COMPARATIVE PERFORMANCE OF THE NEW APTIMA HIV-1 QUANT DX ASSAY

with three commercial PCR-based HIV-1 RNA quantitation assays

INSTRUCTIONS: *Select the text area by each color-coded ▶ on this page to view the respective, full journal text in this document. To return to this infographic page simply tap the ⌂ button on each page.*

OBJECTIVE

Compare the analytical performance of Aptima HIV to 3 PCR-based assays:

- **Abbott Real-Time HIV-1 (RealTime)**
- **Qiagen artus® HI Virus-1 QS-RGQ (Artus)**
- **Roche COBAS® Malaprop/COBAS® Taqman HIV-1 Test v2 (CAP/CTM)**

Focus on samples with low HIV-1 RNA copy number

ASSAY EVALUATIONS

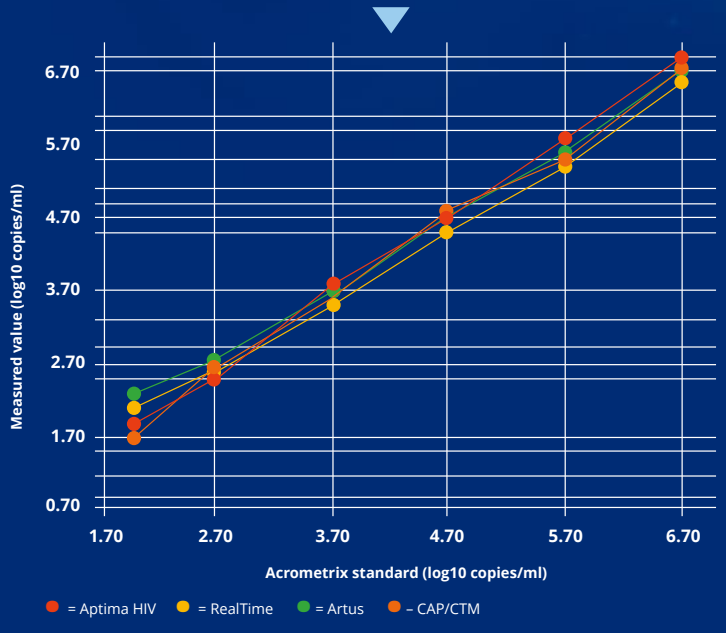
Assay evaluations performed according to manufacturer's instructions

- **AcroMetrix™ Standards (AS)**
- **Third WHO International HIV-1 RNA Standards (IS)**
- **External Quality Assurance (EQA) Standards**
- **Clinical Samples (CS)**

RESULTS

AS performance

Aptima HIV showed high precision, accuracy, and concordance with AS standards across a wide dynamic range (2.00–6.70 log10 copies/mL; R2 > 0.99)



IS performance

Aptima HIV detected HIV-01 RNA in 8/9 and 9/9 replicates with nominal values of 12 and 25 copies/mL

Aptima HIV quantitation of IS did not exceed 20% CV except at 50 copies/mL

	Coefficient of Variation (CV) (%)			
	Aptima HIV	RealTime	Artus	CAP/CTM
50 copies/mL	44	22	83	41
100 copies/mL	20	34	32	22
250 copies/mL	18	13	32	19
500 copies/mL	17	8	26	9

EQA performance

Aptima HIV was fully concordant with expected EQA results

A/G Subtype expected: 4.5 (log copies/mL)

Aptima HIV	4.79
RealTime	4.73
Artis	4.72
CAP/CTM	4.47

CS performance

Aptima HIV detection of HIV-1 RNA in clinical samples was similar to CAP/CTM

Aptima HIV	74%
RealTime	62%
Artis	57%
CAP/CTM	76%

CONCLUSIONS

Plasma viral load is a routine investigation for monitoring individuals infected with HIV-1.

HIV assays exhibit discordance at low HIV-1 RNA copy numbers.

Variation at low-copy number highlights the importance of selecting an assay with precision across the dynamic range, and of consecutive testing to confirm increases in HIV-1 RNA load.

Aptima HIV has excellent comparative performance across the metrics used in this study:

- **Accuracy**
- **Precision**
- **Subtype detection**
- **Clinical sample testing**

and provides a useful new tool for monitoring HV-1 RNA load in clinical laboratories.

Source: Hopkins M, Hau S, Tiernan C, et al. Comparative performance of the new Aptima HIV-1 Quant Dx assay with three commercial PCR-based HIV-1 RNA quantitation assays. J Clin Virol. 2015;69:56-62. doi: 10.1016/j.jcv.2015.05.020

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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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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].

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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].

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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.

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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

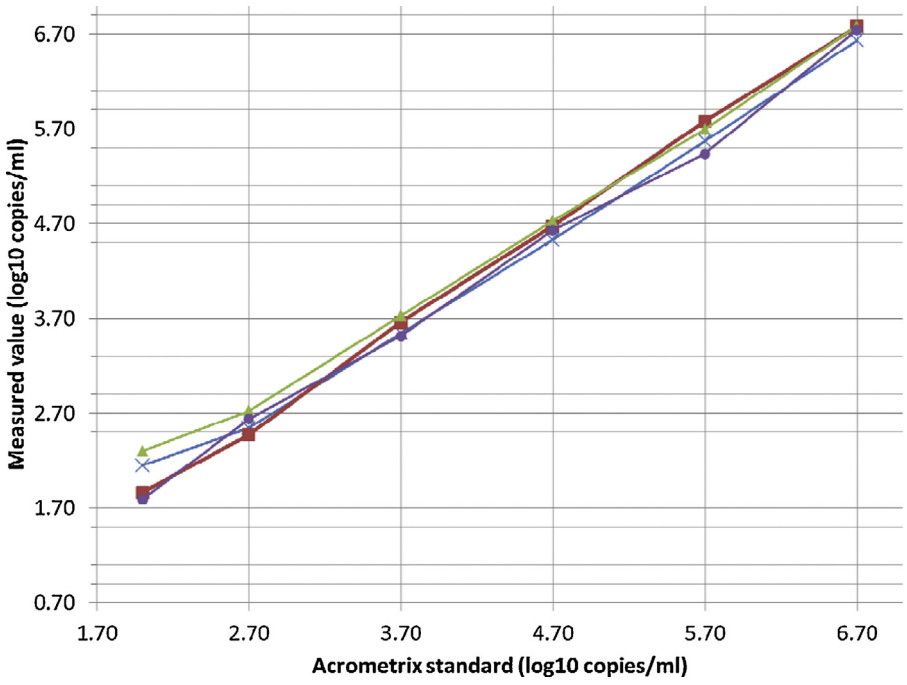


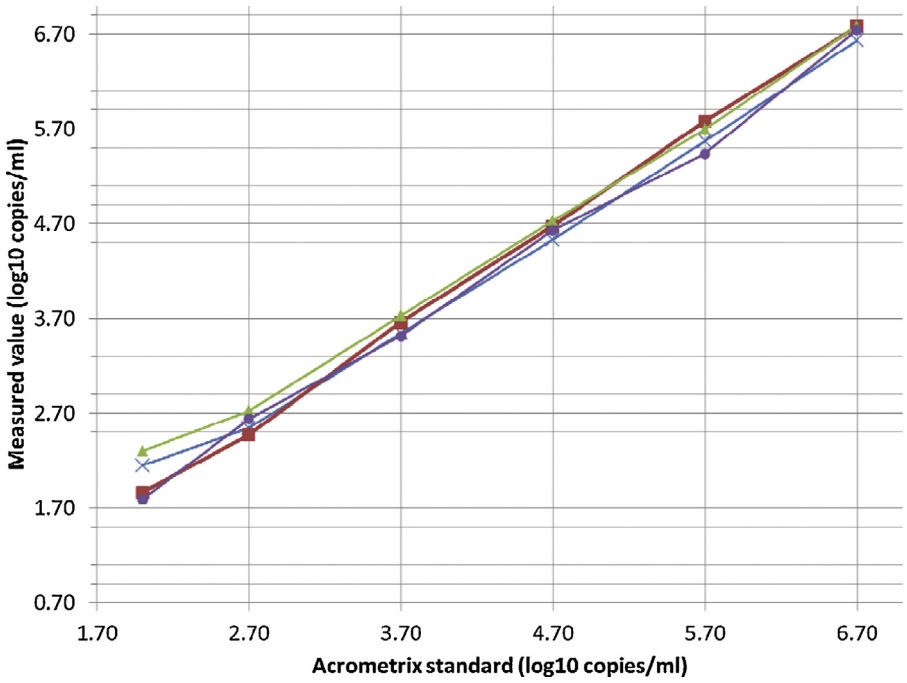
Fig. 1. Viral load measurements with Aptima HIV (square), RealTime (cross), Artus (triangle) and CAP/CTM (circle) for the Acrometrix standard panel ranging from 2.00 to 6.70 $\log_{10}$ copies/ml. The Pearson's correlation coefficient (R^2) = 0.9993, 0.9960, 0.9969 and 0.9966 for Aptima HIV, RealTime, Artus and CAP/CTM, respectively.

points. Analysis of replicates in the range 50–500 copies/ml showed precision decreased close to the LLOQ in all assays (Table 1). Aptima HIV coefficient of variation (CV) values were 18–44% for replicates in the range 250–50 copies/ml, although the lowest CV were seen at 500 copies/ml with RealTime (8%) and CAP/CTM (9%). The corresponding value for Aptima HIV was 17% which related to a 1.3 fold change in 95% CI and this increased to 2.1 at 50 copies/ml. Equivalent CV values using log transformed data of the four IS dilutions (1.7, 2.0, 2.4 and 2.7 $\log_{10}$ copies/ml) were in the range of 2.7–9.1%, 1.3–5.2%, 4.5–15.1% and 1.4–10.2% log copies/ml for Aptima HIV, RealTime, Artus and CAP/CTM.

Table 1
Detection and quantification of replicates (n = 9) of 3rd WHO HIV-1 RNA international standard diluted to nominal low-level copies/ml.

		Aptima HIV	RealTime	Artus	CAP/CTM
50 copies/ml	Quantified/detected (n)	8/9	8/9	7/9	9/9
	Mean (copies/ml)	62	75	109	64
	CV (%)	44	22	83	41
	SD	27	16	90	26
	95% CI	40–85	63–88	26–193	44–84
	Fold change 95% CI	2.1	1.4	7.5	1.9
100 copies/ml	Quantified/detected (n)	9/9	9/9	9/9	9/9
	Mean (copies/ml)	122	137	214	138
	CV (%)	20	34	32	22
	SD (copies/ml)	24	46	68	30
	95% CI	104–141	102–173	162–266	115–161
	Fold change 95% CI	1.4	1.7	1.6	1.4
250 copies/ml	Quantified/detected (n)	9/9	9/9	9/9	9/9
	Mean (copies/ml)	353	311	346	295
	CV (%)	18	13	32	19
	SD (copies/ml)	64	41	112	55
	95% CI	304–402	279–343	260–432	253–338
	Fold change 95% CI	1.3	1.2	1.7	1.3
500 copies/ml	Quantified/detected (n)	9/9	9/9	9/9	9/9
	Mean (copies/ml)	615	573	735	589
	CV (%)	17	8	26	9
	SD (copies/ml)	102	47	194	51
	95% CI	536–693	537–609	585–884	549–628
	Fold change 95% CI	1.3	1.1	1.5	1.1

Mean, 95% confidence intervals (CI), standard deviation (SD), coefficient of variation (CV) calculated only at the four dilutions with nominal values above the LLoQ of all assays.



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Mean, 95% confidence intervals (CI), standard deviation (SD), coefficient of variation (CV) calculated only at the four dilutions with nominal values above the LLoQ of all assays.

4.3. External quality assessment panels

Aptima HIV was fully concordant with expected EQA results (Table 2). Quantitation of the subtype B dilutions was reproducible using the Aptima HIV assay. Quantitation of subtype C samples varied between assays by up to 0.76 $\log_{10}$ copies/ml.

4.4. Comparison of assay sensitivity with clinical samples

A total of 191 samples from HIV-infected patients were analysed. Aptima HIV detected HIV-1 RNA in 74% samples, similar to the proportion reported positive by CAP/CTM (Table 3). When categorising clinical samples above or below the 50 copies/ml threshold, 59%, 59%, 56% and 55% were quantified as <50 copies/ml by Aptima HIV, RealTime, Artus and CAP/CTM, respectively (Table 4). Here, Aptima HIV agreement was highest with RealTime (kappa = 0.924). Further analysis within the range (50–500 copies/ml) demonstrated that 20 (11%), 19 (10%), 25 (13%) and 30 (16%) were quantitated by Aptima HIV, RealTime, Artus and CAP/CTM, respectively. Within these thresholds, Aptima HIV agreement was highest with RealTime (95%, kappa = 0.743) and lowest with CAP/CTM (88%, kappa = 0.497). The number of samples reported between 50 and 200 copies/ml was 16 (8%) Aptima HIV, 17 (9%) RealTime, 18 (9%) Artus and 22 (12%) CAP/CTM. Categorisation of clinical samples at the 200 copies/ml threshold is shown in Supplementary data.

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Linear regression analysis for all clinical results >LLOQ ($n = 87$ for Aptima HIV, 84 for RealTime, 86 for Artus and 107 for CAP/CTM) showed good correlation between Aptima HIV and the three PCR assays ($R^2 > 0.93$) (Supplementary data). Aptima HIV quantified significantly higher than RealTime ($P = 0.001$) with a mean bias of 0.12 $\log_{10}$ copies/ml (Fig. 2A). Overall, >93% of the paired Aptima HIV results fell within the 95% CI levels of agreement when compared with the PCR assays by Bland–Altman analysis, and limits of agreement were narrowest between Aptima HIV and RealTime at 0.49 log copies/ml either side of the mean. There were 5 discrepant samples outside the 95%CI level of agreement between Aptima HIV and RealTime (2 subtypes B, 2 C and 1 A/B), 5 between Aptima HIV and Artus (1 subtype B, 1 C, 1 D/A, 1 CRF01_AE and 1 unknown), and 3 between Aptima HIV and CAP/CTM (2 subtypes C and 1 CRF02_AG). Of these 11 discrepant samples, 5 had low copy numbers (<3 $\log_{10}$ copies/ml) and at least 3 were due to an unexpected result by another assay (Table 5).

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5. Discussion

HIV-1 plasma viral load is a routine investigation for monitoring of HIV-1 infected individuals. In a recent report, 77% (58/75) of laboratories reported using commercial real-time PCR assays [21]. Two of the principal real-time assays, Abbott RealTime and Roche CAP/CTM v2, are both FDA-approved and their performance is well documented in the literature [15,22–29]. Characteristics of the Qiagen Artus HIV-1 real-time PCR test launched in 2010 on the QS-RGQ system have also been described [10,30].

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DHHS refer to optimal suppression below the assay limit of detection, with virological failure as the inability to achieve or maintain HIV-1 RNA load below 200 copies/ml [3]. Both BHIVA and EACS utilise a threshold of 50 copies/ml to define suppression [1,5]. Here virological failure is defined as inability to achieve <50 copies/ml after 6 months of starting ART, or confirmed rebound >400 copies/ml after suppression below 50 copies/ml. A single blip between 50 and 400 copies/ml is not a cause for clinical concern if preceded and followed by viral loads below 50 copies/ml. Thus low level HIV-1 RNA quantitation impact on patient management.

Whilst inter-assay correlation was high overall, concordance was reduced closer to the lower limits of assay performance. This was emphasised with considerable disagreement between tests to designate complete viral suppression to undetectable levels: HIV-1 RNA was detected by at least one assay in 162/191 (85%) samples whereas the four assays agreed HIV-1 RNA was present in only 92 (48%). Hologic states a lower limit of detection of 13 copies/ml (IS) for the Aptima HIV assay and data presented here confirms the test is highly sensitive with clinical samples. Use of ultra-sensitive assays has demonstrated the presence of very low-level HIV-1 replication even in the presence of intensive therapy and the clinical significance of these findings has been reviewed elsewhere [34].

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4.6. Quantitative correlation between Aptima HIV and the PCR assays

Linear regression analysis for all clinical results >LLOQ ($n = 87$ for Aptima HIV, 84 for RealTime, 86 for Artus and 107 for CAP/CTM) showed good correlation between Aptima HIV and the three PCR assays ($R^2 > 0.93$) (Supplementary data). Aptima HIV quantified significantly higher than RealTime ($P = 0.001$) with a mean bias of 0.12 $\log_{10}$ copies/ml (Fig. 2A). Overall, >93% of the paired Aptima HIV results fell within the 95% CI levels of agreement when compared with the PCR assays by Bland–Altman analysis, and limits of agreement were narrowest between Aptima HIV and RealTime at 0.49 log copies/ml either side of the mean. There were 5 discrepant samples outside the 95%CI level of agreement between Aptima HIV and RealTime (2 subtypes B, 2 C and 1 A/B), 5 between Aptima HIV and Artus (1 subtype B, 1 C, 1 D/A, 1 CRF01_AE and 1 unknown), and 3 between Aptima HIV and CAP/CTM (2 subtypes C and 1 CRF02_AG). Of these 11 discrepant samples, 5 had low copy numbers (<3 $\log_{10}$ copies/ml) and at least 3 were due to an unexpected result by another assay (Table 5).

Comparison of RealTime or CAP/CTM to Artus also revealed 5 discrepant samples between each PCR assay compared (Table 5). Artus quantitation was significantly higher on average than RealTime ($P = 0.0001$) and CAP/CTM ($P = 0.002$). Mean differences for RealTime minus Artus, RealTime minus CAP/CTM and CAP/CTM minus Artus were −0.17, −0.05 and −0.14 log copies/ml, respectively (Fig. 2B).

5. Discussion

HIV-1 plasma viral load is a routine investigation for monitoring of HIV-1 infected individuals. In a recent report, 77% (58/75) of laboratories reported using commercial real-time PCR assays [21]. Two of the principal real-time assays, Abbott RealTime and Roche CAP/CTM v2, are both FDA-approved and their performance is well documented in the literature [15,22–29]. Characteristics of the Qiagen Artus HIV-1 real-time PCR test launched in 2010 on the QS-RGQ system have also been described [10,30].

The Aptima HIV-1 Quant Dx assay on the Panther system was approved for European use in the diagnosis and monitoring of HIV-1 infection in November 2014. It is the first commercially available real-time, TMA assay for quantitation of viral RNA levels and the evaluation presented here demonstrated Aptima HIV results are highly correlated with those obtained from real-time PCR technologies. The Aptima HIV test was highly accurate for quantitation of HIV-1 subtype B in the standard panels. Results were similar to those reported by Manak et al. where Aptima HIV quantification was comparable to RealTime and CAP/CTM for all major group M HIV-1 subtypes and four group O isolates [31].

Linearity can be affected by poor precision and this may be more evident close to the LLOQ of an assay. Overall, Aptima HIV quantitation of IS did not exceed 20% CV, except at 50 copies/ml where variation could represent up to two-fold change in viral load. Below 500 copies/ml, imprecision was observed with all assays but only Artus HIV was significantly less reliable. This is similar to the pattern described previously where Artus HIV had lower reproducibility than RealTime and CAP/CTM [15,32]. Whilst a higher number of replicates is needed to give a definitive calculation of total imprecision for each platform, the wide limits of agreement observed in Bland–Altman analysis of Artus measurements supports the greater likelihood of analytical variation using this test [33]. Mean quantitation of clinical samples was similar across all assays with observed bias <0.17 $\log_{10}$ copies/ml. However, it would be prudent not to switch assays interchangeably during patient monitoring where this can be avoided.

DHHS refer to optimal suppression below the assay limit of detection, with virological failure as the inability to achieve or maintain HIV-1 RNA load below 200 copies/ml [3]. Both BHIVA and EACS utilise a threshold of 50 copies/ml to define suppression [1,5]. Here virological failure is defined as inability to achieve <50 copies/ml after 6 months of starting ART, or confirmed rebound >400 copies/ml after suppression below 50 copies/ml. A single blip between 50 and 400 copies/ml is not a cause for clinical concern if preceded and followed by viral loads below 50 copies/ml. Thus low level HIV-1 RNA quantitation impact on patient management.

Whilst inter-assay correlation was high overall, concordance was reduced closer to the lower limits of assay performance. This was emphasised with considerable disagreement between tests to designate complete viral suppression to undetectable levels: HIV-1 RNA was detected by at least one assay in 162/191 (85%) samples whereas the four assays agreed HIV-1 RNA was present in only 92 (48%). Hologic states a lower limit of detection of 13 copies/ml (IS) for the Aptima HIV assay and data presented here confirms the test is highly sensitive with clinical samples. Use of ultra-sensitive assays has demonstrated the presence of very low-level HIV-1 replication even in the presence of intensive therapy and the clinical significance of these findings has been reviewed elsewhere [34].

Table 2
Summary of Qnostics HIV-1 RNA assay evaluation panel results from Aptima HIV, Abbott RealTime, Qiagen Artus and Roche CAP/CTM assays.

		Quantitative result (Log copies/ml)					Log difference			
Sample	HIV subtype	Expected	Aptima	RealTime	Artus	CAP/CTM	Aptima–expected	Aptima–RealTime	Aptima–Artus	Aptima–CAP/CTM
Qn1	A/G	4.5	4.79	4.73	4.72	4.47	0.29	0.06	0.07	0.32
Qn2	Neg	ND	ND	ND	ND	ND				
Qn3	C	4	4.25	3.86	3.49	3.97	0.25	0.39	0.76	0.29
Qn4	C	3	2.86	3.00	2.45	2.97	−0.14	−0.14	0.41	−0.12
Qn5	B	3.5	3.35	3.60	3.52	3.25	−0.15	−0.25	−0.17	0.11
Qn6	B	3.5	3.28	3.54	3.60	3.35	−0.22	−0.26	−0.32	−0.07
Qn7	B	3.1	3.05	3.21	3.27	2.91	−0.05	−0.17	−0.22	0.14
Qn8	B	2.4	2.39	2.20	2.37	2.26	−0.01	0.19	0.19	0.14
Mean							0.00	−0.02	0.07	0.10

Table 3
Comparison between Aptima HIV and the other assays for detection of HIV-1 RNA in clinical samples.

	RealTime		Artus		CAP/CTM		
	Detected	Not detected	Detected	Not detected	Detected	Not detected	Total
Aptima HIV							
Detected	113	28[b]	100	41[d]	130	11[f]	141 (73.8%)
Not Detected	6[a]	44	8[c]	42	15[e]	35	50 (26.2%)
Total	119 (62.3%)	72 (37.7%)	108 (56.5%)	83 (43.5%)	145 (75.9%)	46 (24.1%)	191
Kappa	0.597		0.453		0.638		
Standard error	0.060		0.0622		0.0646		
95% CI	0.479–0.714		0.331–0.575		0.512–0.765		

[a] Discordant results were in the range: <40 copies/ml.
[b] Discordant results were in the range: <30 copies/ml.
[c] Discordant results were in the range: <45 copies/ml.
[d] Discordant results were in the range: <30–507 copies/ml.
[e] Discordant results were in the range: <20–25 copies/ml.
[f] Discordant results were in the range: <30 copies/ml.

Table 4
Comparison between Aptima HIV and the other assays for quantitation of samples < and ≥50 copies/ml.

	RealTime		Artus		CAP/CTM		
	≥50	<50	≥50	<50	≥50	<50	Total
Aptima HIV							
≥50	75	4[b]	72	7[d]	74	5[f]	79 (41.4%)
<50	3[a]	109	13[c]	99	13[e]	99	112 (58.6%)
Total	78 (40.8%)	113 (59.2%)	85 (44.5%)	106 (55.5%)	87 (45.5%)	104 (54.5%)	191
Kappa	0.924		0.787		0.809		
Standard error	0.0281		0.0451		0.0428		
95% CI	0.869–0.979		0.698–0.875		0.725–0.892		

Individual assay results for these samples are given in Supplementary data.
[a] Discordant results were in the range: 57–90 copies/ml.
[b] Discordant results were in the range: 50–77 copies/ml.
[c] Discordant results were in the range: 55–166 copies/ml.
[d] Discordant results were in the range: 50–507 copies/ml.
[e] Discordant results were in the range: 55–419 copies/ml.
[f] Discordant results were in the range: 66–175 copies/ml.

As such, discrepancies in low-level RNA detection may not represent viral escape but could be attributed to random variation within the confidence intervals at the limit of detection in each assay [32,35]. Hence, quantitative values in the region 50–400 copies/ml are often investigated further in UK clinical practice with a follow-up sample requested to confirm viraemia [1]. Aptima HIV identified fewer discordant samples above 50 copies/ml compared to Artus and CAP/CTM. This has potential implications for clinical practice given that fewer patients would be recalled unnecessarily to investigate blips and low-level viraemia.

Similar to previous comparison studies testing multiple subtypes, discrepant samples identified by Bland–Altman analysis included those with lower viral loads and non-B subtypes [7–11,36]. There may be issues relating to detection of low level viraemia with specific assays. Previous investigators reported transition to the CAP/CTM v2.0 assay was followed by an increase of quantifiable viral loads in patients with prior viral suppression, which were then below the limit of detection in subsequent viral load measurements [29]. Similar to CAP/CTM v2.0, Aptima HIV is a highly sensitive dual-target assay. However, Aptima technology differs in that TMA inherently targets RNA molecules for amplification. This reduces the likelihood of proviral DNA amplification contributing to low-level quantitative signals. Likewise, preferential recovery of RNA over DNA has been proposed for the RealTime HIV protocol [37]. These technological factors may account for some of the variation observed between assays at low viral loads.

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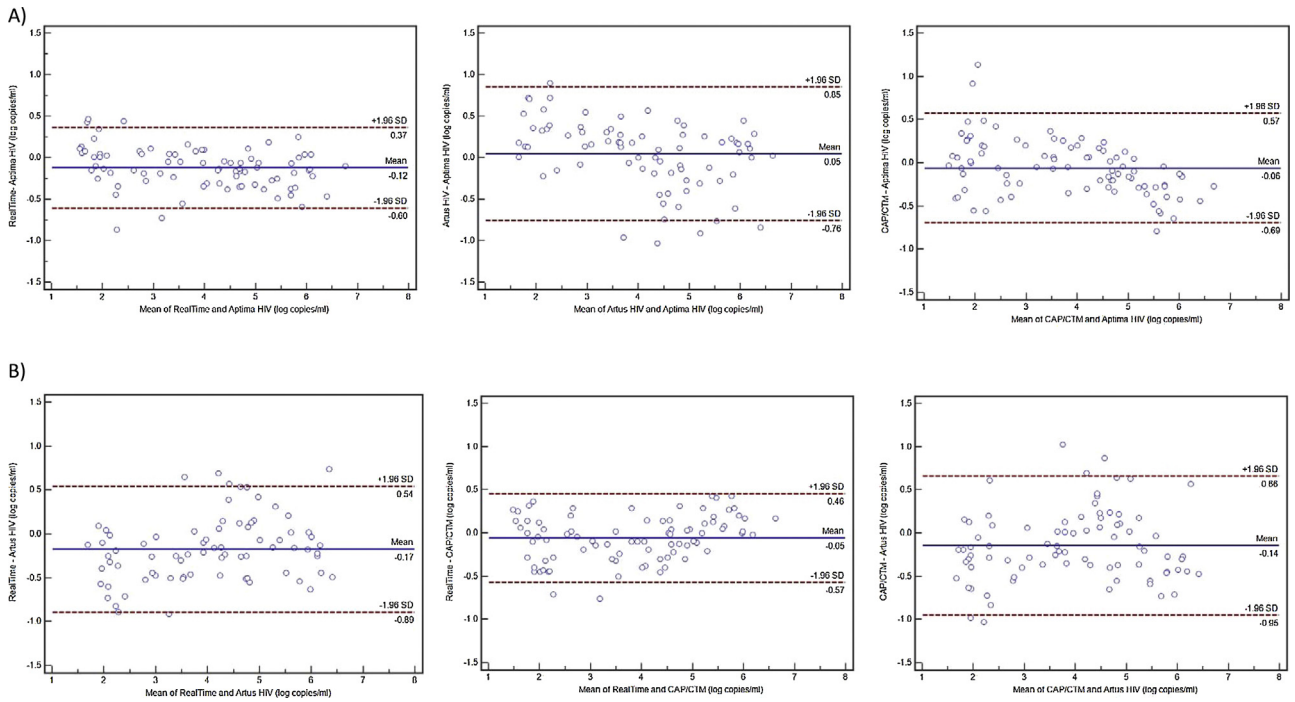

Fig. 2. (A) Comparison of Aptima HIV and each PCR assay by Bland–Altman analysis for samples quantitated above the LLOQ of the assays. (B) Inter-PCR comparison by Bland–Altman analysis for samples quantitated above the LLOQ of the assays. The solid horizontal lines represent the mean differences between the values; the horizontal dotted lines represent the mean differences ±1.96 SD (representing the 95% confidence limits of the agreement) and the values below the dotted lines are the $\log_{10}$ copies/ml values corresponding to the mean ± 1.96 SD. There were 5 discrepant samples outside the 95% CI level of agreement between Aptima HIV and RealTime (2 subtypes B, 2C and 1 A/B), 5 between Aptima HIV and Artus (1 subtype B, 1C, 1 D/A, 1CRF01_AE and 1 unknown) and 3 between Aptima HIV and CAP/CTM (2 subtypes C and 1CRF02_AG).

Variation at low-copy number highlights the importance of selecting an assay with precision across the dynamic range, and of consecutive testing to confirm increases in HIV-1 RNA load. The relationship between variability of low viraemia levels and treatment efficacy is an area of active debate and controlled clinical studies are needed to further understand the significance of blips for patient management [34]. It is vital that dialogue exists between clinic and laboratory to understand and interpret low copy number results. This evidence is needed to guide consensus opinion.

The strength of this study is the use of three commercial comparator PCR assays with Aptima HIV. This allowed direct comparison between the different commercial platforms, easier identification of outlier results in clinical samples and showed that discrepancies can occur with all assays, particularly at low copy

Table 5
Summary of individual quantitative assay results and HIV subtype for all outliers identified for any of the four assays by pairwise Bland–Altman analysis.

		Quantitative result (copies/ml)				
Sample	HIV subtype	Aptima HIV	RealTime	Artus	CAP/CTM	Outlier identified comparing:
51	C	30	82	103	419	RealTime–Aptima CAP/CTM–Aptima RealTime–CAP/CTM
55	C	30	90	159	250	RealTime–Aptima CAP/CTM–Aptima
67	Unknown	66	68	520	49	Artus–Aptima CAP/CTM–Artus
201	B	71	50	207	28	CAP/CTM–Artus
80	A/B	154	427	<45	407	RealTime–Aptima
76	B	507	70	ND	189	RealTime–Aptima
45	B	3343	632	3610	5082	RealTime–Aptima RealTime–Artus RealTime–CAP/CTM
33	C	15, 642	17, 504	1715	18, 200	Artus–Aptima RealTime–Artus CAP/CTM–Artus
21	D/A	74, 718	50, 530	13,417	101, 000	RealTime–Artus CAP/CTM–Artus
196	D/A	77, 870	35, 522	7178	36, 600	Artus–Aptima RealTime–Artus CAP/CTM–Artus
11	CRF01 AE	469, 706	151, 604	57,470	246, 000	Artus–Aptima
17	CRF02 AG	888, 505	390, 707	562,506	145, 000	CAP/CTM–Aptima
204	B	6, 508, 605	5, 143, 319	929,000	3, 460, 000	Artus–Aptima RealTime–Artus

number. A weakness is the absence of follow-up data to establish the clinical significance of Aptima HIV results above 50 copies/ml. A post-implementation clinical audit of low-level viraemia is planned in order to address this.

To summarise, Aptima HIV has excellent comparative performance across the metrics used in this study (accuracy, precision, subtype detection, clinical sample testing) and provides a useful new tool for monitoring HIV-1 RNA load in clinical laboratories. Aptima HIV results for reliable RNA quantitation at low copy number appear promising, although studies with clinical follow-up are required.

Competing interests

MJH has received payment to speak at Hologic and Roche Diagnostic User Group meetings. AMG has consulted for and been part of the speakers' bureau of Roche Diagnostics, Abbott Diagnostics and Qiagen. The authors report no other conflict of interest.

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None.

Ethical approval

The project was reviewed and approved by Royal Liverpool University Hospital Trust Research Governance. National Research Ethics Service approval was not required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2015.05.020

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