

Comparison of Amplicor HIV-1 monitor Test, NucliSens HIV-1 QT and bDNA Versant HIV RNA technologies for quantitation of plasma HIV-1 RNA in HIV-1 infected individuals.

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ABSTRACT

Objective: Quantitation of viral load is an important part of prognosis and effective clinical management of HIV-1 infected individuals. Introduction of new antiretroviral drugs, improved treatment regimens and increase in access to diagnostic services has led to an increase in life span of HIV-1 infected individuals worldwide. Currently various methods are available for measurement of HIV-1 RNA levels with sensitivity limits ranging from 25 to 400 copies per ml and there are various ongoing studies that are evaluating the clinical significance of viral load levels in this range. In the current study, we compared 3 methods of HIV-1 RNA quantitation, Amplicor HIV-1 monitor Test (v1.5, Roche), NucliSens HIV-1 QT (bioMerieux) and Versant HIV-1 RNA bDNA assay (v3.0, Siemens) in parallel from plasma specimens of 72 HIV-1 infected individuals.

Methods: Whole blood EDTA specimens from 72 HIV-1 infected individuals were submitted to the Laboratory for Clinical and Biological Studies, University of Miami, FL for routine quantitation of plasma HIV-1 RNA. Quantitation of plasma HIV-1 RNA was performed in parallel using the three technologies according to protocols provided by the manufacturers. All the statistical evaluations were carried out using the SPSS software (v 14.0).

Results: Among 72 plasma specimens, HIV-1 RNA was detected in 40 each by Amplicor and bDNA (sensitivity, 56%) and 44 by NucliSens (sensitivity, 60%). As expected, HIV-1 RNA levels obtained from all the three methods had a highly significant positive correlation (Pearson Correlation, $P < 0.001$). However, on comparing the technologies on one to one basis we observed that HIV-1 RNA levels obtained with Amplicor and bDNA had higher linear association ($r = 0.995$) versus Amplicor and NucliSens ($r = 0.967$) or NucliSens and bDNA ($r = 0.975$). Comparison of mean HIV-1 RNA levels obtained using the three methods showed no significant difference (One way ANOVA, $P = 0.488$).

Conclusions: Viral load measurement at low copy numbers is subject to constraints imposed by the inherent variability of the assay technology. Our results show that three technologies provide concordant results in terms of sensitivity and Amplicor and bDNA show excellent agreement with each other in quantitation of HIV-1 RNA.

● Monitoring HIV-1 viral load is highly valuable in prognosis and effective clinical management of HIV-1 infected individuals.

● Introduction of new antiretroviral drugs, improved treatment regimens and increase in access to diagnostic services has led to an increase in life span of HIV-1 infected individuals worldwide.

● Currently various methods are available for measurement of HIV-1 RNA levels with sensitivity limits ranging from 25 to 400 copies per ml and there are various ongoing studies that are evaluating the clinical significance of viral load levels in this range.

● Ultra sensitive Amplicor monitor is a reverse transcription linked nucleic acid amplification based test for quantitation of HIV-1 RNA from human plasma. The method can quantitate HIV-1 RNA over a range of 50-75,000 copies/ml.

● NucliSens HIV-1 QT is also a reverse transcription linked nucleic acid amplification based test for quantitation of HIV-1 RNA where the quantity of amplified RNA is measured by means of electrochemiluminescence. The detection range of this method is 25-3,470,000 copies/ml.

● Versant HIV-1 RNA bDNA assay is a chemiluminescence based, sandwich nucleic acid hybridization procedure for quantitation of HIV-1 RNA using synthetic oligonucleotide capture probes. The detection range of this technology ranges from 75-500,000 HIV-1 RNA copies/ml.

ACKNOWLEDGEMENTS

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OBJECTIVES

To compare 3 different technologies, Ultra sensitive Amplicor HIV-1 monitor Test (v1.5, Roche), NucliSens HIV-1 QT (bioMerieux) and Versant HIV-1 RNA bDNA assay (v3.0, Siemens) in parallel to assess their concordance and reproducibility in quantitation of plasma HIV-1 RNA levels.

METHODS

- Whole blood EDTA specimens from 72 HIV-1 infected individuals were submitted to the Laboratory for Clinical and Biological Studies, University of Miami, FL for routine quantitation of plasma HIV-1 RNA.
- Plasma samples were separated in three aliquots for quantitation of viral load using three different methods, Ultra sensitive Amplicor HIV-1 monitor Test (v1.5, Roche), NucliSens HIV-1 QT (bioMerieux) and Versant HIV-1 RNA bDNA assay (v3.0, Siemens) in parallel.
- Results were compared and analyzed statistically using the SPSS software (v 14.0).

RESULTS

- Of the 72 plasma specimens, HIV-1 RNA was detected in 40 each by Amplicor and bDNA (56%) and 44 by NucliSens (60%).
- Comparison of HIV-1 RNA levels obtained using all the three technologies demonstrated a highly significant positive correlation ($P < 0.001$).
- Comparison of technologies on one to one basis showed a higher positive linear correlation between Amplicor and bDNA ($r = 0.995$) versus Amplicor and NucliSens ($r = 0.967$) or NucliSens and bDNA ($r = 0.975$) ($P < 0.001$).
- In case of plasma HIV-1 RNA levels less than 1000 copies/ml a highly significant positive correlation was observed between Amplicor and bDNA ($r = 0.647$, $P = 0.005$) as compared to Amplicor and NucliSens ($r = 0.036$, $P = 0.889$).
- Evaluation of methods for reproducibility showed a low coefficient of variation in all three methods; NucliSens (Mean CV = 1%), Amplicor (Mean CV = 12%) and bDNA (Mean CV = 8%).
- Comparison of mean HIV-1 RNA levels showed no significant difference between the three methods (One way ANOVA, $P = 0.488$).

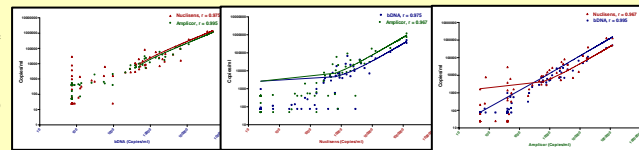


Figure 1: Dot plots showing correlation coefficient and linear regression analyses when plasma HIV-1 RNA levels were compared in parallel using the three technologies. As expected, all of the technologies showed highly significant correlation ($P < 0.001$), with Amplicor and bDNA showing a very good correlation with each other ($r = 0.995$).

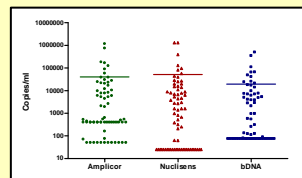


Figure 2: Scatter plots showing distribution of plasma HIV-1 RNA levels obtained in 72 plasma specimens using the three technologies, ultra sensitive Amplicor HIV-1 monitor Test (v1.5, Roche), NucliSens HIV-1 QT (bioMerieux) and Versant HIV-1 RNA bDNA assay (v3.0, Siemens).

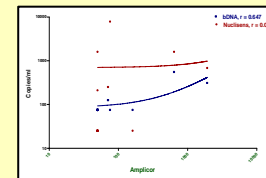


Figure 3: Comparison of 3 technologies when plasma HIV-1 RNA levels were below 1000 copies/ml. Amplicor and bDNA showed a very good concordance with each other versus Amplicor and NucliSens.

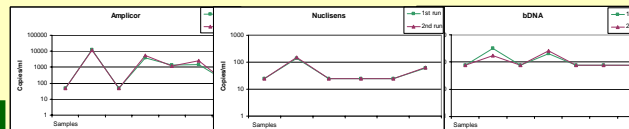


Figure 4: Performance of the three technologies, ultra sensitive Amplicor HIV-1 monitor Test (v1.5, Roche), NucliSens HIV-1 QT (bioMerieux) and Versant HIV-1 RNA bDNA assay (v3.0, Siemens). Plasma samples obtained from six HIV-1 infected individuals were repeated twice in different runs to measure consistency of results.

	N	Percent Detected (%)	Mean (copies/ml)	Std. Deviation (copies/ml)	Std. Error (copies/ml)	Minimum (copies/ml)	Maximum (copies/ml)
Amplicor	72	56	40284	165123	19459	50	1181905
NucliSens	72	60	51506	218356	25733	25	1300000
b DNA	72	56	19308	72576	8553	75	500000

Table 1: Descriptive comparison of plasma viral load values obtained from all of the 72 HIV-1 infected individuals on the three different technologies investigated.

CONCLUSIONS

- Viral load measurement at low copy numbers, especially below 1000 copies/ml is subject to constraints imposed by the inherent variability in the sensitivity of the assay technology.
- Our results have demonstrated that the three technologies produce concordant results at viral loads above 1000 copies/ml. Ultra sensitive Amplicor HIV-1 monitor Test and Versant HIV-1 RNA bDNA assay, in particular show excellent agreement with each other in quantitation of HIV-1 RNA.

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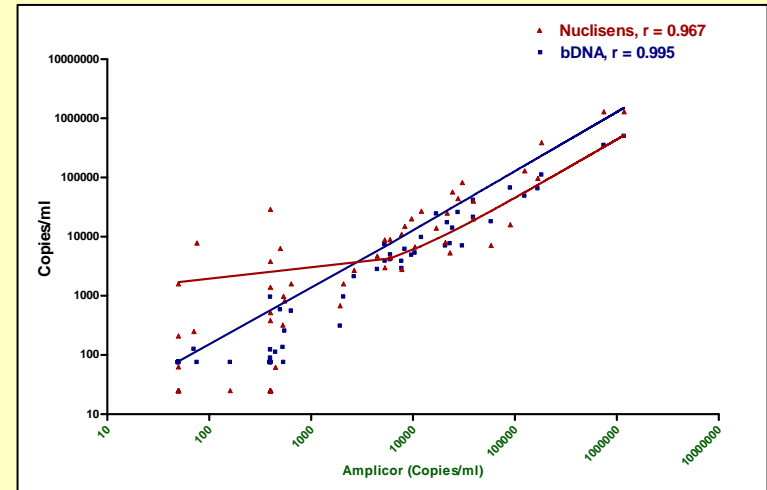
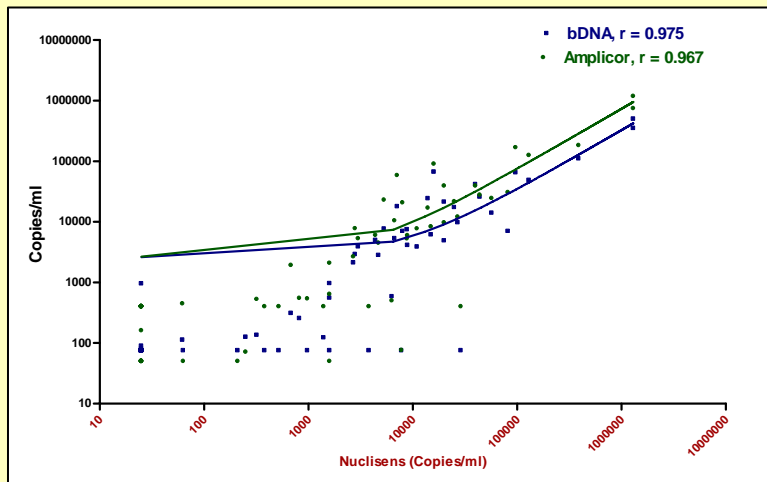
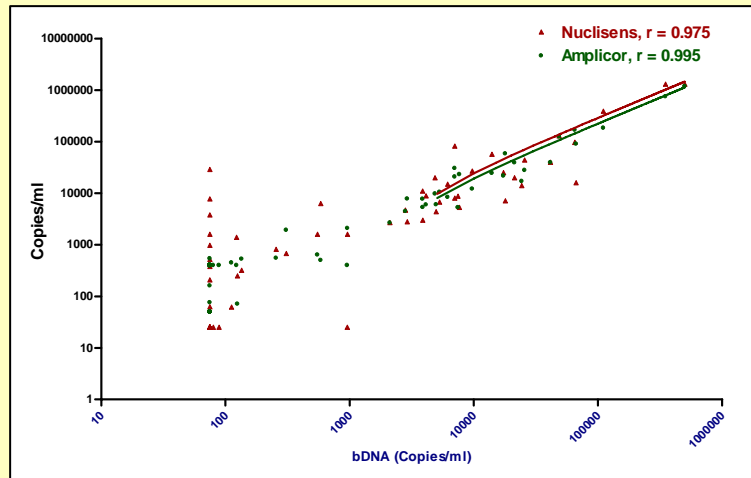
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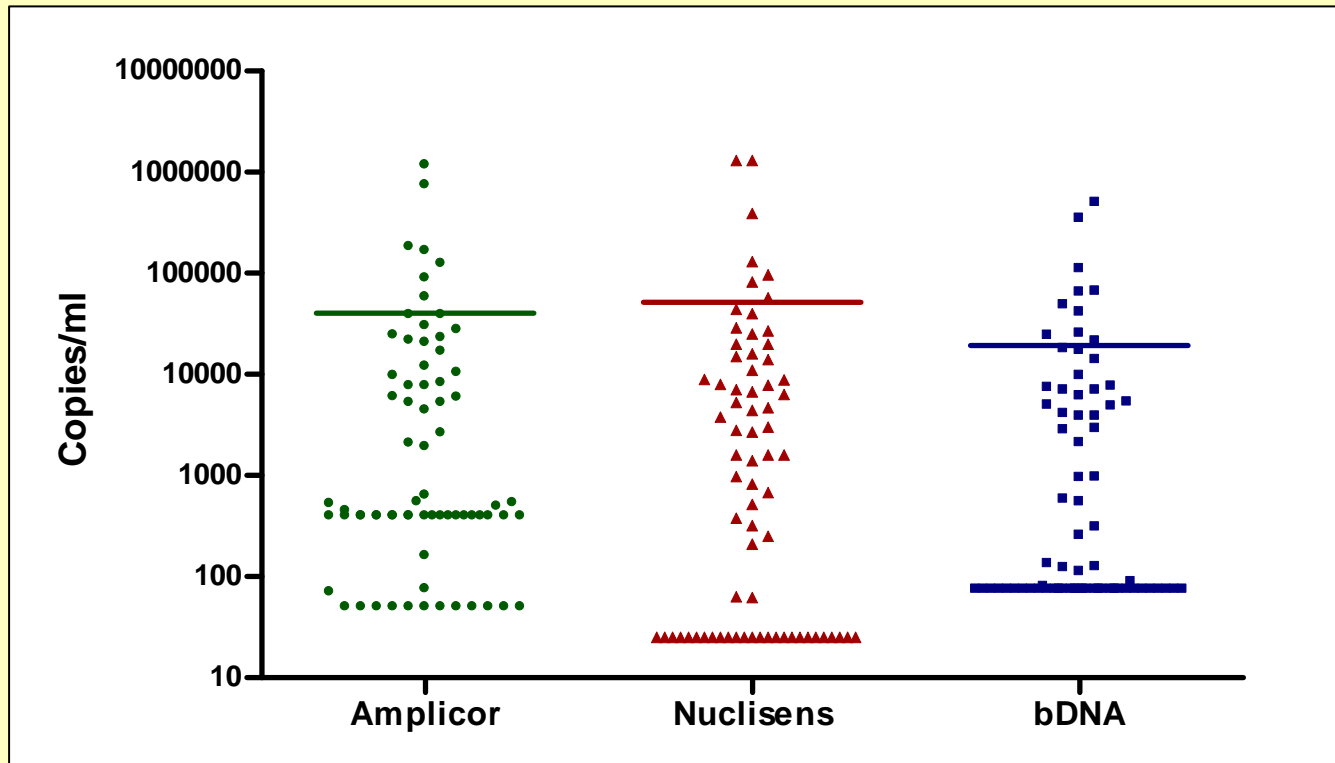
RESULTS

- 1. Of the 72 plasma specimens, HIV-1 RNA was detected in 40 each by Amplicor and bDNA (56%) and 44 by NucliSens (60%).**
- 2. Evaluation of methods for reproducibility showed a low coefficient of variation in all three methods; Nuclisens (Mean CV = 1%), Amplicor (Mean CV = 12%) and bDNA (Mean CV = 8%).**
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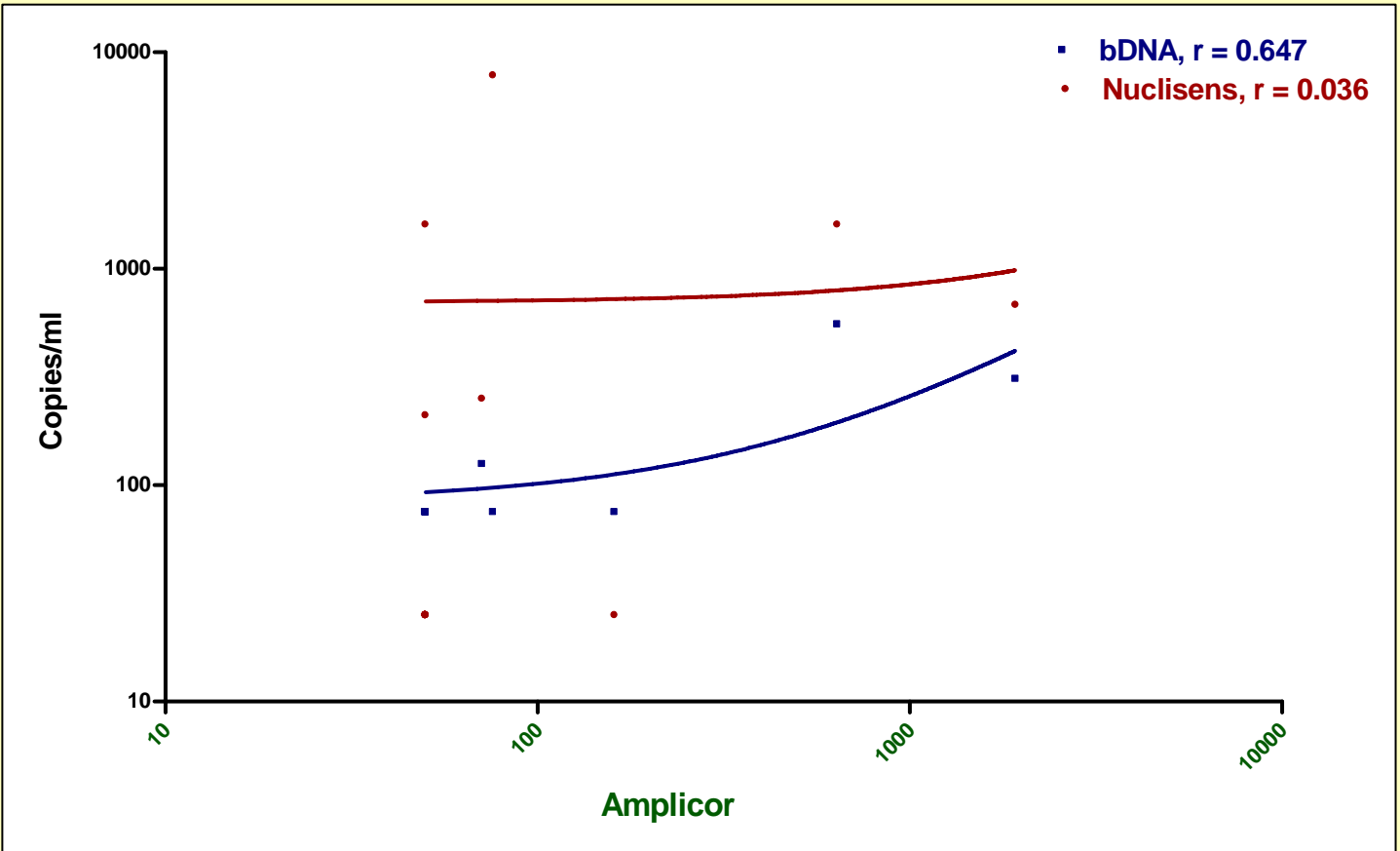
RESULTS



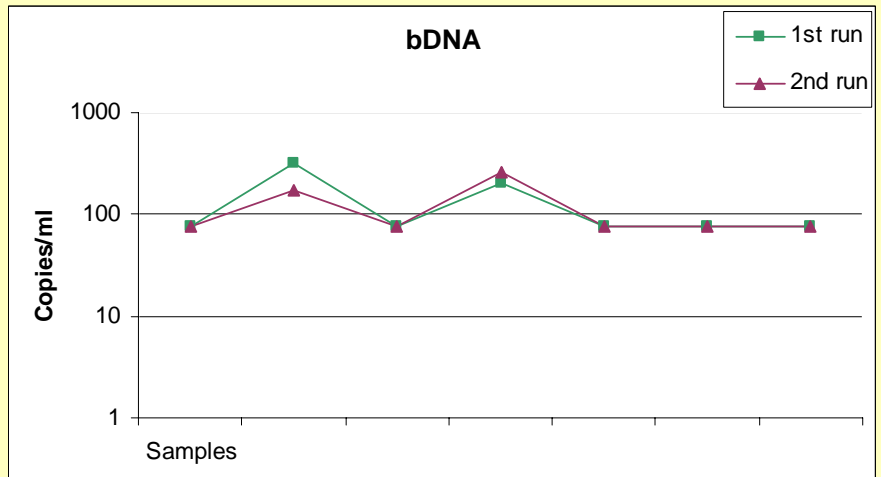
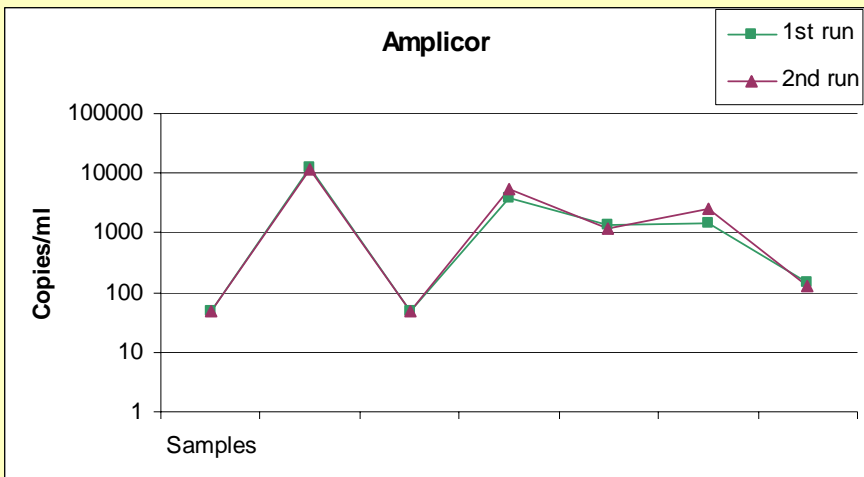
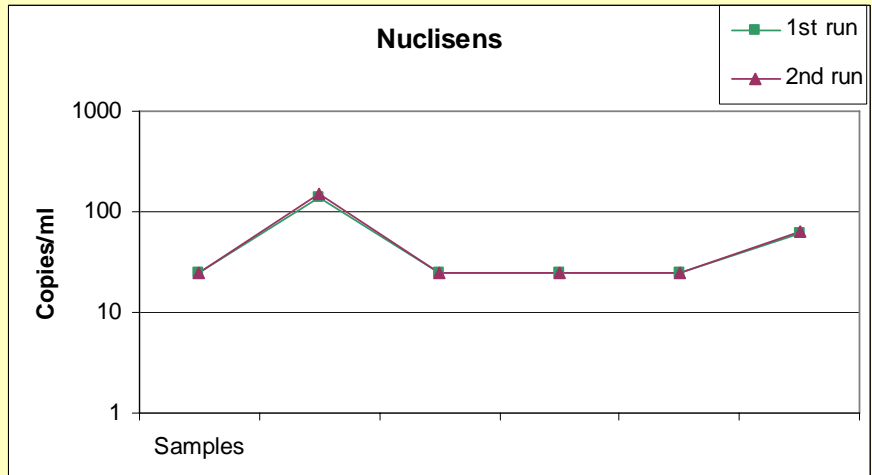
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Scatter plots showing distribution of plasma HIV-1 RNA levels obtained in 72 plasma specimens using the three technologies, ultra sensitive Amplicor HIV-1 monitor Test (v1.5, Roche), NucliSens HIV-1 QT (bioMerieux) and Versant HIV-1 RNA bDNA assay (v3.0, Siemens).



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Performance of the three technologies, ultra sensitive Amplicor HIV-1 monitor Test, NucliSens HIV-1 QT and Versant HIV-1 RNA bDNA assay. Plasma samples obtained from six HIV-1 infected individuals were repeated twice in different runs to measure consistency of results.

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