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Comparative performance of the new Aptima HIV-1 Quant Dx assay with three commercial PCR-based HIV-1 RNA quantitation assays

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ABSTRACT

Background: Quantitative measurement of HIV-1 RNA levels in plasma ('viral load') plays a central role in clinical management. The choice of assay platform can influence results and treatment decisions.
Objective: To compare the analytical performance of the new TMA-based Hologic Aptima® HIV-1 Quant Dx assay with that of three PCR-based assays: Abbott RealTime HIV-1, Qiagen Artus® HI Virus-1 QS-RGQ, and Roche CAP/CTM HIV-1 Test v2.
Study design: Assay performance was evaluated using Acrometrix HIV-1 RNA Standard panels; the 3rd WHO HIV-1 RNA International Standard (12–500 copies/ml; 6 dilutions; 9 replicates); and plasma samples from 191 HIV-positive patients.
Results: Aptima showed high (>0.99) precision, accuracy and concordance with the Acrometrix Standards across a wide dynamic range (2.0–6.7 $\log_{10}$ copies/ml). Variance caused up to 2.1 (Aptima), 1.7 (RealTime), 7.5 (Artus), and 1.9 (CAP/CTM) fold changes in the International Standard quantifications at 50–500 copies/ml. HIV-1 RNA detection rates in plasma samples were 141/191 (74%), 119/191 (62%), 108/191 (57%), and 145/191 (76%) for Aptima, RealTime, Artus and CAP/CTM, respectively. For categorising samples either side of 50 copies/ml, Aptima had excellent agreement with RealTime (kappa 0.92; 95% CI 0.87–0.98); lowest agreement was with Artus (kappa 0.79; 95%CI 0.70–0.88). Aptima quantifications were mean 0.12 and 0.06 $\log_{10}$ copies/ml higher compared with RealTime and CAP/CTM, respectively, and 0.05 $\log_{10}$ copies/ml lower compared with Artus. Limits of agreement were narrowest when comparing Aptima to RealTime.
Conclusions: The new Aptima HIV assay is sensitive, precise, and accurate. HIV assays exhibit discordance at low HIV-1 RNA copy numbers.

1. Background

HIV-1 RNA quantitation (viral load) is used to monitor treatment efficacy, helping clinicians make decisions regarding switching or continuing the current antiretroviral therapy (ART). According to HIV treatment guidelines, ART is considered effective when it leads to undetectable HIV-1 RNA in plasma, whereas results above 50 copies/ml may trigger further investigations [1–5]. Because this threshold is close to the lower limit of quantitation (LLOQ) of most commercially available assays (20–75 copies/ml), assay performance at low HIV-1 RNA levels can significantly influence management decisions during ART.

In the UK, nucleic acid amplification tests are standard practice for monitoring of HIV infection [1–4,6]. These assays are largely based on real-time PCR and share similar performance characteristics [7–11]. Recently the Hologic Aptima HIV-1 Quant Dx assay (Aptima HIV) became commercially available in the UK. Aptima HIV is based on real-time transcription mediated amplification (TMA), a technology with high sensitivity for detection of pathogen RNA [12,13].

While PCR-based assays have been evaluated side-by-side in many studies [10,14,15], the performance of Aptima HIV has not yet been compared with that of other assays. UK clinical laboratories

Abbreviations: AS, Acromterix HIV-1 standards; CV, coefficient of variation; IS, 3rd WHO HIV-1 International Standard; LLOQ, lower limit of quantitation; LTR, long terminal repeat; TMA, transcription mediated amplification.

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are required to validate assay performance prior to implementing any new test in to routine diagnostic use [16].

2. Objectives

The aim of this study was to compare the analytical performance of Aptima HIV with that of three PCR-based assays: Abbott RealTime HIV-1 (RealTime), Qiagen artus® HI Virus-1 QS-RGQ (Artus), and Roche COBAS® AmpliPrep/COBAS® Taqman HIV-1 Test v2 (CAP/CTM), with a particular focus on samples with low HIV-1 RNA copy number.

3. Study design

3.1. Viral load assays

All HIV-1 RNA assays were performed according to the manufacturer's instructions.

3.1.1. Aptima HIV

Plasma (0.75 ml) was transferred into a sample aliquot tube, vortexed and centrifuged at 1000 × *g* for 10 min prior to loading onto the Panther system which extracted HIV-1 RNA from 0.5 ml of plasma using automated target capture technology, followed by amplification and detection of HIV-1 long terminal repeat (LTR) and pol gene targets (Hologic Inc., San Diego, CA, USA). The reported LLOQ is 30 copies/ml with an upper limit of quantitation of 10^7 copies/ml [17].

3.1.2. RealTime

Plasma (1 ml) was aliquoted, vortexed and centrifuged at 431 × *g* for 5 min prior to loading into the Abbott m2000 sample preparation system which extracted HIV-1 RNA from 0.6 ml of plasma, followed by amplification and detection of the HIV-1 integrase gene on the Abbott m2000rt PCR instrument (Abbott Molecular, Inc., Des Plaines, IL, USA). The LLOQ is 40 copies/ml and the upper range of quantitation is 10^7 copies/ml [18].

3.1.3. Artus

RNA was extracted from 1.0 ml of plasma using the Qiagen QIAsymphony SP automated extractor followed by amplification and detection of HIV-1 LTR on the Qiagen Rotor-Gene Q real-time PCR instrument (Qiagen GmbH, Hilden, Germany). The LLOQ is 45 copies/ml and the upper range of quantitation is 4.5×10^7 copies/ml [19].

3.1.4. CAP/CTM v2

Plasma (1.0 ml) was transferred into an input S-tube and loaded onto the Cobas Ampliprep instrument where RNA was extracted from 0.85 ml of sample prior to automated amplification and detection of HIV-1 LTR and gag targets on the COBAS® Taqman Analyser (Roche Molecular Systems, Inc., Pleasanton, CA, USA). The LLOQ is 20 copies/ml and the upper range of quantitation is 10^7 copies/ml [20].

3.2. Evaluation of Acrometrix Standards (AS)

Linearity and accuracy of all four systems was assessed by analysing panels of AS (Acrometrix HIV-1 panel copies/ml, Life Technologies, Carlsbad, CA, USA) in the range 2.00–6.70 $\log_{10}$ copies/ml. Aptima HIV was further evaluated with triplicate samples constructed from an AS panel diluted 1:3 using Basematrix HIV-1 negative human plasma (SeraCare, Lifescience, US). Linear regression analysis was performed and concordance correlation coefficient calculated.

3.3. Evaluation of 3rd WHO International HIV-1 RNA Standards (IS)

Low-level precision of each assay was compared using IS (National Institute for Biological Standards and Control, UK) containing 185,000 IU/ml of HIV-1 subtype B. Nine replicates of 6 IS dilutions in Basematrix were independently extracted and tested on all four systems over three days. The dilutions contained 28, 56, 112, 224, 558 and 1116 IU/ml HIV-1, corresponding to 12.5, 25, 50, 100, 250 and 500 copies/ml, respectively (when using the Qiagen conversion factor of 1 IU/ml = 0.45 copies/ml). Coefficients of variation were calculated at dilution points above the highest LLOQ across the 4 assays (>45 copies/ml).

3.4. Evaluation of assay performance with external quality assurance (EQA) panels

A panel of 8 samples was commissioned in quadruplicate from Qnostics (Glasgow, UK) and analysed by all four HIV assays. The panel contained dilution series of subtypes B and C and a single A/G sample.

3.5. Evaluation of assay performance with clinical samples

3.5.1. Clinical samples

A total of 191 surplus plasma samples from HIV-positive patients attending for care at the Royal Liverpool University Hospital, UK between January and December 2013 were used in this evaluation. Samples were excluded from analysis if less than 5 ml of plasma was available. Plasma was separated within 4–6 h of collection and stored at −80 °C in four separate aliquots with a single freeze–thaw cycle prior to analysis on the four systems. HIV subtype was noted when available from routine HIV genotypic resistance reports. The subtype was assigned from protease (codons 1–99) and reverse transcriptase (codons 1–235) sequences using HIVdb program from Stanford University. HIV subtype was B for 45 patients, non-B for 44 patients (A/B = 1, A/C = 1, A/CRF01_AE = 1, C = 21, CRF01_AE = 7, CRF02_AG = 6, CRF02-AG/B = 1, D/A = 2, G = 2, H = 1, K/F = 1), and unknown for 102 patients.

3.5.2. Pair-wise comparison of assay performance

Agreement for HIV-1 RNA detection and for categorisation above or below the 50 copies/ml threshold was assessed by calculating the kappa value for each pair-wise comparison. Regression and Bland–Altman analysis were performed on quantitative results and differences were tested using paired *t*-tests. All analyses were conducted using Microsoft Excel 2010 and MedCalc software v13.3.0.

4. Results

4.1. Accuracy and linearity of assays across the dynamic range

All Aptima HIV measurements were within 0.24 $\log_{10}$ copies/ml of the Acrometrix target value and data were linear across the dynamic range (precision = 0.9977; accuracy = 0.9972; concordance = 0.9949). Results from all four assays were highly correlated (linear regression analysis; $R^2 > 0.99$) (Fig. 1).

4.2. Precision of HIV-1 RNA quantitation using low-level WHO International Standard (IS)

Aptima HIV detected HIV-1 RNA in 8/9 and 9/9 replicates with nominal values 12 and 25 copies/ml, respectively. Quantitative results were reported for 27% Aptima HIV, 33% RealTime, 38% Artus and 28% of CAP/CTM replicates at these two low-level dilution