

Rates of and Reasons for Failure of Commercial Human Immunodeficiency Virus Type 1 Viral Load Assays in Brazil[∇]

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We examined failures of commercial human immunodeficiency virus type 1 (HIV-1) viral load assays of 1,195 plasma samples from Brazilian patients. Assay failure was assumed for samples in which the virus was undetectable by commercial assay but which tested positive by real-time reverse transcription-PCR of the HIV-1 long terminal repeat (LTR) region or if the viral load differed by $>2 \log_{10}$ from that determined by LTR assay. Failure rates for Bayer Versant bDNA 3.0, Roche Amplicor Monitor v1.5, and bioMerieux NucliSens QT were 0.68, 0.47, and 4.33%, respectively. NucliSens may be inadequate for use in Brazil.

Worldwide, over 38 million people are estimated to be infected with human immunodeficiency virus type 1 (HIV-1) (15). About 630,000 of them live in Brazil. In Brazil, HIV-infected individuals receive treatment free of charge, which creates one of the largest populations on antiretroviral therapy worldwide (10). Determination of viral load is an integral part of therapy, necessary to recognize treatment failure and to prevent transmission of resistant strains. However, commercial viral load assays were originally designed to detect subtype B viruses, which are most prevalent in North America and Western Europe (14). It is likely that such assays will be less appropriate for other virus populations, and several earlier studies focused on this problem. Unfortunately, most studies used small numbers of clinical samples, in the range of 100 specimens (1, 4, 12, 13). No study has prospectively evaluated a routine testing program, including all major viral load assays, and none used a uniform gold standard method for comparison.

In view of the large numbers of patients under monitored therapy in Brazil, the significance of wrong viral load quantification was determined in this specific setting. We analyzed 1,195 samples from three different regions of Brazil. In a recent multicenter study on HIV-1 quantification (6), viral loads in these samples were tested with either the Bayer Versant bDNA 3.0, bioMerieux NucliSens HIV-1 QT, or Roche Amplicor Monitor v1.5 assay. Viral loads in all samples were also determined with a new real-time reverse transcription-PCR test for the highly conserved 5' long terminal repeat (LTR) region. This assay detected non-B subtypes with improved efficiency (6). It served as our gold standard.

We selected all samples that tested negative in any of the three commercial assays and showed viral loads above 5,000 copies/ml by real-time LTR reverse transcription-PCR. In ad-

dition, samples that were underquantified by at least a factor of 100 in the commercial assays compared to results with LTR PCR were also included.

To make sure that underlying data were correct, all LTR viral loads originally determined in the previous study were confirmed by retesting the original plasma samples with the LTR assay. Viral loads determined by commercial assays were not retested, since these were issued during ongoing treatment by certified Brazilian viral load laboratories, which are subjected to strict quality control standards by the Brazilian Ministry of Health.

As shown in Table 1, the Bayer Versant bDNA 3.0 and Roche Monitor v1.5 showed significant discrepancies in 0.68% and 0.47% of samples, respectively. The NucliSens assay, however, gave discrepant results for 4.33% of the tested samples. This rate was significantly higher than those with the other commercial methods ($\chi^2 = 20.36$, $P < 0.0001$). To exclude a systematic technical error in the gold standard method, 10 of the 14 samples with discrepant results by NucliSens were retested with Roche Amplicor (no material was left from the remaining four samples). Indeed, the virus was detected by Amplicor in 7 out of 10 samples. The median discrepancy between LTR and Amplicor results was $0.95 \log_{10}$ for the positive samples. For comparison, the median difference between LTR and NucliSens was $4.44 \log_{10}$.

The reasons for failure were determined by sequencing the *gag* and *pol* genes directly from the samples. Phylogenetic analysis was performed using TreeCon 1.3. Phylogenetic typing was confirmed with Simplot 3.5.1 (8), using the subtype reference alignment of the Los Alamos HIV database supplemented by extra Brazilian B-, C-, and F-subtype sequences (three per subtype).

The numbers of mismatches for both the Monitor v1.5 and the NucliSens assays were highest for F, BF, C, and CF samples (Fig. 1). Both samples in which the virus was not detected by Monitor v1.5 had subtype F signatures in their amplicon target regions (*gag*) and revealed a common mismatch pattern in the hybridization domain of the reverse primer. To our surprise, the majority of samples with results discrepant by

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TABLE 1. Comparison of undetected or underquantified viral load as determined by the Bayer Versant bDNA 3.0, Roche Amplicor Monitor v1.5, and Biomerieux NucliSens QT assays

Assay	Subtype ^a (p24/pol)	No. of samples	Log deviation ^c (range)	No. of deviant samples/samples tested
Roche Amplicor Monitor v1.5	F/F	1	4.68	2/430
Bayer Versant bDNA v3.0	FB/BF	1	4.23	3/442
	B/B	1	5.58	
	C/C	1	2.40	
Biomerieux NucliSens HIV-1 QT	F/C	1	4.49	14/323
	B/B	11	4.54 (2.3–5.7)	
	F/rec ^b	3	3.99 (1.7–5.5)	

^a Phylogenetic analysis comprised HXB2 genome residues 1345 to 1839 and HXB2 2266 to 3257. The primer sequences used for amplification and sequencing were HXB2 886 to 908, 1307 to 1328, 1344 to 1363, 1344 to 1369, 2155 to 2174, 2220 to 2242, 2600 to 2620 (sense) and 2176 to 2152, 1843 to 1822, 1736 to 1760, 1654 to 1675, 3334 to 3311, 3308 to 3288, and 3298 to 3276 (antisense). Typing of the circulating recombinant form was based on SimPlot analysis of *gag* (p24)/*pol* genes.

^b Includes one F/B, one F/BF, and one F/FB *gag* (p24)/*pol* recombinant sample.

^c The median expressed as log₁₀(LTR assay – commercial assay).

NucliSens contained pure subtype-B viruses (11 of 14) (Table 1); the remainder were also F recombinants. Oligonucleotide mismatches are shown in Fig. 1 for all BF recombinants and four subtype-B samples. For the remaining subtype-B samples,

such mismatches could not be confirmed. Interestingly, there are other recent reports of such observations, suggesting that oligonucleotide mismatches may not be the only reason for assay failure with the NuciSens system (1, 12).

For the bDNA assay, it was not possible to determine the contribution of virus variability to misquantification because its 98 probes in the *pol* gene are unpublished. Its design should be relatively stable against nucleotide mismatches (7, 13). Interestingly, for two of the three samples misquantified with this assay, the alignment in Fig. 1 suggests that they would predictably test negative with the NucliSens and Monitor assays (no retesting was possible due to lack of plasma). These viruses were C subtypes or C recombinants.

This is the first study to permit a quantitative estimation of HIV-1 viral load assay failure within Brazil, one of the largest HIV-1 treatment settings worldwide. Approximately 83% of Brazilian HIV-1 strains are subtype B, 14% are F, and 3% are C. Subtype C is predominant, occurring at a rate of up to 50%, in the south of Brazil, whereas subtype F and B/F recombinants are predominant in the north, occurring at rates of up to 50% (2, 3, 11, 17). The conformity of our samples with this spectrum of HIV-1 subtypes and the large number of samples analyzed justify extrapolation of our study to the Brazilian HIV-1 population. Currently, NucliSens is being applied for HIV-1 viral load determination in 32 of 73 Brazilian Ministry

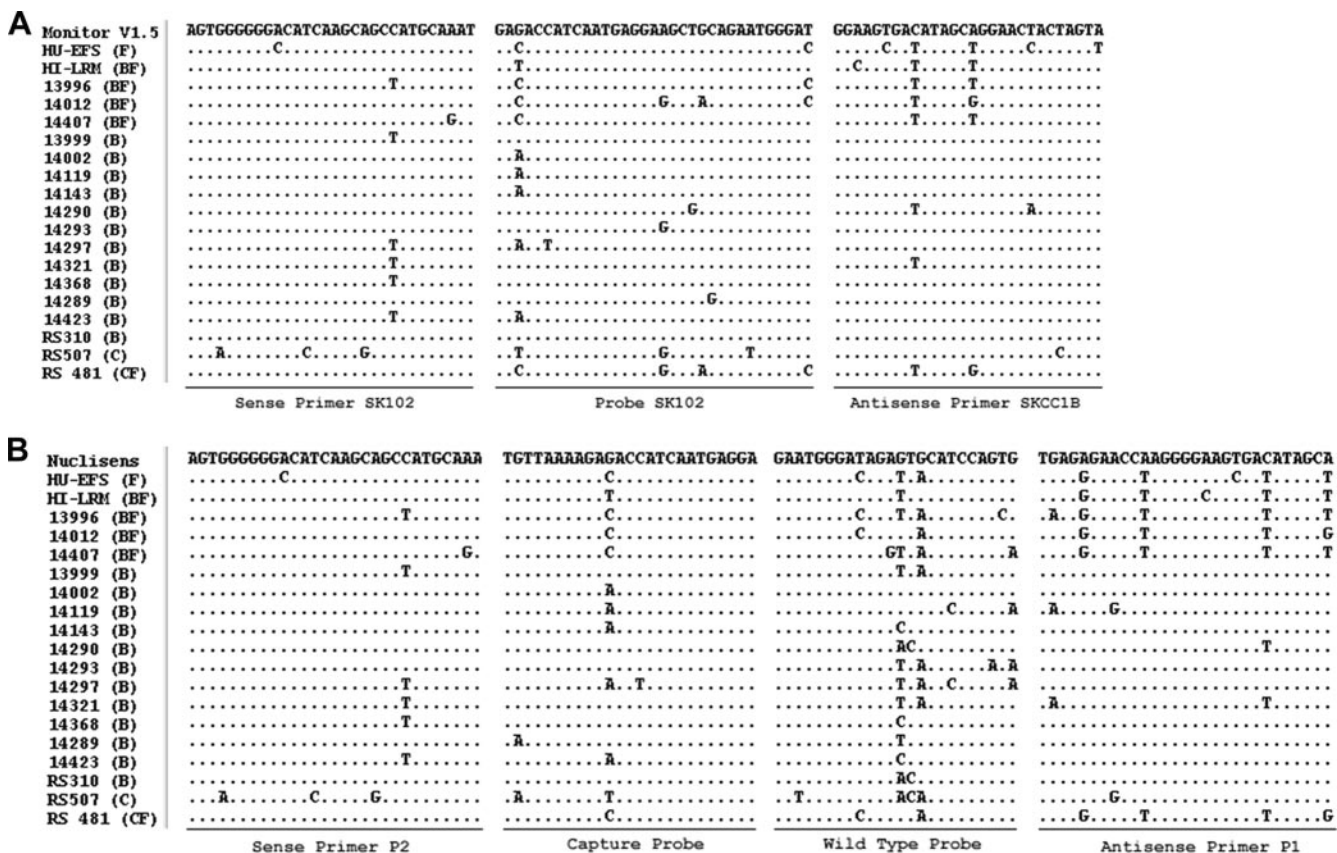


FIG. 1. Nucleotide mismatches of Amplicor Monitor v1.5 (A) and NucliSens HIV-1 QT (B) assays at oligonucleotide binding sites in the *gag* p24 gene. The viral loads in samples HU-EFS and HI-LRM were underquantified by Monitor v1.5. The viral loads in samples 13996 to 14423 were underquantified by NucliSens. Samples RS310 to RS481 were underquantified by bDNA v3.0. Primer and probe sequences were obtained from N. L. Michael et al. (9) and C. Christopherson et al. (5) for Monitor v1.5 and B. van Gemen et al. (16) and I. von Truchsess et al. (18) for NucliSens, respectively.

of Health reference laboratories, many of which are situated in the regions with the highest prevalence of subtype-F and F-recombinant viruses. One hundred eighty thousand individuals are on antiretroviral therapy in Brazil, receiving up to four viral load determinations per year. According to these numbers, the observed rate of 4.3% samples for which the virus was undetectable or seriously underquantified with the Biomerieux NucliSens assay can be extrapolated to at least 2,580 patients annually. Whether this assay is appropriate for Brazil at all should be reexamined.

Nucleotide sequence accession numbers. All nucleotide sequences determined in this study are available from GenBank, accession numbers EF075946 to EF075983.

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