast to our assay, which detects all HIV-1 groups and their simian precursors in chimpanzees and gorillas as previously reported (Etienne et al., 2013).

When we used the threshold of 3.00 log copies ml, which defines VF in resource-limited countries, according to WHO guidelines (WHO, 2016), the concordance between the two assays values slightly decreased to 94.7%. The new assay detected 360 samples versus 339 for the Abbott RealTime HIV-1 assay. Among the 25 discordant samples, genotypes and drug resistance patterns were available for 20 with 13 carrying resistance to at least one drug of their first-line ART. From the 13 drug resistant strains, 11 were not detected by Abbott RealTime HIV-1, and two not by the new assay and as a consequence, the corresponding patients would thus not have switched to second-line ART. However, seven non-resistant strains were also detected with the new assay, and the corresponding patients probably need support to improve adherence to their ART. Indeed, as the genotyping is not available in RLCs, the WHO recommends a second VL after at least one month of adherence support to avoid unnecessary switch to second-line (WHO, 2016). It is thus likely that part of the non-resistant samples will be negative after this intervention (Guichet et al., 2016).

5. Conclusions

Routine VL monitoring clearly allows early detection of treatment failure and when it is correctly associated with timely ART switches it will thus limit the emergence of drug resistance, reduce transmission of resistant strains and virological failure in second-line due to high viral load at baseline. Overall, our study showed that the new assay could be easily implemented in a laboratory in RLCs with VL test experience. Despite absence of divergent HIV-1 group O, N or P samples in our panel and the higher lower detection limit, the new VL assay showed good performance on a wide diversity of HIV-1 group M subtypes and CRFs, as the Abbott RealTime HIV-1 assay. The capacity of the new assay to detect all

HIV-1 groups and their simian ancestors will also allow a timely detection of emerging new HIV-1 variants. However, the new assay can be improved, by using higher input volumes of plasma for a better sensitivity and a lower detection limit, but also by including an internal control to identify technical errors. Nevertheless, scale-up of VL testing and capacity should also be accompanied with improvement of program management to ensure continuous supply of VL tests as well as training and participation in external quality programs.

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Abbreviations

VL viral load

RLC	resource-limited countries
VF	virological failure
CRF	circulating recombinant form
URF	unique recombinant form

Fig. 1. Correlation between the two assays for HIV-1 RNA quantification. HIV-1 group M RNA viral load quantified by the new universal HIV-1/SIVcpz/SIVgor RT-qPCR and Abbott RealTime HIV-1 (Abbott Molecular, IL, USA). Samples ($n = 356$) detected with both assays were plotted on this linearity plot. The solid line represents the Pearson correlation, $r=0.84$; $P < 0.001$.

Fig. 2.

Bland-Altman difference plot comparing the quantifiable HIV-1 RNA viral loads measured with the two assays among a large diversity of HIV-1 non-B variants ($n = 356$). The vertical axis indicates the difference between the universal HIV-1/SIVcpz/SIVgor RT-qPCR and Abbott RealTime HIV-1 assays, and the horizontal axis shows the mean viral load between the two assays. The mean bias on the difference (solid line, $+0.47 \log_{10}$ copies/mL) and limits of agreement (dashed lines) are shown on the graphic, as well as values of the 95% CI. (nd: genotyping was not done).

Table 1

Discordant VL results between the new universal HIV-1/SIVcpz/SIVgor RT-qPCR and Abbott RealTime HIV-1 assays. The clinical consequence related to presence of HIV drug resistance mutations ($n = 25$) on the number of the three first-line ART drugs failing (0–3) is shown. The lower detection limit is 1.60 and ~2.70 log₁₀ copies/mL for Abbott RealTime HIV-1 and the universal HIV-1/SIVcpz/SIVgor RT-qPCR, respectively.

Sample ID	Subtype	Abbott RealTime HIV-1 VL log ₁₀ copies/mL	Universal HIV-1/SIVcpz/SIVgor RT-qPCR VL log ₁₀ copies/mL	VL universal - VL Abbott log ₁₀ copies/mL	Number of first-line antiretroviral drugs failing
Y1/286	CRF02_AG	. ^a	3.26	nd	0
C1/147	na	-	3.30	nd	na
C1/1025	na	-	3.38	nd	na
Y1/241	na	1.73	3.45	1.72	na
Y1/208	na	1.99	3.35	1.36	na
Y1/621	CRF02_AG	2.06	3.57	1.51	0
Y1/642	CRF09_cpx	2.08	3.62	1.54	0
Y1/623	CRF02_AG	2.17	3.39	1.23	0
Y1/236	CRF02_AG	2.34	3.42	1.08	1
Y1/629	G	2.34	3.47	1.13	0
Y1/131	CRF11_cpx	2.35	3.23	0.88	2
Y1/613	F2	2.42	3.56	1.13	0
Y1/263	CRF37_cpx	2.46	3.78	1.32	2
Y1/251	CRF02_AG	2.46	3.28	0.81	1
Y1/609	CRF02_AG	2.58	3.97	1.39	2
Y1/651	CRF02_AG	2.60	3.83	1.24	0
Y1/311	na	2.67	3.64	0.96	na
Y1/102	CRF02_AG	2.70	4.17	1.47	2
Y1/066	CRF02_AG	2.74	4.09	1.35	3
Y1/003	CRF02_AG	2.85	4.04	1.20	2
Y1/079	CRF02_AG	2.95	3.68	0.72	2
Y1/183	G	2.95	3.73	0.78	2
Y1/290	CRF02_AG	2.96	3.35	0.39	3
Y1/129	CRF13_cpx	3.67	-	nd	2
Y1/153	CRF22_01A1	4.65	-	nd	2

means that HIV VL is not detectable or below lower limit of detection for each assay; na: not amplified by PCR; nd: not done.