

Significant Differences Between Plasma HIV-1 RNA Assays in HIV-1 Subtype E Infected Patients Treated with Antiretroviral Therapy

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Initial CD4+ T-lymphocyte counts and plasma HIV-1 RNA are independently strong predictors of survival both in HIV-1 subtype B and non-B infected populations.¹⁻³ More importantly, both HIV-1 RNA plasma levels and CD4+ T-lymphocyte counts are the major markers for initiating and monitoring Antiretroviral (ARV) therapy.⁴⁻⁷ Thus plasma HIV-1 RNA assays should be accurate and reproducible. Of note, different plasma HIV-1 RNA assays perform differently even in HIV-1 subtype B infection, which is the bases for the development of most quantitative nucleic acid-based assays.^{1,2,8} Amplicor HIV-1 Monitor test version 1.0 values are consistently 2-2.5 folds higher than Quantiplex bDNA 2.0.^{1,2} Moreover, it is of importance for the international use that the assays detect the various HIV subtypes. Undetected viral subtypes lead to underestimation of the viral loads and therefore to suboptimal treatment. In Thailand the predominant HIV subtype is E.⁹⁻¹¹ In fact, all isolates of subtype E that have been sequenced to date represent

SUMMARY A total of 72 HIV-1 infected Thai patients treated with didanosine (ddI) or stavudine (d4T) plus ddI at the time of interim analysis were analyzed. Sixty patients (83%) carried subtype E documented by HIV-1 V3 serotyping. HIV-1 RNA levels were measured using three commercial viral load assays. At baseline (n = 57), Quantiplex 2.0 and NucliSens 2.0 showed mean log₁₀ HIV-1 RNA of 0.7 log₁₀ or 5 fold lower than Amplicor 1.5 (mean 4.29 versus 5.0 log₁₀, respectively, *p* < 0.001). At week 20 of treatment (n = 29), HIV-1 RNA levels were detected in 55.2%, 31%, and 33.5% of subjects tested by Amplicor 1.5, Quantiplex 2.0, and NucliSens 2.0, respectively. In conclusion: plasma HIV-1 RNA analyses showed comparable values with Quantiplex 2.0 and NucliSens 2.0 assays. In contrast, Amplicor 1.5 resulted in approximately 5 folds higher HIV-1 RNA levels and a 25% higher rate of detection of plasma HIV-1 RNA as compared to the other two assays. As the current goal of therapy is to suppress plasma viral load below the detection limit of the assays, the significant differences between the assays may influence antiretroviral efficacy evaluation and management.

mosaic genomes⁵ and are currently named CRF01_AE. The cytoplasmic domain of gp 41 is from subtype A.⁹⁻¹¹ The HIV regulatory regions and the long terminal repeat appear to be A/E mosaic and mainly A-associated in *gag* and *pol*.⁹⁻¹¹ The capacity for HIV subtypes to recombine or otherwise generate mosaic forms, such as the A/E mosaic, may affect the reliability of the commercially available assays. Most of the commercial plasma HIV-1 RNA assays were developed when little sequence information on non-B HIV-1 subtypes was available. Sev-

eral studies have shown that commercial plasma HIV-1 RNA assays vary in their ability to quantify different HIV-1 subtypes.¹¹⁻¹⁴

The first generation Amplicor HIV-1 Monitor test did not ac-

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curately quantify plasma HIV-1 RNA levels in individuals infected with subtypes A, E, F, G and H.¹⁴⁻¹⁶ The addition of a new primer set, designed for non-B HIV-1 subtypes, to the Amplicor HIV-1 Monitor assay v1.0 significantly improved the efficiency of quantification for these subtypes.¹⁵⁻¹⁶ Nevertheless, most of the plasma HIV-1 RNA assay comparisons in non-B subtypes were cross-sectional studies.¹⁷⁻¹⁹

We investigated the abilities and characteristics of 3 different plasma HIV-1 RNA quantification test kits, i.e. Quantiplex bDNA version 2.0, NucliSens NASBA version 2, and Amplicor HIV-1 Monitor test version 1.5 (added-in primer set) in a prospective Thai cohort, predominantly infected with HIV-1 subtype E, by comparing plasma HIV-1 RNA levels before and after initiation therapy with didanosine (ddI) or didanosine and stavudine (d4T).

MATERIALS AND METHODS

Patient population

For this study, plasma samples of patients who provided informed consent and participated in HIVNAT 002²⁰ were used. All patients were recruited from the Thai Red Cross Society's Anonymous STD/HIV screening clinic and the HIV outpatient immune clinic of King Chulalongkorn Memorial Hospital, Bangkok, Thailand. In HIV-NAT 002,²⁰ a randomized open-label dose-ranging study to assess the efficacy and optimal dosing schedule of didanosine (ddI) and stavudine (d4T), 72 HIV-infected, treatment-naïve Thai patients were enrolled. A safety interim analysis of the HIV-NAT 002 trial was done when the first patient reached week 24. Plasma samples were collected from all patients before therapy (week 0) and after initiation of therapy at weeks 4, 8, 16, and 20 up

until the time for interim analysis was reached. Whole blood was collected into blood collection tubes containing ethylenediamine tetra-acetic acid (EDTA) anticoagulant. Within 6 hours of collection, the plasma was separated by centrifugation at 400 x g for 10 minutes. Plasma samples were immediately stored at -70°C until processing.

HIV-1 serotyping

Of all patients in HIVNAT 002, HIV-1 subtypes were determined by HIV-1 V3 peptide-based enzyme-linked immunosorbent assays (ELISA) as previously described.²¹ Three different HIV-1 V3 loop peptides: TSITIGPGQVFYRTGD (subtype E, residues 309-324), KSIHLGPGQAWYTTQE (subtype B', residues 309-324), and YNKR-KRIHIGPGRAFYTTKN (subtype B_{MN}, residues 305-324) were used for the assays.

HIV-RNA quantification

Only HIV-1 subtype E infected samples, as determined by HIV-1 serotyping, were used for the comparison between the three plasma HIV-1 RNA assays. At the time of the interim analysis of HIV-NAT 002 trial, evaluable plasma samples for all three assays at week 0, 4, 8, 16 and 20 were 57, 50, 49, 39 and 29, respectively. A separate aliquot of plasma sample from each individual at each visit was analyzed for plasma HIV-1 RNA by different HIV-RNA quantification test kits, i.e. Amplicor HIV-1 Monitor test 1.5, Quantiplex bDNA 2.0, and NucliSens NASBA 2.0, according to the manufacturers' instructions. Each of the tests has a dynamic range of 400-750,000; 500-1,000,000; and 400-10,000,000 HIV-1 RNA copies/ml, respectively. All samples were analyzed in a blinded-fashion by the same person in the Cellular Immunology

laboratory of Department of Medicine, Faculty of Medicine, Chulalongkorn University. These samples had been stored for less than 9 months at -70°C.

Statistical analysis

For the analysis, data of only subtype E infected patients and samples tested with all three assays at each visit were used. Data are described as means (standard deviations). Spearman's Rho correlation coefficients for non-parametric data were calculated to describe the relation between the assays. Difference between assays in log₁₀ plasma HIV-1 RNA and percentages of undetectable patients were tested by paired t-tests and Chi-Square test, respectively. A *p* value < 0.05 was considered as significant.

RESULTS

Three hundred and nineteen blood samples from 72 participants in HIV-NAT 002 were obtained. HIV-1 subtypes were E in 60 (83%) and both E and B_{MN} in 5 (7%) patients. In 7 (10%) patients the HIV-1 subtype could not be identified by the HIV-1 V3 peptide-based serotypic assays. The baseline characteristics of the patients are listed in Table 1. Prior to commencement of the antiretroviral therapy the correlation coefficients between the three assays varied from 0.78 to 0.85 (*p* < 0.001) (Fig. 1). Among subjects infected with HIV-1 subtype E (*n* = 57), the corresponding mean ± SD log₁₀ HIV-1 RNA levels were 5.00 ± 0.63, 4.29 ± 0.72, and 4.29 ± 0.76 with Amplicor HIV-1 Monitor test 1.5 assay, Quantiplex bDNA 2.0, and NucliSens NASBA 2.0, respectively.

Plasma HIV-1 RNA levels measured with the Amplicor HIV-1 Monitor test 1.5 assay were signifi-

cantly higher than measured with the Quantiplex bDNA 2.0 (mean difference 0.71, 95% CI: 0.62 to 0.80) and NucliSens NASBA 2.0 (mean difference 0.71, 95% CI: 0.60 to 0.83). The mean difference of 0.70 log₁₀ of HIV-1 RNA copies/ml is approximately a 5-fold difference. Viral load measurements with Quantiplex bDNA 2.0

and NucliSens v2.0 were comparable (mean difference 0.01, 95% CI: -0.11 to 0.14).

The percentages of patients with undetectable plasma HIV-1 RNA at baseline (n = 57) were 0%, 5.3%, and 3.5% with Amplicor HIV-1 Monitor test 1.5, Quantiplex bDNA 2.0, and NucliSens NASBA

2.0, respectively. At week 20 of antiretroviral therapy (n = 29), the percentages of patients with undetectable plasma HIV-1 RNA with the Amplicor HIV-1 Monitor test 1.5, Quantiplex bDNA 2.0, and NucliSens NASBA 2.0 were 44.8%, 69%, and 66.5%, respectively (Table 2). Both, before and after treatment, the Amplicor HIV-1 Monitor test 1.5 yielded consistently higher plasma HIV-1 RNA results than Quantiplex bDNA 2.0 and NucliSens NASBA 2.0 assays (Fig. 2). The mean log reduction of plasma HIV-1 RNA from baseline at week 20 after treatment determined by Amplicor HIV-1 Monitor test 1.5, Quantiplex bDNA 2.0, and NucliSens NASBA 2.0 was 1.71, 1.14, and 1.28 log₁₀ copies/ml, respectively ($p < 0.001$, Amplicor 1.5 compared to the other tests).

DISCUSSION

In this HIV-1 subtype E infected cohort, the prospective com-

Table 1 Baseline characteristics of the study population (N = 72)

Characteristics	
Age, years	
mean (SD)	31 (7.6)
Gender, n (%)	
male	34 (47%)
female	38 (53%)
CDC stage, n (%)	
A	47 (65%)
B	25 (35%)
CD4 count, cells/ml	
median [IQR]	253 [208-302]
Viral subtype(s), n (%)	
E	60 (83%)
E + B _{MN}	5 (7%)
Unknown by serotyping	7 (10%)

Table 2 Characteristics of the assays, percentages undetectable and log₁₀ viral load at week 0, week 8, and week 20 of the treatment (Based on the same evaluable subjects for all 3 assays at each visit)

	Amplicor 1.5	Quantiplex 2.0	NucliSens 2.0
Dynamic range of the assay (copies/ml)	400-750,000	500-1,000,000	400-10,000,000
Week 0 (evaluable samples = 57)			
% undetectable	0.0 %	5.3%	3.5%
Log₁₀ copies/ml, mean ± SD	5.00 ± 0.63 ^{1,2}	4.29 ± 0.72 ³	4.29 ± 0.76
Week 8 (evaluable subjects = 53)			
% undetectable	30.6% ^{1,2}	73.5% ³	69.4%
Week 20 (evaluable subjects = 29)			
% undetectable	44.8%	69%	66.5%
log₁₀ copies/ml, mean ± SD	3.27 ± 0.88 ^{1,2}	3.06 ± 0.70 ³	2.95 ± 0.64

¹Difference between Amplicor and Quantiplex < 0.001

²Difference between Amplicor and Nuclisens < 0.001

³Difference between Quantiplex and Nuclisens is not significant

Difference between assays in log₁₀ viral loads and percentages of undetectable patients were tested by paired t-tests and Chi-Square test, respectively

parison of three assays for the determination of HIV-1 RNA in plasma demonstrated that the viral loads differ depending on the choice of assay. Amplicor HIV-1 Monitor test 1.5 with the added primer set, resulted in consistently and significantly higher plasma HIV-1 RNA levels and higher rates of detectable patients than Quantiplex bDNA 2.0 and NucliSens NASBA 2.0 assays, before and after initiation of therapy. The difference was much higher than those reported in studies performed with HIV-1 subtype B samples.^{1,2,15,16}

In subtype B studies, Amplicor HIV-1 Monitor test v1.0 values are consistently 2 to 2.5-fold or 0.3-0.4 \log_{10} higher than Quantiplex bDNA 2.0 values.^{1,2} Whereas, our results showed that Amplicor HIV-1 Monitor assay v1.5 yields a 0.7 \log_{10} higher level of plasma HIV-1 RNA or an approximately fivefold greater mean amount of copies/ml compared to Quantiplex bDNA 2.0 and NucliSens NASBA 2.0 assays. After 20 weeks of nucleoside therapy, the proportion of patients with plasma HIV-1 RNA levels below the limit of detection measured with Quantiplex bDNA 2.0 or NucliSens NASBA 2.0 assays was approximately 1.5 folds higher than measured with Amplicor HIV-1 Monitor test 1.5 (67% and 69% versus 44%, respectively (Table 2 and Fig. 2)). The Quantiplex bDNA 2.0 and NucliSens NASBA 2.0 assays were comparable, both in plasma HIV-1 RNA levels and percentages of samples with undetectable virus. However, the correlation coefficients between the three assays varied significantly from 0.78 to 0.87 ($p < 0.001$). Our study supports the results of Segondy *et al.*²² that a valid comparison of the virological efficacies obtained with different antiretroviral drug regimens requires the use of the same viral load quantitation procedure.

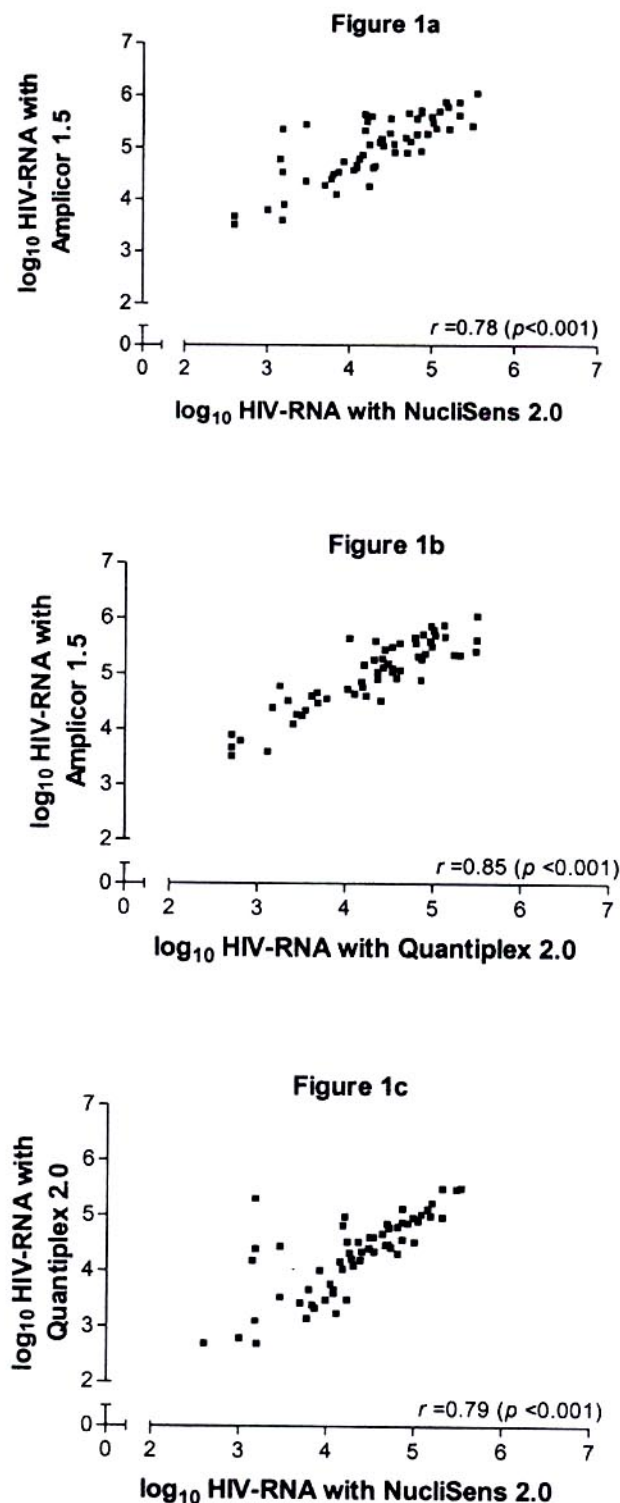


Fig. 1 The correlation of three commercial plasma HIV-1 RNA assays in patients infected with subtype E (N = 60).

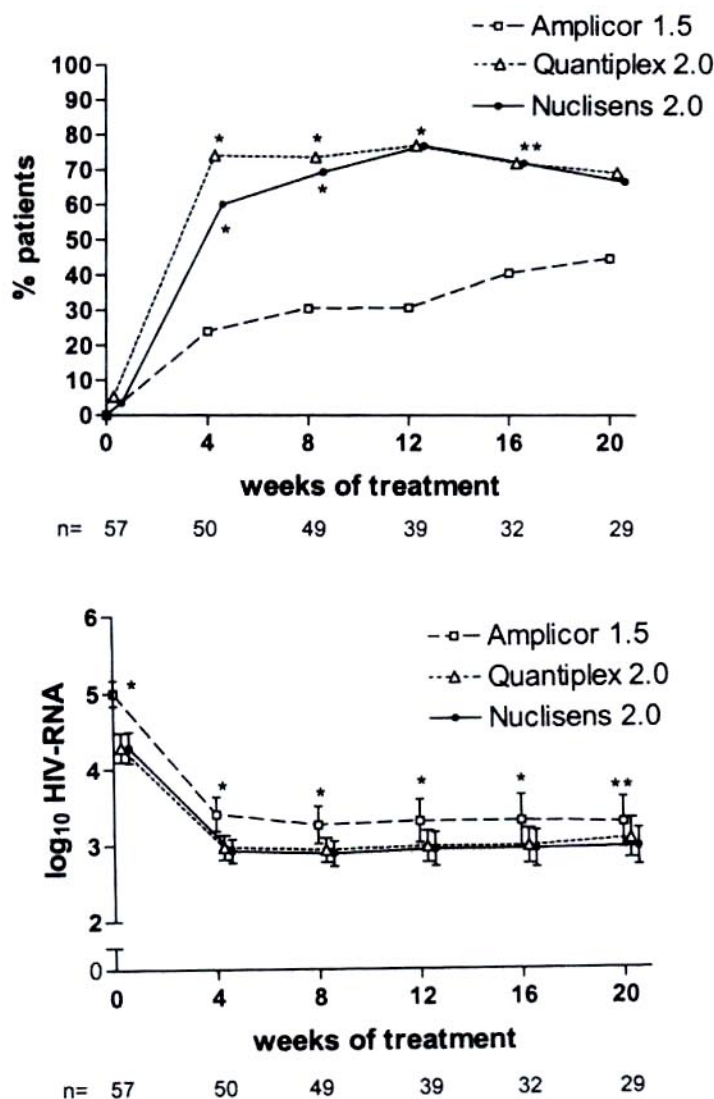


Fig. 2 Percentage of patients with plasma HIV-1 RNA below the limit of the detection of the assays (upper figure, * $p < 0.001$, ** $p < 0.05$, Chi-square test as compared with the Amplicor 1.5 results) and changes in \log_{10} HIV-1 RNA in copies/ml (lower figure, * $p < 0.001$, ** $p < 0.05$; paired Student t-test as compared to the Amplicor 1.5 results).

Based on 20 subtype E infected plasma specimens, Parekh *et al.*²³ showed that the correlation among different assays, especially between Quantiplex bDNA 2.0/NucliSens NASBA and Amplicor HIV-1 Monitor v1.0 versus NucliSens NASBA, was poor. The mean \log_{10} difference for subtype E

specimens was 0.4 between Quantiplex bDNA 2.0 and Amplicor HIV-1 Monitor v1.0 and only 0.08 between Quantiplex bDNA 2.0 and NucliSens.²³ This indicates that the less sensitive Amplicor HIV-1 Monitor v1.0 assay for subtype E resulted in a mean \log_{10} difference of 0.4 or approximately 2.5

folds higher than with Quantiplex bDNA 2.0. Triques *et al.*^{16,24} found that for samples infected with subtypes A and E, Amplicor HIV-1 Monitor 1.5 yielded a significant increase of more than 1 \log_{10} plasma HIV-1 RNA copies/ml (approximately 10 fold) compared to version 1.0 and 1.5.^{16,24}

Currently several more sensitive plasma HIV-1 RNA assays, some of which are commercially available (detection limit of 50 copies/ml) such as the Roche Amplicor Ultrasensitive assay and the Bayer Quantiplex HIV-1 RNA 3.0 (bDNA 3.0), are being used to monitor HIV-1 infected individuals on highly active anti-retroviral therapy (HAART). In a U.S. cohort, Elbeik *et al.*²⁵ found that bDNA 3.0 was approximately 2 times more sensitive than Quantiplex bDNA 2.0 and was comparable to the Roche Amplicor Ultrasensitive assay. In a study of 127 plasma samples from 57 Swiss patients infected with HIV-1 subtypes A, B, C and E, Schmid *et al.*²⁶ also demonstrated that the Amplicor HIV-1 Monitor Test 1.5 and the Bayer bDNA 3.0 assay were comparable. Based on the Swiss HIV cohort study, Amplicor HIV-1 monitor 1.5 appears to be the most sensitive assay compared to the ultra-sensitive reverse transcriptase assay, p24 antigen enzyme immunoassay, NucliSens NASBA, and Quantiplex bDNA 2.0 in persons infected with various subtypes.¹⁹ A study in Greece (HIV-1 subtype was not specified) showed that results generated with Quantiplex bDNA 3.0 or with Roche Amplicor Ultrasensitive 1.5 were approximately two-fold greater than those generated with Quantiplex bDNA 2.0. In contrast, Quantiplex bDNA 3.0 and Roche Amplicor Ultrasensitive 1.5 measurements were highly correlated ($r = 0.96$) and in good agreement (92.7%).²⁷

However, another study found that despite the improvements made to both the Roche Amplicor and the Chiron Quantiplex assays discordant results were detected between the two assays in 25.7% of cases, occurring with samples from all subtypes (A-E).²⁸

In conclusion, our results confirm that commercial plasma HIV-1 RNA assays vary in their ability to quantify different HIV-1 subtypes. Countries with a high prevalence of HIV-1 subtype E (or recently called CRF01_AE) such as Thailand, the Amplicor HIV-1 Monitor 1.5 resulted in an approximately fivefold higher mean plasma HIV-1 RNA level compared to Quantiplex bDNA 2.0 and NucliSens NASBA 2.0. Nonetheless, some studies suggest that Roche Amplicor HIV-1 Monitor 1.5 and the newest Quantiplex bDNA version 3.0 generated comparable results in non-B subtype infections including E. Thus, it is important to evaluate the performance of each assay before use in the quantification of plasma samples from patients infected with non-subtype B HIV-1 RNA. More importantly, beside the effort to improve the sensitivity of viral load assays against various HIV-1 subtypes, there is also the urgent need to make the tests accessible in harmony with the current effort of making HAART widely accessible to patients worldwide.

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