Performance of point-of-care Xpert HIV-1 plasma viral load assay at a tertiary HIV care centre in Southern India

Chinnambedu Ravichandran Swathirajan,1 Ramachandran Vignesh,1,2 Jayaseelan Boobalan,1 Sunil Suhas Solomon,1,3 Shanmugam Saravanan1,* and Pachamuthu Balakrishnan1

Abstract

Background. Sustainable suppression of HIV replication forms the basis of anti-retroviral therapy (ART) medication. Thus, reliable quantification of HIV viral load has become an essential factor to monitor the effectiveness of the ART. Longer turnaround-time (TAT), batch testing and technical skills are major drawbacks of standard real-time PCR assays.

Methods. The performance of the point-of-care Xpert HIV-1 viral load assay was evaluated against the Abbott RealTime PCR m2000rt system. A total of 96 plasma specimens ranging from 2.5 log_{10} copies ml^{-1} to 4.99 log_{10} copies ml^{-1} and proficiency testing panel specimens were used. Precision and accuracy were checked using the Pearson correlation coefficient test and Bland–Altman analysis.

Results. Compared to the Abbott RealTime PCR, the Xpert HIV-1 viral load assay showed a good correlation (Pearson r=0.81; P<0.0001) with a mean difference of 0.27 log_{10} copies ml^{-1} (95% CI, −0.41 to 0.96 log_{10} copies ml^{-1}; so, 0.35 log_{10} copies ml^{-1}).

Conclusion. Reliable and ease of testing individual specimens could make the Xpert HIV-1 viral load assay an efficient alternative method for ART monitoring in clinical management of HIV disease in resource-limited settings. The rapid test results (less than 2 h) could help in making an immediate clinical decision, which further strengthens patient care.

INTRODUCTION

Clinical management of HIV-1-infected individuals has always been of immense importance because of the absence of an effective vaccine or possible cure. With India being home to 2.1 million HIV-infected individuals [1], where HIV-1 subtype C is more predominant at >98% [2, 3], laboratory monitoring and quicker diagnosis are always demanded for better clinical management of the infected individuals. WHO estimates that at the end of 2015, 15 million HIV-infected individuals were receiving anti-retroviral therapy (ART) and further acceleration programmes have also been initiated for wider access to ART with the ‘test and treat’ strategy [4]. Since ART programmes are expanding, continued monitoring of HIV-infected individuals becomes significant to ensure treatment efficacy and to tackle HIV-1 drug resistance [5]. Poor sensitivity and specificity of clinical and immunological monitoring of HIV-infected individuals provides misleading information on treatment responses and delayed switching of anti-retrovirals [5, 6]. Hence, plasma viral load (PVL) quantification becomes an integral part of HIV clinical management.

PVL quantification provides information on ART efficacy and treatment failure [5, 7], and erroneous reports affect clinical decisions [8]. Hence, it becomes particularly important for accurate and sensitive PVL quantification assays. Data suggest that PVL of <50 copies ml^{-1} (1.7 log_{10} copies ml^{-1}) in plasma to be the best outcome of ART. Additional investigation is required if the PVL is >50 copies ml^{-1}, and a PVL of >1000 copies ml^{-1} is considered an indication of possible HIV resistance or treatment failure for the undergoing regimen [4, 8, 9].

There are many commercial PVL assays available with new assays being continuously introduced which differ in their detection ranges, turnaround-time (TAT), degrees of automation, efficiencies and costs. When switching from one assay to another, parameters such as inter-assay reproducibility and accuracies between the assays should be addressed. Most PVL assays require expensive laboratory infrastructure and well-trained individuals. Therefore, a PVL quantification assay which is simple, easy-to-perform and has short TAT is highly desired. Hence, in this study we...
evaluated the performance of the Xpert HIV-1 viral load assay on the GeneXpert Instrument Systems platform (Cepheid) with the Abbott RealTime PCR HIV-1 assay (Abbott Molecular), which is simple and easy to use, and could easily be an alternative for current larger analytical platforms [10].

METHODS
Specimens
This evaluation study was conducted in specimens collected between July 2015 and June 2016 from HIV-1 patients attending the Y. R. Gaitonde Centre for AIDS Research and Education (YRG CARE), a tertiary care centre for HIV-infected individuals in Chennai, Southern India. A total of 103 specimens that were tested by Abbott RealTime PCR as part of patient care services and had remaining samples stored in the freezer at \(-75\pm 5\) °C, were utilized for this validation anonymously without using patient identifiers. Of the 103 specimens, 11 specimens had a PVL of <3 \(\log_{10}\) copies ml\(^{-1}\) (<1000 copies ml\(^{-1}\)), 72 had a PVL of \(3 \leq \log_{10}\) copies ml\(^{-1}\) to \(<4 \log_{10}\) copies ml\(^{-1}\) (1000 copies ml\(^{-1}\) to <10,000 copies ml\(^{-1}\)), and 20 had a PVL of \(4 \leq \log_{10}\) copies ml\(^{-1}\) (\(\geq 10,000\) copies ml\(^{-1}\)). It was ensured that all the specimens were subjected to a single freeze-thaw cycle prior to testing using the Xpert HIV-1 viral load assay.

Plasma viral load quantification
Both assays were performed as per the manufacturers’ instructions. The Abbott RealTime HIV-1 assay combines automated plasma RNA extraction on the m2000sp system and real-time PCR amplification of the integrase gene fragment, with non-competitive fluorescence detection, on the fully automated m2000rt PCR system. For the Xpert HIV-1 viral load assay, 1 ml plasma specimen was transferred into the cartridge and loaded into the GeneXpert instrument (single module); RNA extraction, purification, reverse transcription and cDNA real-time quantification were performed in the completely automated cartridge system. Both systems have a linear detection range of 1.6–7.0 \(\log_{10}\) copies ml\(^{-1}\) (40 to 10,000,000 copies ml\(^{-1}\)).

Three samples from the Virology Quality Assurance (VQA) Program (Rush University Medical Center, Chicago, USA) which had viral loads of 50, 200 and 1500 copies ml\(^{-1}\), respectively, were tested. To check assay precision, two plasma specimens were tested five times each using the Xpert HIV-1 viral load assay. Ten plasma samples were tested using the recently installed GeneXpert four module system and the GeneXpert single module system in order to perform an intra-assay comparison. Similar evaluation criteria were applied for this comparison.

Statistical analysis
The Pearson correlation coefficient (\(r\)) was calculated to determine the linear relationship between the assays [11]. Bland–Altman analysis was used to assess the agreement between the two different assays for quantification of PVL, as well as to check the agreement between two GeneXpert instruments. All statistical analyses were performed using GraphPad Prism, Ver5.0.

RESULTS
A significant positive correlation (Pearson \(r=0.81; P<0.0001\)) was observed between Abbott RealTime PCR and Xpert HIV-1 viral load assays (Fig. 1). The Bland–Altman plot showed a positive bias with a mean difference of 0.27 \(\log_{10}\) copies ml\(^{-1}\) (95% CI, –0.41 to 0.96 \(\log_{10}\) copies ml\(^{-1}\)) and standard deviation (SD) of 0.35 \(\log_{10}\) copies ml\(^{-1}\) between the two assays (Fig. 2).

Out of 103 specimens tested, 98 (95.1%) were successfully quantified using the Xpert HIV-1 viral load assay, while there were no evaluable results available for five specimens (three with ‘error’ code and two with ‘invalid’ results). HIV-1 was not detected in one specimen and a repeat testing of a different aliquot of the same specimen using the Xpert HIV-1 viral load assay provided a result of 1.6 \(\log_{10}\) copies ml\(^{-1}\) (<40 copies ml\(^{-1}\)). Excluding the five specimens with no evaluable results and the specimen which was retested, a total of 96 specimens were subjected to validation analyses. A difference of >0.5 \(\log_{10}\) copies ml\(^{-1}\) between the assays was observed in 18 (18.75%) specimens. Compared to the Abbott RealTime PCR assay, the Xpert HIV-1 viral load assay over-estimated PVL levels in 85.5% of specimens tested.

The Xpert HIV-1 viral load assay quantified 83 (97.6%) specimens as \(3 \leq \log_{10}\) copies ml\(^{-1}\) (1000 copies ml\(^{-1}\); a threshold for clinical monitoring of ART) out of 85 specimens which were quantified in the same range by the Abbott RealTime HIV-1 assay. Similarly, only five (45.45%) specimens were quantified by the Xpert HIV-1 viral load assay to be within the detection limit of >1.6 \(\log_{10}\) copies ml\(^{-1}\) to <3 \(\log_{10}\) copies ml\(^{-1}\) (40–1000 copies ml\(^{-1}\)) out of 11 specimens quantified by the Abbott RealTime HIV-1 assay.

![Fig. 1. Correlation between Xpert HIV-1 viral load assay and Abbott RealTime PCR, m2000RT assay.](attachment:image)
Certified reference materials of the VQA Program were tested and the Xpert assay yielded results of 69, 162 and 1260 copies ml\(^{-1}\) for specimens containing 50, 200 and 1500 copies ml\(^{-1}\), respectively. Precision testing was performed with two plasma specimens repeatedly tested five times each, and showed excellent coefficient of variation (CV) of 1.9 and 1.07 %, respectively.

When GeneXpert single module and four module systems were compared, a strong positive correlation (Pearson \(r=0.95; \ P<0.0001\)) (Fig. 3) and a mean difference of 0.04 \(\log_{10}\) copies ml\(^{-1}\) (95 % CI, \(-0.13\) to 0.20 \(\log_{10}\) copies ml\(^{-1}\); SD, 0.08 \(\log_{10}\) copies ml\(^{-1}\)) were observed (Fig. 4).

**DISCUSSION**

Measuring HIV-1 RNA in plasma is an important marker to ensure successful treatment, identify adherence problems and determine whether ART regimens should be switched as it provides an early and more accurate indication of treatment failure. Hence, the continuous development of PVL assays which are accurate, automated, sensitive, easy to use, require less expertise, are less prone to contamination and have short TAT is highly desirable. In order to implement a new HIV-1 PVL assay for patient care, greater concordance and better determination of its analytical performance with the established quantification assays is required before putting the new assay into patient care.

The Xpert HIV-1 viral load assay is one such assay which matches the above criteria and, considering these credentials, evaluation of this assay is warranted. Overall correlations between the Xpert HIV-1 viral load assay and the Abbott RealTime PCR assay were good, with high significance with a better mean difference and concordance. In agreement with other studies, the Xpert HIV-1 viral load assay quantified 95.1 % of the cases [7, 10]. In 85.5 % of the cases, higher quantification levels were observed in Xpert HIV-1 viral load assay than Abbott RealTime PCR, which is consistent with other studies [8, 12, 13]. This raises a concern when the Xpert HIV-1 assay results in a viral load of >3 \(\log_{10}\) copies ml\(^{-1}\) since it is a clinically relevant threshold for detecting virological failure, which in turn might affect treatment decisions. However, as per WHO guidelines, a switch in ART regimen is recommended only when the PVL is above >3 \(\log_{10}\) copies ml\(^{-1}\) based on two consecutive visits after 3 months, with good adherence support [4]. This might rule out the scenario of misinterpreting virological failure when viral load is quantified using Xpert HIV-1 viral load assay which
also has significant inter-assay comparability with the Abbott RealTime assay. Delays in getting PVL reports by conventional assays lead to complications such as accumulation of resistance and exposure to failing therapy. As the Xpert HIV-1 viral load assay has the ability to provide results within 2 h of specimen collection, those circumstances are avoided, resulting in effective clinical decisions being made.

The Xpert HIV-1 viral load assay has an advantage over the Abbott RealTime PCR assay where the specimens can only be analysed in batches, which in turn affects TAT and patient management. Both extraction and amplification being automated and achieved in a single cartridge in shorter TAT makes the Xpert HIV-1 viral load assay an able diagnostic tool for demanding situations [10]. Moreover, the testing cost of the Xpert HIV-1 viral load assay does not differ significantly from the existing Abbott RealTime assay. The single-use cartridge-based Xpert HIV-1 viral load assay is unique and its simplified technical methodology, specimen requirement and ease of use signify this assay as a potent candidate for field testing and in locations where access to virological monitoring is difficult. This feature might help in decentralizing HIV care, especially in resource-limited settings where HIV patient management remains a complex issue [13].

In conclusion, factors such as simplicity, rapid results and reliability based on higher concordance with an existing assay, make the Xpert HIV-1 viral load assay a sought-after methodology for quantifying viral load in plasma specimens in resource-limited settings.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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