Comparison of LCx with Other Current Viral Load Assays for Detecting and Quantifying Human Immunodeficiency Virus Type 1 RNA in Patients Infected with the Circulating Recombinant Form A/G (CRF02)

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LCx was compared to other assays in measuring human immunodeficiency virus type 1 (HIV-1) CRF02 viremia. LCx showed significant but low correlation with the other methods. Values of $<2.60 \log_{10}$ cp/ml were observed in 29.6% of specimens with LCx and in only 14.8% with bDNA and PCR, suggesting suboptimal performance of LCx with CRF02.

Clinical management of human immunodeficiency virus type 1 (HIV-1) infection and implementation of treatment strategies rely on routine measurement of levels of HIV-1 RNA in plasma (23, 24). A variety of commercial assays, employing different molecular technologies, are available for measuring HIV-1 RNA levels in plasma. They differ in sensitivity, dynamic range, target region, and methods of nucleic acid detection (15, 16, 18, 25).

Most of these assays have been optimized for the detection of subtype B of HIV-1 (13), which predominates in the United States and Europe. However, increasing numbers of non-subtype-B infections are being identified in Europe (1, 4, 9, 12, 17) and the United States (6, 22). In developing countries, non-B strains are widespread and often predominant (20).

Therefore, it is important that viral load (VL) assays detect and accurately quantify all the known HIV-1 subtypes, inter-subtype recombinants, and emerging variants to avoid under-quantification or even complete lack of detection. HIV-1 sequence variation within target regions used for VL quantification can directly impact the performance of diagnostic assays (2, 21).

It has been reported that some commercial assays either underevaluate or fail to detect HIV RNA subtypes A, E, and G and other minor strains (7, 8, 10, 11, 14, 19). Poor performance of the NucliSens HIV RNA QT assay in quantifying viremia for the circulating recombinant form of HIV-1 A/G (CRF02) has been recently described (3, 27). Here we extended the analysis to the LCx HIV RNA quantitative system (Abbott Laboratories, North Chicago, Ill.). VL results obtained by the LCx assay, based on competitive PCR, were compared with those obtained by three other commercial assays (Versant HIV-1 RNA version 3.0 [based on branched DNA (bDNA) technology], Bayer Diagnostics, Emeryville, Calif.; Amplicor HIV-1 Monitor, version 1.5 [standard proce-

dures, based on PCR technology], Roche Molecular Systems, Alameda, Calif; and NucliSens HIV-1 RNA QT [based on nucleic acid sequence-based amplification (NASBA), Organon Teknika, Durham, N.C.], by using plasma samples from a selected group of 27 individuals infected by HIV-1 CRF02-A/G (3, 27) and from 21 patients infected by HIV-1 subtype B.

TABLE 1. Individual values of HIV-1 VL measured by four commercially available methods on samples from HIV-1 CRF02-infected patients

Patient no. ^a	VL (copies/ml) in assay:			
	Versant	LCx	NucliSens	Amplicor
1	31,380	<200	18,000	168,000
2	15,270	11,840	820	25,500
3 ^b	<50	<200	<80	7,100
4	6,592	14,872	470	6,190
5	94,056	<200	26,000	166,000
6	44,788	23,243	7,200	100,000
7	16,194	3,030	<80	10,900
8	24,316	28,450	540	20,200
9	90,647	186,397	22,000	744,000
10	104,070	30,969	3,400	128,000
11	28,642	48,519	150,000	213,000
12 ^b	143	616	<80	678
13	1,120	811	1,100	677
14	115,755	157,589	18,000	443,000
15	380,220	1,079	50,000	747,000
16	27,019	63,630	2,600	125,000
17	5,768	<200	<80	4,530
18	3,597	3,333	230	6,980
19	1,497	2,572	710	5,150
20	9,639	18,393	150	12,200
21	108,674	<200	1,500	267,000
22	51	<200	<80	<400
23	9,900	10,269	270	<400
24 ^b	<50	<200	<80	<400
25	404	1,913	<80	<400
26	6,340	3,307	560	9,870
27	7,045	272	360	13,000

^a At the time of blood sampling, all patients were naive for antiviral treatment; three of them were under treatment with two nucleoside analogs or two nucleoside analogs plus a protease inhibitor.

^b Patient under treatment at the time of blood sampling.

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FIG. 1. Comparison of HIV-1 VL values measured by four assays (Versant HIV RNA 3.0, Amplicor HIV Monitor 1.5, NucliSens HIV RNA QT, and LCx HIV RNA) for 27 patients infected by HIV-1 recombinant form A/G (CRF02), by means of the method described by Bland and Altman (5). Plots of difference versus average of VL values obtained by Versant HIV RNA 3.0 and LCx HIV RNA (A), NucliSens HIV RNA QT and Versant HIV RNA 3.0 (B), Versant HIV RNA 3.0 and Amplicor Monitor 1.5 (C), NucliSens HIV RNA QT and LCx HIV RNA (D), NucliSens HIV RNA QT and Amplicor Monitor 1.5 (E), LCx HIV RNA and Amplicor Monitor 1.5 (F) are shown. The resulting linear regression curves are indicated. Because of different LLD of the four assays, all samples with HIV RNA levels lower than 400 (2.60 log₁₀) cp/ml in the bDNA, LCx, and NASBA assays were given a value of 2.60 log₁₀ cp/ml (the LLD of the Roche procedure) to make the LLD equal among methods.

FIG. 2. Correlation between plasma HIV RNA levels measured by Versant HIV RNA version 3.0, Amplicor HIV-1 Monitor 1.5, NucliSens QT HIV RNA, and LCx HIV RNA in samples from 27 patients infected by HIV-1 recombinant form A/G (CRF02). (A) NucliSens QT HIV RNA compared to LCx HIV RNA, (B) NucliSens QT HIV RNA compared to Amplicor HIV-1 Monitor 1.5, (C) Versant HIV RNA version 3.0 compared to Amplicor HIV-1 Monitor 1.5, (D) Versant HIV RNA version 3.0 compared to LCx HIV RNA, (E) LCx HIV RNA compared to Amplicor HIV-1 Monitor 1.5, (F) NucliSens QT HIV RNA compared to Versant HIV RNA version 3.0. As in Fig. 1, the LLD for all tests was set to 2.60 log₁₀ cp/ml. Linear regression lines with 95% confidence limits are shown.

Isolates from all the patients had their HIV genotype assignment based on the nucleotide sequence of *gag* and/or *pol* regions, as described previously (3). The patients were all in the chronic phase of the infection, and most of them had not been treated by the time of sampling (Table 1). Each patient was sampled once, and all specimens were tested once. Assays were conducted in parallel with an external subtype B standard (Pelysyp-97; CLB Viral Diagnostic Laboratory, Amsterdam, The Netherlands).

Individual values obtained by the four assays are reported in Table 1. Plasma HIV-1 RNA levels were $\log_{10}$ transformed before being subjected to statistical analysis. Values were compared by nonparametric tests, as indicated. Data were also analyzed by plotting the difference between measurements obtained by two methods against their average, to assess the correlation between this difference and the magnitude of measurement (5). Furthermore, individual values obtained in different assays underwent linear regression analysis, and Pearson's correlation coefficient (r) was calculated. To avoid biases in statistical analyses because of the difference in the lower limits of detection (LLD) of the assays, all samples with HIV RNA levels undetectable by Amplicor, or $<2.60 \log_{10}$ copies (cp)/ml by the bDNA, LCx, and NASBA methods, were assigned the value of the Amplicor LLD, i.e., $2.60 \log_{10}$ cp/ml.

The mean values of VL, obtained by the four assays with the whole group of samples harboring HIV-1 CRF02, were statistically different (Friedman test, $P < 0.001$): $4.21 \pm 1.07 \log_{10}$ cp/ml by Amplicor, $3.98 \pm 0.89 \log_{10}$ cp/ml by Versant, $3.58 \pm 0.90 \log_{10}$ cp/ml by LCx, and $3.21 \pm 0.80 \log_{10}$ cp/ml by NucliSens. Mean values obtained by LCx were significantly different from those obtained by Amplicor but not from those obtained by Versant and NucliSens (Wilcoxon sign rank test, $P = 0.004$, $P = 0.184$, and $P = 0.069$, respectively).

Comparisons conducted according by the method of Bland and Altman (5) showed that in NucliSens-Amplicor and Versant-Amplicor comparisons, absolute values of difference with respect to average values, between the members of each paired value, significantly increased with the magnitude of VL ($r = -0.324$ and $P = 0.022$, and $r = -0.197$ and $P = 0.045$, respectively) (Fig. 1). The other pairs of methods considered did not show such a trend, indicating a constant difference in measuring VL.

When paired values obtained for each sample was compared by using different assays were compared the correlation was significant for each pair of assays considered (Fig. 2). However, Amplicor and Versant showed the highest correlation coefficient ($r = 0.894$ and $P < 0.001$), while the correlation coefficients involving LCx and the other assays were notably lower: $r = 0.334$ and $P = 0.088$ for LCx-NucliSens, $r = 0.431$ and $P = 0.025$ for LCx-Amplicor, and $r = 0.464$ and $P = 0.015$ for LCx-Versant.

The proportion of samples with values of $\leq 2.60 \log_{10}$ cp/ml was different among the assays ($P = 0.015$ by the Cochran test): only 4 (14.8%) of 27 samples were undetectable by Amplicor and Versant, while 8 (29.6%) and 11 (40.7%) samples were undetectable by LCx and NucliSens, respectively. The proportion of samples with undetectable VL was significantly different when considering NucliSens-Versant and NucliSens-Amplicor ($P = 0.016$ by the McNemar test), as expected, while for LCx-Versant, LCx-Amplicor, and LCx-NucliSens the dif-

ferences were not statistically significant ($P = 0.219$, $P = 0.289$, and $P = 0.508$, respectively). The values of the kappa statistic were lower for pairwise comparisons involving LCx (0.279 and $P = 0.135$ for LCx-NucliSens, 0.169 and $P = 0.334$ for LCx-Amplicor, and 0.337 and $P = 0.031$ for LCx-Versant) than in the other cases (0.413 and $P = 0.032$ for Amplicor-Versant, and 0.404 and $P = 0.009$ for Amplicor-NucliSens and Versant-NucliSens).

Samples from patients infected with HIV-1 subtype B were also analyzed. All assays gave comparable results, with an excellent correlation among the methods (data not shown), thus confirming high performance and strong correlation of all these methods in detecting subtype B.

Although the LCx assay employs primers targeting a specific and highly conserved sequence in the *pol* region, ensuring consistent results for all HIV-1 M and O subtypes (26), this study demonstrated a certain degree of inability to efficiently quantitate VL from CRF02. This inability is less pronounced than that previously demonstrated for NucliSens (27). Although the number of samples used in this study is small, these results can have important implications. In fact, measurement of VL is a key issue in clinical management of HIV-1-infected patients. Since infections by non-B strains and intersubtype recombinant forms of HIV-1 are spreading worldwide, it will be worthwhile to extend our observation to other circulating non-B HIV-1 subtypes in order to obtain information about the actual performance of VL assays, particularly in respect to their use in developing countries.

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