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Can HIV reverse transcriptase activity assay be a low-cost alternative for viral load monitoring in resource-limited settings?

Soham Gupta,[1] Riya Palchaudhuri,[1] Ujjwal Neogi,[2] Hiresave Srinivasa,[1] Per Ashorn,[3] Ayesha De Costa,[4] Clas Källander,[5] Anita Shet[6]

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For numbered affiliations see end of article.

Correspondence to
Soham Gupta;
soham.gupta@ki.se and
Ujjwal Neogi; ujjwal.neogi@ki.se

ABSTRACT

Objective: To evaluate the performance and cost of an HIV reverse transcriptase-enzyme activity (HIV-RT) assay in comparison to an HIV-1 RNA assay for routine viral load monitoring in resource limited settings.

Design: A cohort-based longitudinal study.

Setting: Two antiretroviral therapy (ART) centres in Karnataka state, South India, providing treatment under the Indian AIDS control programme.

Participants: A cohort of 327 HIV-1-infected Indian adult patients initiating first-line ART.

Outcome measures: Performance and cost of an HIV-RT assay (ExaVir Load V3) in comparison to a gold standard HIV-1 RNA assay (Abbott m2000rt) in a cohort of 327 Indian patients before (WK00) and 4 weeks (WK04) after initiation of first-line therapy.

Results: Plasma viral load was determined by an HIV-1 RNA assay and an HIV-RT assay in 629 samples (302 paired samples and 25 single time point samples at WK00) obtained from 327 patients. Overall, a strong correlation of r=0.96 was observed, with good correlation at WK00 (r=0.84) and at WK04 (r=0.77). Bland-Altman analysis of all samples showed a good level of agreement with a mean difference (bias) of 0.22 $\log_{10}$copies/mL. The performance of ExaVir Load V3 was not negatively affected by a nevirapine/efavirenz based antiretroviral regimen. The per test cost of measuring plasma viral load by the Abbott m2000rt and ExaVir Load V3 assays in a basic lab setting was $36.4 and $16.8, respectively.

Conclusions: The strong correlation between the HIV-RT and HIV-1 RNA assays suggests that the HIV-RT assay can be an affordable alternative option for monitoring patients on antiretroviral therapy in resource-limited settings.

Trial registration number: ISRCTN79261738.

Strengths and limitations of this study

- To the best of our knowledge, the current study is the most thoroughly evaluated study of ExaVir Load V3 HIV reverse transcriptase-enzyme activity (HIV-RT assay) from India to date.
- This study was performed in a large number of patients in a longitudinal manner looking into the effects of the non-nucleoside reverse transcriptase inhibitors-based therapy and drug resistance mutations on the performance of the HIV RT-enzyme activity assay.
- Though the assay has been validated before in other non-C subtype settings, in this study the HIV-RT assay was validated on a larger scale in a subtype C predominant setting and subtype C is the most predominant subtype globally, more so affecting the resource-limited settings.
- This study was limited to patients hailing from Southern India and a performance evaluation and cost-effectiveness of the HIV-RT assay needs to be accessed on a national level.

survival.[1 2] Expanding access to ART in resource-limited settings along with close monitoring is needed for successful treatment outcomes. In high income settings, this is achieved by performing quantitative viral load monitoring every 3–6 months,[3] as viral load monitoring detects early treatment failure. However, in resource-limited settings, therapeutic outcome is evaluated either on the basis of a CD4 T cell count or clinical findings,[4] neither of which accurately predicts viral suppression.[5] Early detection of viral failure by monitoring the viral load also provides the opportunity to intensify adherence counselling to improve adherence to ART, potentially leading to resuppression of viral load before the evolution of drug-resistant virus can take place.[6]

The currently used viral load assays are based on the amplification of HIV-1 virion

INTRODUCTION

The principal aim of antiretroviral therapy (ART) is durable suppression of replicating plasma virus to undetectable levels, thereby delaying disease progression and prolonging

RNA, which is considered impractical for wide-scale use in resource-limited settings, as it requires infrastructure, facilities for molecular diagnostics, expensive equipment and skilled technicians, which are often unavailable.[7] Simpler, less expensive viral load assays would be very useful in the resource-limited environments that are most impacted by this epidemic.

An alternative to measure the HIV-1 RNA is to measure the activity of the viral reverse transcriptase (RT) enzyme. The ExaVir Load assay (Cavidi, AB, Sweden), a low-cost and technically less demanding assay using an ELISA-based method to measure RT enzyme activity, has shown promising results.[8–19] Although previous versions of the test have been evaluated against several PCR-based HIV tests that measure viral RNA, there are few comparative studies between ExaVir Load V3 and such molecular real-time assays.[14 16 18] The most recent V3 of the ExaVir Load assay has an enhanced sensitivity (lower detection limit 200 copies/mL) and reduced turnaround time compared to V2.[14 18]

HIV-1 subtype C (HIV-1C) is the dominant strain in most low and middle income countries like India, South Africa and Ethiopia,[20] and the need for a simple low cost viral load monitoring tool is a priority in these settings. Data available from countries dominated by the HIV-1C epidemic are limited. Where available, evaluations have been mainly performed with earlier version assays (ExaVir Load V1 and V2), which had lower detection limits of 400 copies/mL.[7 11 21 22] Studies evaluating Exavir Load V3 have been mainly performed in non-C dominated countries.[14 16 18 19]

Thus, we aimed to evaluate the performance of the HIV RT-enzyme activity (HIV RT) assay (ExaVir Load V3) in comparison to a 'gold standard' HIV RNA load (Abbott m2000rt real-time PCR) assay in a cohort of patients before and after initiation of first-line ART in Indian settings. We also compared viral load measurements from both assays in a subset of patients with drug-resistant mutations at baseline. Further, we studied the difference in costs of the two viral load assays in the context of our laboratory setting from a provider perspective.

MATERIALS AND METHODS
Study participants and samples
Between April 2010 and September 2011, EDTA plasma samples were collected from HIV-1-infected adult patients attending the Infectious Disease Clinic, St. John's Medical College and Hospital, Bangalore (main site) and ART centre, Krishna Rajendra Hospital, Mysore (peripheral site) enrolled in the HIVIND randomised controlled trial (Trial registration: ISRCTN79261738).[23] All the patients included in the study initiated ART with reverse transcriptase inhibitor (RTI) drugs, that is, two nucleoside reverse transcriptase inhibitors (NRTI), zidovudine (AZT) or stavudine (d4T)

with lamivudine (3TC) + one non-nucleoside reverse transcriptase inhibitor (NNRTI), either nevirapine (NVP) or efavirenz (EFV) as per the standard national AIDS programme guidelines.[24]

Plasma samples were collected at two time points; (1) prior to ART initiation (not longer than 3 months prior) and (2) 4 weeks after ART initiation. The plasma samples were separated within 6 h of EDTA whole blood collection, aliquoted and stored at −80°C in the main site. Plasma samples aliquoted in the peripheral site were stored at −20°C and transported to the main site on dry ice (every 2 weeks) and then stored at −80°C prior to testing.

Ethical statement
Ethical approvals for the conduct of the trial were obtained from the Institutional Ethical Review Board of St John's Medical College Hospital, Bangalore (IERB 1/ 369/08-92/2008) and Krishna Rajendra Hospital, Mysore (NO/PS/173/2010). All patients participating in the HIVIND study have given their written consent.

Plasma HIV-1 RNA assay
Plasma HIV-1 RNA load was measured in the patient cohort using the Abbott Real-Time PCR, m2000rt system with a manual RNA extraction procedure on an m2000sp sample preparation system as per the manufacturer's instructions. The assay was performed using an initial volume of 0.2 mL plasma, which provides limits of quantification between 150 copies/mL (lower limit of detection) and 10 000 000 copies/mL (upper limit of detection). In every run, a negative control, a low positive control and a high positive control supplied in the Abbott Realtime HIV-1 control kit were included. This measure using Real-Time PCR was considered as the gold standard. This protocol was validated by an external quality control programme by the Quality Control for Molecular Diagnostics, Glasgow, Scotland (QCMD, http://www.qcmd.gov) on a 2010 panel (Consisting of four HIV-1B samples, two HIV-1C samples, one HIV-1A/ G sample and one HIV-1 negative sample) and obtained a highly satisfactory score.

Plasma HIV RT enzyme activity assay
The viral RT enzyme activity was quantified using Cavidi ExaVir Load V3 as per the instructions of the manufacturer.[14] In an ELISA-based format, the RT activity of the RT enzyme recovered from 1 mL of patient plasma was determined and compared to a standard curve based on known amounts of an HIV 1 recombinant RT constructed from the BH10 isolate. The RT activity in the sample was expressed as femtogram HIV-1 RT activity/mL (fg/mL) plasma that was translated into RNA copies/mL equivalent by the ExaVir Load analyser software. The lower detection limit of the assay was ≥1fg/mL that is, ≥200 copies/mL. In every run, a single positive control and a negative control were used to monitor the performance of the RT extraction process

and to access between run variations. The positive control was prepared by pooling the EDTA plasma from a high HIV-1 viral load sample and HIV-1 negative plasma; 1.2 mL was aliquoted and stored in a −80°C freezer. The laboratory personnel running the ExaVir Load V3 assay were blind to the plasma HIV-1 RNA values.

HIV-1 subtyping and baseline drug resistance

Genotypic resistance testing (GRT) was performed on the baseline plasma samples. Briefly, the reverse transcriptase (RT) region of the HIV-1 pol gene was amplified and sequenced using the primers described by us previously.[25] HIV-1 subtyping was determined on the basis of the *pol* gene as well as the *env* gene (wherever sequence data were available).[26–28] using the maximum likelihood (ML) phylogenetic tree based on reference sequences downloaded from the Los Alamos Database (http://www.hiv.lanl.gov). Primary drug resistance analysis was evaluated using the WHO list of mutations from 2009 (WHO_SDRM 2009).[29]

Comparative cost analysis

We did an analysis comparing the costs between Abbott m2000rt and ExaVir Load V3 from a provider (laboratory service provider) perspective. We used costs from our lab for this purpose. Costs considered included annuitised capital costs for the two different instruments including operator-supplied instruments. These are instruments which are necessary in case of a new laboratory. In case of Abbott, the operator-supplied instruments included single-channel micropipettes, two dry baths and a vortex. For ExaVir Load V3, they included micropipettes (both single-channel and multichannel), an ELISA plate reader, an incubator, a rocker and a vortex.

Costs for start-up kits, human resource costs (including time for training), annual maintenance, reagents and other consumables were also considered. We assumed the working life for Abbott m2000rt and ExaVir Load V3 to be 5 years, and a discount rate of 5% was applied.

Assumptions

Number of patients: There are at present 1500 ART-experienced patients in our ART centre. Assuming that viral load monitoring of these patients will require to be performed every 6 months, there will be 3000 samples a year.

Maintenance costs: In the case of Abbott m2000rt the costs for servicing, maintenance of instrument and calibration of laser head. In the case of Abbott m2000rt the annual maintenance cost included the costs for servicing, maintenance of instrument and calibration of laser heads.

Human resource skills and training requirements: From our experience, 1 month was required to train a technician on Abbott m2000rt. One week was required for training on ExaVir load V3. We also considered that the technician handling Abbott m2000rt would require to be more senior and experienced (salary $300 per month) compared to the technician working with ExaVir Load V3 (salary $200 per month).

Time for each method of testing: For Abbott m2000rt, a batch of 24 reactions which comprise 21 samples and 3 controls will involve a total time of 8 h (from the beginning of RNA extraction up until obtaining results), of which 5 h involve the technician's time. For Exavir Load V3, the assay is performed in batches of 30 samples. Though the turnaround time is 48 h, it involves 5 h of actual hands-on time per batch. Costs for time of the technicians were calculated against the salaries mentioned above.

In the cost comparison analysis, we did not consider costs associated with the sample collection, storage and transportation as these are common to both tests.

Statistical analysis

All statistical analyses were performed after the HIV RT and HIV-1 RNA level values were $\log_{10}$ transformed. For analysis, the lower limit of detection of the HIV RT assay (≤200 copies/mL) was considered; samples showing <200 copies/mL by any of the assays were assigned a value of 199 copies/mL. With Exavir Load V3, we achieved a varying upper detection limit ranging from >360 000 to >770 000 in different runs. Thus, samples with a viral load of >360 000 (the lowest range of the upper detection limit obtained for ExaVir Load V3) by any of the assays were assigned a viral load of 360 000 copies/mL. The diagnostic agreement between the HIV RT assay and the HIV-1 RNA assay at different viral load cut-offs was determined from the κ statistic. Pearson's correlation coefficient (r) was calculated to study the correlation between $\log_{10}$ HIV RT activity (copies/mL equivalents) and $\log_{10}$ HIV RNA (copies/mL). However, since this coefficient does not take into account the possibility that one measure may differ consistently from the other, we further assessed the level of agreement using pairwise Bland-Altman plots. This plot compares the measures between the two tests by plotting the difference in the two VL measures against the average of the two measures.

RESULTS

Sample characteristics

Plasma viral load was obtained from 629 samples collected from 327 HIV-1-infected adult patients, of which 302 were paired (before ART and 4 weeks after ART initiation). HIV-1C was the predominant subtype observed in 98.1% (313/319) of the patients. Six out of 319 genotyped patients (1.9%) showed the presence of non-C subtype strains, namely BC recombinant (1), BD recombinant (1), A1C recombinant (2) and HIV-1A1 (2). Eleven patients (3.4%) showed the presence of single primary drug-resistant mutations, with six samples

Table 1 Comparison of HIV-1 plasma VL levels measured by the HIV RT and HIV-1 RNA assays by ART status, HIV-1 subtypes and RT-drug resistant mutations

Sample type	Number of samples	Mean viral load±SD in $\log_{10}$copies/mL		Mean $\log_{10}$ viral load difference±SD in $\log_{10}$copies/mL
		ExaVir Load V3	Abbott m2000rt	
All samples	629	3.98±1.3	4.19±1.3	0.22±0.3
ART Status				
Naïve (baseline at WK00)	327	5.07±0.6	5.33±0.5	0.25±0.3
Experienced (WK04)	302	2.79±0.5	2.97±0.6	0.19±0.4
Subtype at WK00 (n=319)*				
C	313	5.08±0.6	5.33±0.4	0.25±0.3
Non-C	6	5.05±0.5	5.3±0.4	0.25±0.3
DRMs at WK00 (n=319)*				
Wild type (no DRM)	308	5.07±0.6	5.32±0.5	0.25±0.3
NRTI mutations	6	5.41±0.3	5.55±0.01	0.15±0.3
NNRTI mutations	5	5.26±0.2	5.62±0.2	0.36+0.2

*Genotyping performed only in baseline samples.
ART, antiretroviral therapy; DRM, drug-resistant mutations, NNRTI, non-nucleoside reverse transcriptase inhibitors; NRTI, nucleoside reverse transcriptase inhibitors; RT, reverse transcriptase; VL, viral load.

harbouring NRTI-associated drug-resistant mutations (DRMs) and five samples with NNRTI-associated mutations (table 1).

Comparison between HIV RT activity and HIV-1 RNA load assay

There were 54 samples (8.5%) that were quantifiable by the HIV-1 RNA assay but were below the detection limit of the HIV RT assay (table 2). At a lower limit of quantification of 200 copies/mL, 90.7% of the samples showed a quantifiable virus by the HIV RT assay. The percentage of the samples with a quantifiable viral load by the HIV RT assay increased with higher viral load cut-offs by the HIV-1 RNA assay as shown in table 2. Overall, there was acceptable agreement observed between the HIV RT and HIV-1 RNA assays, with excellent agreement observed at higher values of plasma viral load ≥3.0 $\log_{10}$-copies/mL (κ=0.76). Of all the samples, 81.7% (514/629) had viral load values by the HIV RT assay, which differed by<0.5 $\log_{10}$ units from the HIV-1 RNA values, while 99.2% (624/629) of the samples differed by <1.0 $\log_{10}$ units.

A strong positive correlation was observed between the plasma viral load values by the HIV RT and HIV-1 RNA assays (r=0.96) in all the samples. A good correlation was

noted in ART-naïve samples (r=0.84) as well as in samples at week 04 of ART (r=0.77; figure 1).

Bland-Altman plots for all the samples showed good levels of agreement with a mean difference (bias) of 0.22 $\log_{10}$copies/mL, with acceptable limits of agreement (−0.45 and +0.89 $\log_{10}$copies/mL). A good level of agreement was also observed separately at baseline (mean difference bias of 0.25; range of acceptable limit of agreement: −0.39 and +0.89 $\log_{10}$copies/mL) and at WK04 (mean difference bias of 0.19; range of acceptable limit of agreement: −0.52 and +0.89 $\log_{10}$copies/mL; figure 2).

## Influence of current ART and drug-resistant mutations on RT-enzyme activity

Table 1 shows that the mean $\log_{10}$ difference between the HIV RT and HIV-1 RNA assays both before and after initiation of ART was not significantly different (<0.25 $\log_{10}$ copies/mL) but was well within the clinically accepted limit of 0.5 $\log_{10}$copies/mL. Thus, the performance of the HIV RT assay is not affected by the presence of the NNRTI (nevirapine/efavirenz)-based ART regimen. Also, the presence of either NRTI-associated DRMs (n=6; M41L: 1, D67N: 1, T69D: 1, M184I: 1 and T215S: 2) and NNRTI-associated DRMs (n=5; Y181C: 1, K101E: 1 and K103N: 2) showed an acceptable change

Table 2 Agreement between the HIV RT assay and HIV-1 RNA assay at different PVL levels

| PVL by HIV-1 RNA In copies/mL ($\log_{10}$ copies/mL)	Agreement	κ Value	Number of samples detected by Abbott m2000rt	Percentage of Samples detected by ExaVir Load V3
≥200 (2.3)	89.1	0.46	580	90.7
≥400 (2.6)	88.1	0.57	550	93.5
≥1000 (3.0)	89.7	0.76	458	97.6
≥5000 (3.7)	94.4	0.89	344	99.7
≥10 000 (4.0)	96.8	0.94	324	100

PVL, plasma viral load; RT, reverse transcriptase.
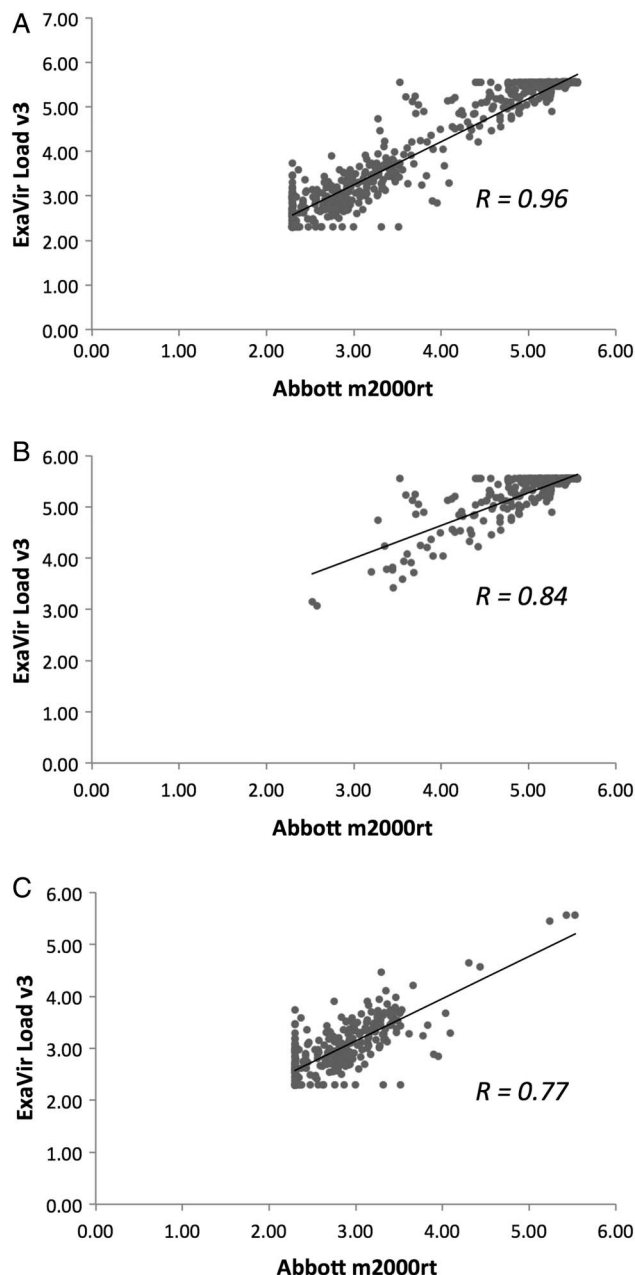
Figure 1 Correlation between the Abbott m2000rt and ExaVir Load V3 assays for (A) all 629 samples showing r=0.96 (B) 327 baseline (WK00) samples showing r=0.84 and (C) 302 4-weeks post-ART (WK04) samples showing r=0.77. ART, antiretroviral therapy.

(<0.4 $\log_{10}$ copies/mL) in mean $\log_{10}$ difference from the corresponding value among wild types. Although the samples with mutations are small, it indicates that the presence of NRTI and NNRTI DRMs did not negatively impact the test performance.

Cost comparison of the assays

The laboratory cost of viral load monitoring of HIV-infected patients analysed in our cohort by both Abbott m2000rt (HIV-1 RNA) and ExaVir Load V3 (HIV RT) is shown in table 3. The per test cost of the
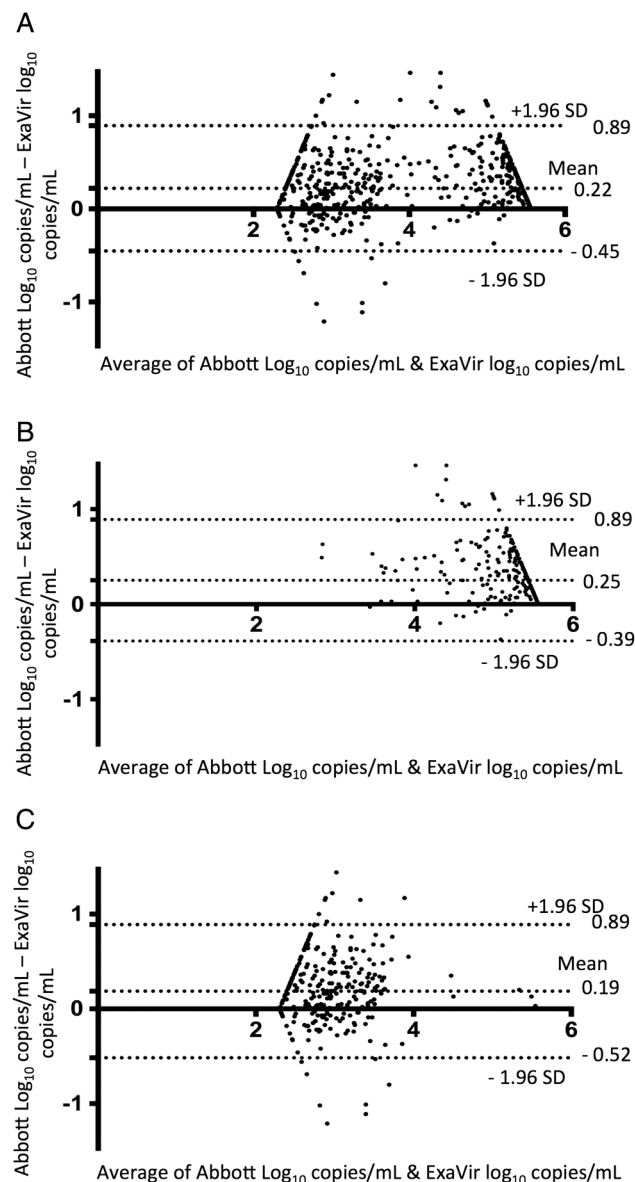
Figure 2 Bland-Altman plot with 95% CI of limits of agreement between HIV-1 viral loads measured with the Abbott Real-Time m2000rt assay and the ExaVir Load V3 assay for (A) all 629 samples showing a mean bias of 0.22 with 95% limits of agreement ranging from −0.45 to 0.89 (B) 327 baseline (WK00) samples showing a mean bias of 0.25 with 95% limits of agreement ranging from −0.39 to 0.89 (C) 302 4 weeks post-ART (WK04) samples showing a mean bias of 0.19 with 95% limits of agreement ranging from −0.52 to 0.89. ART, antiretroviral therapy.

plasma viral load measured by Abbott m2000rt and ExaVir Load V3 was $36.4 and $16.8, respectively. Thus, by using ExaVir Load V3, $19.6 per test can be saved. In a laboratory with a pre-existing basic set-up for ELISA-based assays, ExaVir Load V3 can be performed at $16.1, saving $20.2 per test. Most of the expense saved by using ExaVir Load V3 was due to (1) lower capital costs (instruments $37 750 against $2000) (2) the lower cost of the assay reagents ($15/test against $31/test; table 3).

Table 3 Cost comparison between the HIV RT assay and HIV-1 RNA assay for a laboratory doing 3000 tests/year

Cost items ($)	Abbott m2000rt Cost ($)	ExaVir Load V3 Cost ($)
Annuitised costs of capital instruments	8719	462
Annuitised cost of operator supplied instruments	293	1540
Annual maintenance cost	1126	666
Costs of kits per year	93 000	45 000
Consumables per year	4616	1846
Training time for lab staff to run the test	300	50
Salary costs (3000 tests per year)	1200	1000
Total ($)	109 634	50 534
Cost/test ($)	36.4	16.8

US$1=60 INR.
RT, reverse transcriptase.

DISCUSSION

A good correlation between the HIV RT and HIV-1 RNA assays was observed in the current HIV-1C predominant setting in India. The agreement between the tests was not significantly affected by the NRTI/NNRTI-based antiretroviral regimen used. Earlier studies performed on panels of different subtypes and recombinants have suggested that the HIV RT assay detects all HIV-1 and HIV-2 subtypes with similar efficiency.[14 15 30] This assay, ExaVir Load V3, can therefore be an attractive option for viral load monitoring in Indian settings.

This study compared the ExaVir Load V3 assay with the Abbott m2000rt HIV-1 RNA assay and observed an excellent correlation (r=0.96). An earlier study from London comparing the same tests observed a similar correlation (r=0.94).[14] Strong correlations between the ExaVir Load V3 and Roche HIV-1 RNA-based assays have also been observed by two other studies, by Greengrass et al[18] from Australia (Roche Cobas Amplicor; r=0.85) and Huang et al[16] from China (Roche Cobas TaqMan 48; r=0.95). Neither of these studies was performed in HIV-1C dominated settings. The HIV RT assay showed a good agreement with the HIV-1 RNA assay at the clinically important viral load threshold of 1000 copies/mL, which is used by the WHO to define viral failure to first-line therapy and is also most often used as the cut-off for drug resistance genotyping.[31] The performance of the Exavir Load below 1000 copies/mL is moderate.

In general, we observed an underestimation of viral load of 0.22 $\log_{10}$ RNA copies by the HIV RT assay, which is similar to what has been observed in other studies.[14 16 32] These two surrogate assays use very different methods for quantifying the plasma viral load. The HIV-1 RNA assays quantify the amount of viral RNA irrespective of RNA functionality, while the HIV RT assay quantifies the amount of active RT enzyme. The calibration constant used to translate RT activity into RNA copies was estimated from a study of an Australian cohort[18] and is not completely accurate for all combinations of HIV RNA assays and cohorts with varying subtype compositions. The variation observed is, however, well within the acceptable limit of <0.5 $\log_{10}$ copies.

NNRTI drugs bind to the RT enzyme, inhibit its activity and prevent viral replication. Several articles have discussed the possibility that enzymatically inactive RT drug complexes could result in under quantification of RT in relation to RNA.[29 30 33] These studies were, however, cross-sectional and never found any evidence for reduced RT activity during NNRTI therapy. In contrast to previous cohorts, the longitudinal sampling in our study provides optimal material for evaluation of the effects of NNRTI-based drug regimens. When comparing HIV viral load data from the same patient cohort before and after onset of ART, we found a mean $\log_{10}$ difference between ExaVir Load V3 and Abbott m2000rt of 0.25 for naïve patients and 0.19 for experienced patients (table 1). Thus, the difference between the tests did not increase after onset of therapy. This supports evidence that the current NNRTI containing therapy does not adversely influence the recovery of RT enzyme activity.

On a small number of samples, we assessed if the presence of drug-resistant mutations decreased the RT fitness so as to influence the performance of the HIV RT assay. We had six samples with single NRTI mutations and five samples with single NNRTI mutations and observed no evidence that their presence caused any significant difference in the association between RT enzyme activity and HIV-1 RNA load. These results were not unexpected and support evidence from previous studies by Napravnik et al and Rooijen et al indicating that the presence of NRTI or NNRTI mutations do not affect the relationship between RT enzyme activity and HIV-1 RNA load.[34 35] Resistance to NRTIs is mediated by a primitive DNA editing function that is introduced into the HIV RT by certain mutations. An energy dependent base excision reaction removes the last base in the growing DNA chain. This requires an energy donator, usually ATP or GTP, and might decrease RT reaction velocity. This happens readily in vivo, but the reaction conditions in the current RT assay do not support this reaction.[36]

To the best of our knowledge, this is the most thoroughly evaluated study of ExaVir Load V3 from India to date. Thus far, there have been three comparative studies from India that have been reported from the states of Andhra Pradesh (Anantpur),[37] Tamil Nadu (Chennai)[7] and New Delhi.[32] Iqbal et al[7] from Chennai cross-sectionally evaluated the ExaVir Load assay V1 and Roche Amplicor Monitor assay. They found a good agreement between the two tests and a significant inverse correlation between ExaVir Load and CD4+ T-cell count. Alvarez-Uria et al[37] from Anantpur

compared the accuracy of ExaVir V3 with Roche Cobas TaqMan HIV-1 test and Roche Amplicor HIV-1 DNA assay for early infant diagnosis. ExaVir performed well showing 100% sensitivity and 99% specificity, but no quantitative correlations were evaluated. A more recent study by Kokkayil et al[32] compared ExaVir Load V3 with Roche Cobas TaqMan among 75 ART naïve patients and reported no statistically significant difference between the two assays.

There are a few drawbacks of the HIV RT assay. The long turnaround time of 48 h makes it appear labour intensive, though the actual hands-on time is approximately 5 h. The prolonged incubation time is critical to achieve assay sensitivity. For standard performance, the assay requires 1 mL of plasma, which is high in comparison to the requirement for HIV-1 RNA assays, thus limiting its possible usefulness in paediatric populations. However, a recent study by Greengrass et al[19] observed that sample volumes down to 0.25 mL with VL >800 copies/mL can be utilised for paediatric monitoring. The ExaVir Load assay does not provide a standard positive and negative control, thus requiring the lab to prepare its own controls, which may compromise the quality assurance of the assay. Additionally, we noted that the quality of the water used for washing is important as impurities and bacterial contaminants present in water may contain polymerases which can create background noise and increase the level of the lower detection limit.

In spite of these caveats, the HIV RT assay has advantages over the HIV-1 RNA assays in resource-limited settings because it is an ELISA-based assay and can be performed in any routine lab at a lower cost. The ExaVir Load assay requires a cheaper and maintenance free start-up kit as compared to real-time assays. We observed that performing the HIV RT assay routinely in our centre would save us $20.2 per sample as compared to an HIV-1 RNA assay. A more basic laboratory, which requires installing basic ELISA equipment, would save $19.6 per sample (table 3).

The use of CD4 cell count as a prognostic marker has been debated; it is argued that this count may not reflect the actual viral load status of the patient.[38] The cost associated with viral load monitoring using HIV-1 RNA assays, despite being lower than PCR assays, is a major limiting factor for its implementation. Currently, in India, viral load testing has been phased in to support patients failing first-line ART. In the year 2012, about 4157 viral load tests were performed under the National AIDS Control Organisation (NACO).[39] Considering that $19.6 could have been saved per sample by performing an HIV RT assay, the cost saving for these 4157 viral load tests could have amounted to $ 81 477 if an HIV RT assay had been used.

In our cost-comparison analysis, we have used a provider perspective (lab service). We acknowledge that this is a narrower perspective than a societal one, which would include patient costs, opportunity costs among other costs. However, the purpose of our analysis was to provide information to laboratories in resource constrained settings, often faced with decisions in the face of tight budgets, and thus a societal perspective was not considered necessary. A laboratory manager faced with a limited budget would concentrate entirely on costs that have an immediate impact on her/his own budget; this is the perspective adopted in this study.

NACO is now considering taking up the monitoring approach recommended by the WHO to diagnose and confirm ART failure. Considering that there are currently 604 987 HIV-1-infected individuals receiving first-line ART at 380 centres spread across the country, the cost reduction of utilising HIV RT compared to HIV RNA plasma load can be substantial.[40]

Scaling up ART requires the critical support of HIV-1 viral load monitoring. Evidence from the comparative performance of the HIV RT assay with HIV-1 RNA assays from ours and other studies from India indicates that the ExaVir Load assay could serve as an affordable alternative to monitor patients on ART.

Author affiliations
[1]Division of Clinical Virology, Department of Microbiology, St. John's Medical College, Bangalore, India
[2]Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden
[3]Department for International Health, University of Tampere School of Medicine, Tampere, Finland
[4]Department of Public Health Sciences, Karolinska Institute, Stockholm, Sweden
[5]Cavidi AB, Uppsala, Sweden
[6]Department of Pediatrics, St John's Medical College Hospital, Bangalore, India

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