

Evaluation of the Aptima[®] HIV-1 Quant Dx Assay for HIV-1 RNA Viral Load Detection and Quantitation in Plasma of HIV-1-Infected Individuals: A Comparison With Abbott RealTime HIV-1 Assay

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The Hologic Aptima[®] HIV-1 Quant Dx assay (Aptima HIV) is a real-time transcription-mediated amplification method CE-approved for use in diagnosis and monitoring of HIV-1 infection. The analytical performance of this new assay was compared to the FDA-approved Abbott RealTime HIV-1 (RealTime). The evaluation was performed using 220 clinical plasma samples, the WHO 3rd HIV-1 International Standard, and the QCMD HIV-1 RNA EQA. Concordance on qualitative results, correlation between quantitative results, accuracy, and reproducibility of viral load data were analyzed. The ability to measure HIV-1 subtypes was assessed on the second WHO International Reference Preparation Panel for HIV-1 Subtypes. With clinical samples, inter-assay agreement for qualitative results was high (91.8%) with Cohen’s kappa statistic equal to 0.836. For samples with quantitative results in both assays ($n = 93$), Lin’s concordance correlation coefficient was 0.980 ($P < 0.0001$) and mean differences of measurement, conducted according to Bland–Altman method, was low ($0.115 \log_{10}$ copies/ml). The Aptima HIV quantified the WHO 3rd HIV-1 International Standard diluted from 2000 to 31 cp/ml (5,700–88 IU/ml) at expected values with excellent linearity ($R^2 > 0.970$) and showed higher sensitivity compared to RealTime being able to detect HIV-1 RNA in 10 out of 10 replicates containing down to 7 cp/ml (20 IU/ml). Reproducibility was very high, even at low HIV-1 RNA values. The Aptima HIV was able to detect and accurately quantify all the main HIV-1 subtypes in both reference panels and clinical samples. Besides excellent performance, Aptima HIV shows full automation, ease of use, and improved workflow compared to RealTime. **J. Med. Virol. 88:1535–1544, 2016.**

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INTRODUCTION

Plasma levels of HIV-1 RNA (viral load [VL]) along with CD4 T lymphocyte (CD4) cell count are the two major laboratory tests that play a role in assessing HIV-1-infected individuals before antiretroviral therapy (ART) is initiated and then monitoring their treatment during the course of HIV management. The management of HIV-infected patients has changed substantially with the availability of newer, more potent, and less toxic therapies [NIH Panel on Antiretroviral Guidelines for Adults and Adolescents, 2016]. Although monitoring CD4 count along with VL is still part of clinical practice and supported by various guidelines, VL is now the most important indicator of initial and sustained response to ART and should be measured in all patients at entry into care, initiation of therapy, and on a regular basis

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thereafter (3–12 months) [WHO Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, 2014; NIH Panel on Antiretroviral Guidelines for Adults and Adolescents, 2016]. Further, pre-treatment VL measurements are gaining importance as poor response to some therapies is associated with higher baseline VL levels [Sax et al., 2009; Cohen et al., 2013]. Not only does monitoring promptly detects unsuccessful treatment, but it can help identify adherence problems and determine when to switch ART regimens during treatment failure [WHO Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, 2014].

Various international guidelines and clinical trials define optimal viral suppression as a VL persistently below the limit of detection (20–75 copies/ml depending on the assay used) with a consensus value of 50 copies/ml [Thompson et al., 2012; WHO Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, 2014; Williams et al., 2014; EACS Guidelines, 2015; NIH Panel on Antiretroviral Guidelines for Adults and Adolescents, 2016]. Patients with levels below this threshold have the lowest probabilities of morbidity and mortality. There is more variation in the definition of virologic failure among guidelines, with thresholds ranging from >48 copies/ml at the low end to >1,000 copies/ml at the high end [Thompson et al., 2012; WHO Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, 2014; Williams et al., 2014; EACS Guidelines, 2015; NIH Panel on Antiretroviral Guidelines for Adults and Adolescents, 2016]. Isolated blips (transiently detectable and often less than 400 copies/ml) are not uncommon in patients undergoing successful treatment and have been shown to be not predictive of treatment failure [Havlir et al., 2001]. Because of this type of data, these guidelines recognize treatment failure as confirmed VL greater than the threshold after initial successful suppression. An analysis of ACTG studies indicated that a threshold of 200 copies/ml eliminated most cases of apparent viremia caused by blips or assay variability [Ribaudo et al., 2009].

It is clear that as low VL continues to be of importance in clinical decision making, the assay chosen to monitor patients must be accurate and precise between its limit of detection and 1,000 copies/ml. Changes in VL of $0.5 \log_{10}$ copies/ml are considered statistically significant (2 standard deviations) and often used to determine if a change is needed in ART regimen [NIH Panel on Antiretroviral Guidelines for Adults and Adolescents, 2016]. Most studies indicate that currently available real-time PCR tests are similar, with high sensitivity, specificity, and reproducibility [Sire et al., 2011; Wall et al., 2012; Garcia-Diaz et al., 2013]. However, the performance characteristics at low VL is often not equivalent, with lower inter-assay concordance seen at 50 copies/ml compared to

200 copies/ml and may lead to overestimation or underestimation of HIV-1 RNA levels [Amendola et al., 2014; Swenson et al., 2014].

Although PCR-based technology continues to be the most prevalently used for VL monitoring, newer technologies are entering the market that improve upon the current PCR assays. There is a need for assays with increased accuracy and precision at low VLs to address many of the remaining questions on the impact of low VL in the clinical setting. Further, increasing lab efficiency is mandated by changes in healthcare; necessitating an increase in throughput and automation while maintaining high assay performance [Melanson et al., 2007; Amendola et al., 2011a].

Here, the performance of the recently approved Hologic Aptima HIV-1 Quant Dx assay (Aptima HIV) was compared to the test routinely used in the Lazzaro Spallanzani Hospital in Rome for HIV-1 VL assessment, that is, the Abbott RealTime HIV-1 assay (RealTime). In some cases, the comparison was extended to Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 version 2.0 the (CAP;1;/CTM) assay. Aptima HIV is based on real-time transcription-mediated amplification (TMA) technology using a dual target detection: it amplifies both the *pol* integrase and the long terminal repeat (LTR) regions. To fully understand the variability and concordance between the assays in the low VL range, dilutions of the WHO 3rd HIV-1 International Standard (WHO-IS), from 2,000 to 31 copies/ml (5,700–88 IU/ml) and clinical samples (from 1,000 copies/ml to “not detected” HIV-1 RNA) were analyzed. In addition, it was focused on the detection and quantification of multiple HIV-1 subtypes, by using clinical samples and two external quality assessment panels: the WHO 2nd International Reference Panel Preparation for HIV-1 Subtypes for NAT (WHO-ST) and the QCMD HIV-1 RNA EQA panel (Quality Control for Molecular Diagnostics [QCMD], Scotland, UK).

MATERIALS AND METHODS

Clinical Samples Collection

The study included residual plasma samples obtained from HIV-1-infected patients attending the out-patient care facility of the “Lazzaro Spallanzani” Hospital in Rome for routine monitoring of HIV-1 VL in a time lapse of 2 months (from June to August 2014). A total of 220 clinical samples, spanning the full range of HIV-1 viral load values based on the initial RealTime results, were chosen that contained sufficient plasma volume to allow the subsequent testing with both RealTime and Aptima HIV assays. The comparison was run in the same working day on the thawed aliquots, and only these results, irrespective of initial RealTime results, were used to evaluate the inter-assay concordance. In addition, 25 clinical samples containing non-B subtypes (2 subtype A1, 5 subtype C, 4 subtype F1, 1 subtype F2, 1 subtype G)

and CRFs (1 subtype CRF01 AE, 6 subtype CRF02 AG, 1 subtype CRF12 BF, 2 subtype CRF29 BF, 1 subtype CRF31 BC, and 1 subtype CRF31 BC+F1) were tested with both assays: 5 were from the 220 clinical samples (with known non-B subtype) and 20 were supplementary clinical samples chosen for their non-B subtype and availability of aliquots of plasma necessary for the comparison. Plasma from whole-blood samples collected in EDTA-containing tubes was separated by centrifugation (1,100g for 5 min) and stored at -80°C and never thawed until the day of assay comparison, that occurred within 6–8 months. All samples were de-identified prior to testing. In case of difference between Aptima HIV and RealTime greater than $0.5 \log_{10}$ copies/ml, a new aliquot (if available) was thawed and tested with CAP/CTM assay, in the attempt of resolving the observed discordance.

Ethics Statement

The study was approved by the local institutional review board of the “Lazzaro Spallanzani” Hospital. Written informed consent was deemed unnecessary for retention and testing of residual plasma samples from these patients.

HIV-1 Subtype Establishment on Clinical Samples

HIV-1 subtype was retrieved from the HIV-1 *pol* sequences, in the context of drug-resistant mutation pattern assessment, and was available for 80% of patients. For subtype establishment, the HIV-1 *pol* sequences were aligned in BioEdit and compared to reference sequences for the major HIV-1 subtypes and circulating recombinant forms (CRFs), available on the Los Alamos database (<http://www.hiv.lanl.gov>). Subtype classification was confirmed using the REGA (<http://www.bioafrica.net/regagenotype/html/subtypinghiv.html>) and the COMET (<http://comet.retrovirology.lu/>) subtyping tools. In case of discordant results, the subtype was assigned on the base of RDP4 (<http://rdp4.software.informer.com/4.1/>) and of phylogenetic analysis, using MEGA6 (<http://www.megasoftware.net/>).

HIV-1 RNA Assays

All viral load assays were performed according to the manufacturers' instructions following the product inserts (Hologic, Inc., San Diego, CA: Aptima HIV-1 Quant Dx assay; Abbott Molecular: Real Time HIV-1; Roche Molecular Diagnostics. COBAS[®] AmpliPrep/COBAS[®] TaqMan[®] HIV-1 Test, v2.0). All the assays used in this comparison (RealTime, Aptima HIV, and CAP/CTM) automatically report the results in copies/ml according to their respective conversion factor (as declared by the manufacturer).

The Aptima HIV assay (Aptima[®] HIV-1 Quant Dx; Hologic, Inc.) was performed on the fully automated

Panther system. Aptima HIV has a linear quantification range of 30–10,000,000 copies/ml (with 0.5 ml plasma) and the reported LOD is 13 copies/ml based on the 3rd HIV-1 WHO-IS.

The RealTime assay (Abbott RealTime HIV-1; Abbott Molecular, Inc., Des Plaines, IL) was performed on the *m2000* sp/rt instruments. The linear quantification range of the assay is 40–10,000,000 copies/ml (0.6 ml protocol) and the reported LOD is 40 copies/ml.

Roche CAP/CTM (Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 version 2.0; Roche Molecular Systems, Inc., Pleasanton, CA) was performed on the docked configuration of the Cobas AmpliPrep and TaqMan 96 instruments. The linear quantification range of the assay is 20–10,000,000 copies/ml (1.1 ml plasma) with a LOD of 20 copies/ml.

Quality Control Material

Four external quality assessment (EQA) panels were utilized to establish the performance (linearity, precision, accuracy) of the Aptima HIV.

Linearity and accuracy was assessed by analyzing the Acrometrix HIV-1 panel (Life Technologies, Carlsbad, CA) whose assigned HIV RNA content spans the range 2.00 – $6.70 \log_{10}$ copies/ml. In addition, a dilution series panel was created from the WHO 3rd HIV-1 International Standard (WHO-IS; NIBSC code: 10/152 [www.nibsc.ac.uk]) containing $5.27 \log_{10}$ IU/ml of HIV-1 subtype B virus. Ten replicates spanning the range 5,700–88 IU/ml, or 2,000–31 copies/ml according to the Aptima conversion factor (1IU = 0.35 cp/ml), were made by serially diluting WHO-IS in HIV-1-negative human plasma (Basematrix 53, Defibrinated human plasma, Sera Care, Milford, MA). Moreover, 10 and 3 additional replicates with 15 and 7 copies/ml (43 and 20 IU/ml) were tested with Aptima HIV and RealTime, respectively.

To assess the performance of Aptima HIV against multiple subtypes, the Qnostics HIV-1 Evaluation panel (Qnostics; QCNM14-038-HIV-1), the QCMD HIV-1 RNA EQA panel (QCMD; HIVRNA 14B [www.qcmd.org]), and the WHO 2nd International Reference Panel Preparation for HIV-1 Subtypes for NAT (WHO-ST; NIBSC code: 12/224) were used. The Qnostics and QCMD panels contain each seven members spanning subtypes B (four samples), C (two samples), AG (one sample), and a negative control. The WHO-ST panel consists of heat inactivated virus from 10 samples representing the subtypes A, B, C, D, AE, F, G, AA-GH, group N, and group O. Each panel member was considered equivalent for both amount and HIV-1 subtypes to that stated for the 1st WHO-ST panel, according to the manufacturer instructions.

The comparison with the Acrometrix panel and the Qnostics HIV-1 Evaluation panel included the CAP/CTM assay in addition to Aptima HIV and RealTime.

TABLE I. Correlation in Qualitative Results From 220 Clinical Samples at the 40 copies/ml Threshold of the RealTime Assay

RealTime	Aptima HIV		Total
	<40 cp/ml	≥40 cp/ml	
<40 cp/ml	109	3	112
≥40 cp/ml	15	93	108
Total	124	96	220

Data Analysis

All VL data were analyzed as \log_{10} -transformed values. Concordance on qualitative results between the Aptima assay and the reference test was established by Cohen's kappa statistic and differences in detection rates by Fisher's exact test. In the correlation analysis, only the VL data in which both Aptima HIV and RealTime had quantitative values were considered. The correlation between quantitative results was evaluated by using the linear regression analysis, the Lin's concordance correlation coefficient (ccc) of the measurements [Lin, 1989] and Bland-Altman [Bland and Altman, 1986] plot.

RESULTS

Comparison on Clinical Samples

A comparative evaluation of the sensitivity and concordance between Aptima HIV and RealTime on their ability to quantitate VL of HIV-1 RNA was performed using 220 residual clinical samples, with HIV-1 RNA levels ranging from not detected to greater than 100,000 copies/ml in the RealTime assay. The degree of inter-assay concordance for qualitative results at the RealTime 40 copies/ml HIV-1 RNA threshold was very good ($\kappa=0.836$; $SE=0.037$; $95\%CI=0.764-0.908$). There was 91.8% agreement in the observations, with 18 (8.2%)

samples having discordant results (Table I). Of the 18 discordant samples, 15 were quantified with the RealTime (mean VL: 61 copies/ml; range 41–96 copies/ml) and resulted <30 copies/ml with Aptima HIV, and three were quantified with the Aptima HIV (mean VL: 246 copies/ml; range 50–438 copies/ml) but resulted <40 copies/ml with the RealTime. Twelve samples (6%) had greater than a $0.5 \log_{10}$ copies/ml difference between assays, with seven of these having quantifiable results in both assays, as shown in Table II. The HIV-1 subtype was known for 10 out of these 12 discordant samples and included B, C, CRF31 BC + F1, and CRF02 AG subtypes. Interestingly, samples n[#] 3 and n[#] 11 (both subtype B) resulted at lower level of VL both with RealTime and the dual target CAP/CTM assay (Table II), but due to limited sample volumes, these specimens could not be tested by sequencing of the viral genome targets. Further work is needed to verify these difference with a larger data set.

When breaking down the population into samples that were “not detected,” detected using assay specific LLOQ (<40 copies/ml RealTime; <30 copies/ml Aptima HIV) and quantitated, although the proportion of samples quantitated by the two methods was not significantly different (101 samples, 46%, for Aptima HIV vs. 108 samples, 49%, for RealTime), there were significant differences concerning the rate of detection. In fact, the proportion of samples with HIV-1 RNA “not detected” was significantly higher for RealTime versus Aptima HIV (29.1% vs. 19.5%; $P=0.003$, $OR=2.62$, McNemar test). Further, more samples were detected (but not quantitated) with Aptima HIV than with RealTime (34.5% vs. 21.8%; $P<0.001$, $OR=2.75$, McNemar test).

There were 93 samples with quantifiable results in both assays using the RealTime threshold of 40 copies/ml (four additional samples were quantitated by Aptima HIV using 30 copies/ml threshold described above and not included in correlation). The distribution of these samples is shown in Figure 1A.

TABLE II. For Samples With Difference Between Aptima HIV and RealTime Greater Than a $0.5 \log_{10}$ copies/ml, a New Aliquot was Thawed and Analyzed With CAP/CTM

Sample number	RealTime	Aptima HIV	CAP/CTM	HIV-1 subtype
1	1.98	Detected <1.48	2.01	B
2	1.89	Detected <1.48	1.68	–
3	Detected <1.60	2.64	Detected <1.30	B
4	1.98	1.40	1.58	C
5	1.73	2.26	2.15	C
6	3.19	2.60	3.21	B
7	4.36	5.16	5.02	C
8	4.47	5.04	4.55	–
9	4.26	4.78	4.64	B
10	4.72	5.48	4.82	CRF31 BC + F1
11	Detected <1.60	2.40	1.73	B
12	1.75	Not detected	NA	CRF02 AG

RealTime LOD: $1.60 \log_{10}$ copies/ml; Aptima HIV LOD: $1.48 \log_{10}$ copies/ml; CAP/CTM: $1.30 \log_{10}$ copies/ml. NA, not analyzed.

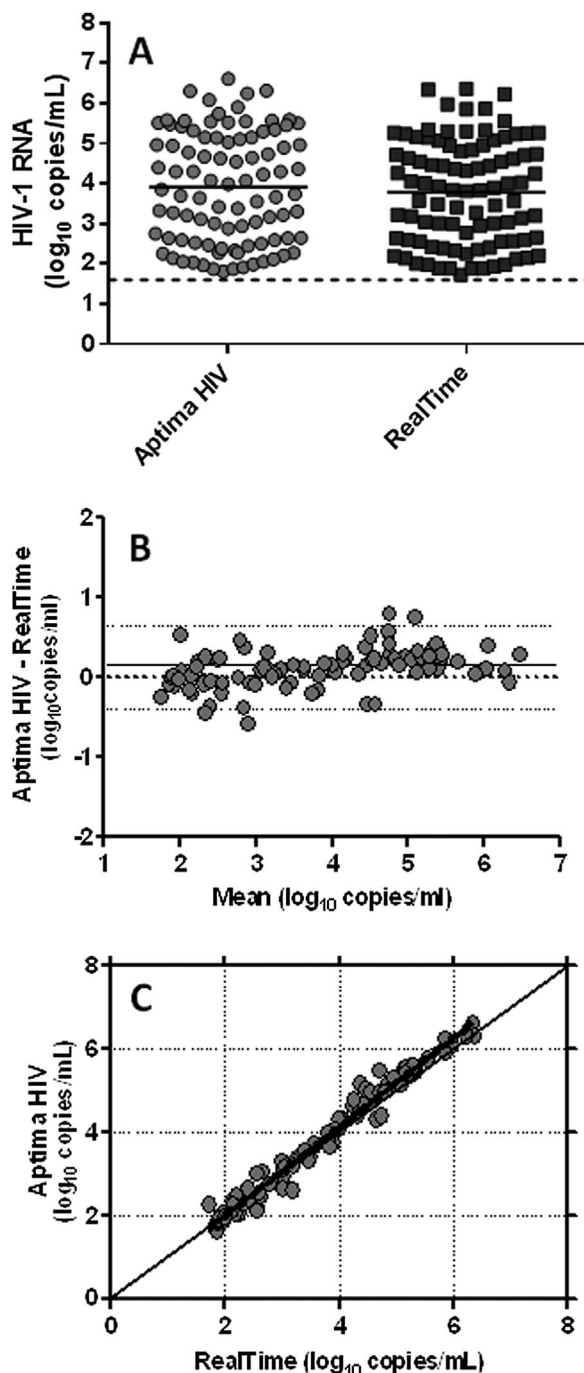


Fig. 1. Comparison of Aptima HIV and RealTime performances on clinical samples. Clinical samples ($n=93$) with quantifiable results (\log_{10} -transformed with $>1.60 \log_{10}$ copies/ml) were tested with both assays. (A) Scatter-dot plot showing the distribution of samples with quantifiable results in the Aptima HIV (light gray circles) and RealTime (dark gray squares) assays. The mean value is indicated with a solid horizontal line. (B) Bland-Altman plot of the mean values and differences between Aptima HIV and RealTime. The 95% limits of agreement are indicated as fine dotted lines and the bias as a solid line. (C) Correlation of Aptima HIV results with RealTime. The thick black line indicates the Deming linear regression best fit and the thin black line the line of perfect concordance. Lin's $c_{cc}=0.980$, $P < 0.0001$.

The mean values obtained with the Aptima HIV assay (mean \pm SD, $3.91 \pm 1.39 \log_{10}$ copies/ml) were $0.115 \log_{10}$ copies/ml (95% limits of agreement, -0.3619 , 0.6118) higher than the mean values from the RealTime assay (mean \pm SD, $3.79 \pm 1.29 \log_{10}$ copies/ml), especially at high VL levels (Fig. 1B). Values obtained in each assay were highly correlated with Lin's c_{cc} of 0.980 ($P < 0.0001$; Fig. 1C). Overall, 66 (71%) of the quantified samples had slightly higher levels in Aptima HIV assay. From the discordant samples described above (Table II), a difference of $>0.5 \log_{10}$ copies/ml was observed in six samples (6.5%) with quantifiable results in both assays. Of these six samples, four were outside the 95% limits of agreement of the assays. Upon retesting of these samples (11 out of 12) with CAP/CTM, the results from Aptima HIV were within $0.5 \log_{10}$ copies/ml of difference for four out of six samples, as expected, as CAP/CTM assay gives VL results higher than RealTime [Bourlet et al., 2011; Karasi et al., 2011; Sire et al., 2011; Sollis et al., 2014]. These samples were subtype B and CRF31 BC. Two additional samples were outside of the 95% limits of agreement, but had less than $0.5 \log_{10}$ copies/ml difference (-0.45 and $-0.37 \log_{10}$ copies/ml).

Twenty-five clinical samples harboring known non-B subtypes and CRFs (ranging from HIV-1 RNA "not detected" to $6.0 \log_{10}$ copies/ml HIV-1 RNA) were also analyzed in both assays. The differences in results varied from 0.00 to $0.80 \log_{10}$ copies/ml and are shown in Figure 2, with the Aptima HIV results on average $0.17 \log_{10}$ copies/ml higher than RealTime; 16 samples had quantitative values in both assays and were highly correlated (Pearson $r=0.9843$, $P < 0.0001$). Six samples (four subtype C, one subtype CRF31 BC + F1, and one subtype CRFO2 AG) had greater than a $0.5 \log_{10}$ copies/ml difference between assays, indicating that the Aptima assay is less affected by non-B subtype variability. In addition, Aptima HIV detected (<30 cp/ml) one sample belonging to subtype CRFO1 AE that was undetected with RealTime. On the contrary, RealTime quantified one CRFO2 AG sample that resulted "not detected" with Aptima HIV. Unfortunately, an additional aliquot of this sample was not available for repeat testing.

Assay Comparison With the WHO Reference Standard

To assess each assay's ability to accurately and precisely quantify low VL, serial dilutions of the WHO-IS were tested in replicates of 10, from 31 to 2,000 copies/ml ($88-5700$ IU/ml) (or, if expressed in \log_{10} /cp/ml, from 1.5 to $3.3 \log_{10}$ cp/mL; Fig. 3A). The results demonstrate high precision for both assays ($<12.5\%$ CV) and high agreement with the target values ($<0.03 \log_{10}$ copies/ml mean difference). Distribution of differences between the assays were not significantly different over the concentrations of

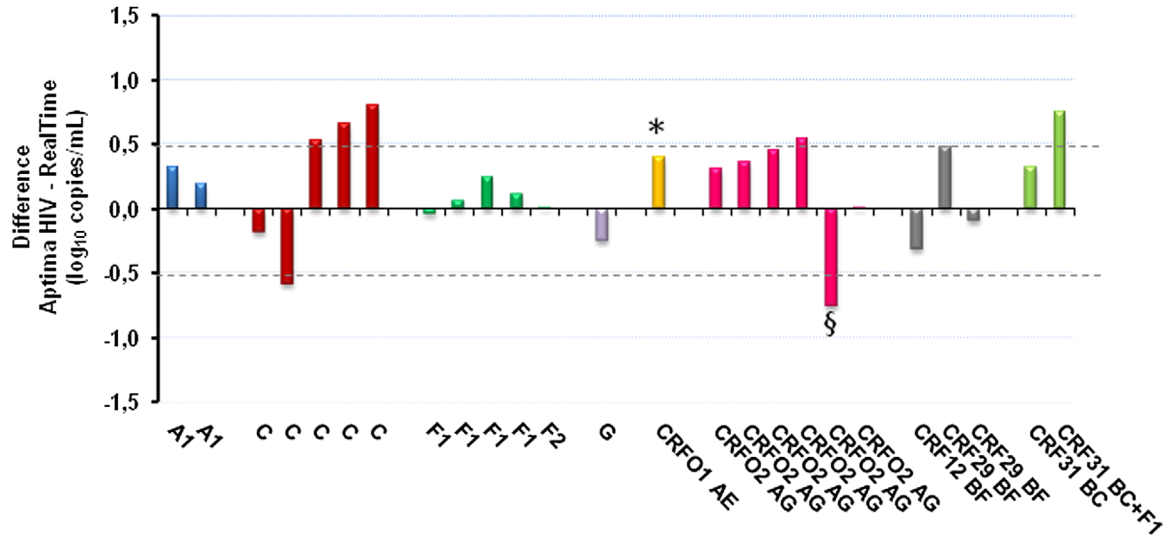


Fig. 2. Comparison of Aptima HIV and RealTime performances on clinical samples harboring HIV-1 non-B subtypes. Clinical samples ($n=25$) with HIV-1 non-B subtypes or CRFs were analyzed with both assays and differences in measurement for each sample are shown. Dotted lines indicates difference >0.5

\log_{10} copies/ml of clinical relevance. The sample with * was given as “not detected” by RealTime and $<30 \log_{10}$ copies/ml with Aptima HIV. The sample with § resulted “non detected” with Aptima HIV assay. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com].

the WHO-IS ($P=0.698$, Kruskal–Wallis test). At each concentration, the assays had similar coefficients of variation, leading to no significant differences between the assays. The assays were highly correlated across the range of samples tested (Lin’s ccc 0.978, $P<0.0001$). Mean values for Aptima HIV were slightly higher than for RealTime ($0.018 \log_{10}$ copies/ml; 95% limits of agreement, -0.3193 to 0.2832). Both assays showed excellent linearity upon dilution ($R^2=0.970$) and concordance of the observed data obtained with Aptima HIV versus expected values was shown in Figure 3B. Interestingly, in additional serial dilutions of the WHO-IS containing 15 and 7 copies/ml, corresponding to 43 and 20 IU/ml respectively, HIV RNA was assessed with Aptima HIV: the assay was able to detect HIV RNA (given as detected <30 cp/ml) in 10 out of 10 (100%) replicates containing 15 and 7 copies/ml HIV RNA. With RealTime, HIV RNA was detected in two out of three (66%) replicates and in one out of three (33%) replicates containing 15 and 7 copies/ml, respectively.

Assay Comparison With External Quality Control Material

Four panels were tested to compare the linearity of the two assays (Fig. 4A), as well as to assess their ability to detect different HIV subtypes (Fig. 4B–D).

For the linearity, the seven-member Acrometrix panel was tested by Aptima HIV, RealTime, and also CAP/CTM (Fig. 4A). Each assay accurately quantified the panel members that ranged from 100 to 5,000,000 copies/ml. The mean differences from the target value for CAP/CTM ($0.14 \log_{10}$ copies/ml) were

numerically higher than either Aptima HIV ($-0.024 \log_{10}$ copies/ml) or RealTime ($0.008 \log_{10}$ copies/ml), but did not reach statistical significance.

Assay Comparison for the Ability to Detect HIV-1 Subtypes

The ability to detect different HIV-1 subtypes was assessed with three distinct panels. First, the Qnostics panel was tested in all three assays (Fig. 4B). All of them accurately quantified all of the samples ($<0.5 \log_{10}$ copies/ml difference) of the panel. On average, results from Aptima HIV and CAP/CTM were closer to the target values than RealTime, with mean differences of 0.099, 0.104, and $0.249 \log_{10}$ copies/ml, respectively. The largest contributing factors to these differences for Aptima HIV were one of the subtype B members ($0.5 \log_{10}$ copies/ml) and for RealTime both subtype C panel members (0.49 and $0.46 \log_{10}$ copies/ml).

The QCMD and the WHO-ST panels were tested with both Aptima HIV and RealTime (Fig. 4C and D). Overall, the QCMD panel members were quantified by both assays with little difference compared to the consensus target values: $0.151 \log_{10}$ copies/ml for Aptima HIV and $-0.075 \log_{10}$ copies/ml for RealTime). The major difference between Aptima HIV and the target values was observed for one member containing the recombinant A/G form ($+0.34 \log_{10}$ copies/ml), while RealTime sub-estimated a subtype B member ($-0.27 \log_{10}$ copies/ml; Fig. 4C).

Regarding the WHO-ST panel (Fig. 4D), all 10 of the panel members are formulated in the same concentration range of the 1st WHO panel:

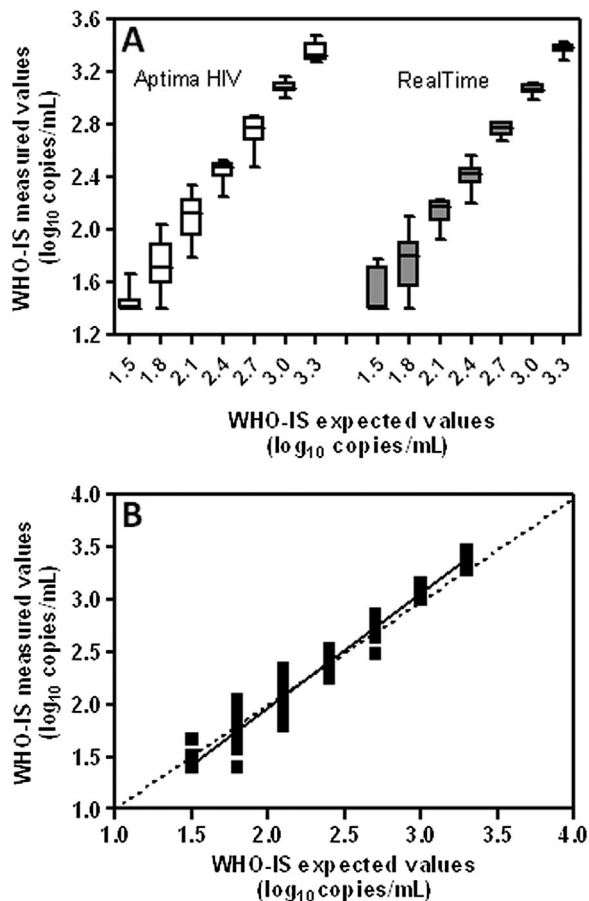


Fig. 3. Comparison of Aptima HIV and RealTime performances on serial dilutions of the WHO-IS. Dilutions of the WHO-IS were prepared to cover a range of concentration between 31 and 2,000 copies/ml ($1.5\text{--}3.3\log_{10}$ copies/ml) according to the Aptima conversion factor (IU=0.35 cp/ml) and tested with both assays. (A) Whisker bar plot of Aptima HIV (black) and RealTime (gray) showing the 5–95 percentiles. The horizontal line in each box is the median. (B) Concordance graph of the observed data obtained with Aptima HIV versus expected values, with linear regression line (solid) and line of perfect concordance (dashed). Lin's ccc=0.978, $P<0.0001$. For the analysis of data, samples that were detected but <40 or <30 copies/ml were assigned a value of 25 ($1.40\log_{10}$) copies/ml.

$2.18\text{--}3.66\log_{10}$ copies/ml [Holmes et al., 2003]. Agreement between the two assays was good ($<0.33\log_{10}$ copies/ml difference) with the exception of the Group N ($1.73\log_{10}$ copies/ml) and the subtype D ($0.68\log_{10}$ copies/ml) samples, being under quantitated in the RealTime assay. Further, agreement between the Aptima HIV and RealTime results with the target consensus value was substantially similar, with a mean differences of 0.22 and $-0.14\log_{10}$ copies/ml, respectively.

DISCUSSION

This study has compared the performance for the quantification of HIV-1 RNA of the Hologic Aptima HIV assay with the Abbott RealTime assay, with a

focus on values critical for therapeutic decision making. A combination of clinical samples and external quality assessment panels was utilized to determine the agreement between assays.

A total of 220 clinical samples were selected based upon previous testing with the RealTime assay. Correlation was high for samples with quantifiable results, as well as when analyzing samples at the 40 copies/ml RealTime detection threshold. The Aptima HIV assay was more likely to detect samples (including those quantitated and those detected but not quantitated) than the RealTime assay, likely owing to the lower LOD of the Aptima HIV assay of 13 copies/ml. Overall, there was excellent agreement between the assays, with Aptima HIV results being slightly higher than RealTime. The results here described are in line with those previously reported [Hopkins et al., 2015; Mor et al., 2015; Schalasta et al., 2015], where Aptima HIV was comparable to RealTime and CAP/CTM assays.

In general, HIV-1 subtype did not affect the performance of the assays. In our comparison, although few discordant clinical samples with greater than a $0.5\log_{10}$ copies/ml difference were observed (that could be explained by the different target region of the assays), the subtypes were spread between B, C, and two CRFs and did not show a bias. For all major group M HIV-1 subtypes and CRFs, our findings indicate that Aptima HIV, as well as RealTime, can accurately quantify non-B subtypes in a clinical population. In fact, 25 clinical samples harboring non-B subtypes and CRFs (ranging from HIV-1 RNA “not detected” to $6.0\log_{10}$ copies/ml HIV-1 RNA) were analyzed to challenge the ability of the assays to quantify difficult subtypes and both assays resulted equally sensitive for subtype A1, F1, G, and also for the CRFs that are spreading all over the world: AG, AE, and BF (with exception of one CRF AG sample, not confirmed by a second test due to lack of plasma). Interestingly, with subtype C and the CRF31 BC samples, Aptima HIV seems to be less affected in the viral load quantification than RealTime, providing higher results (with $>0.5\log_{10}$ copies/ml difference) accordingly to Mor et al. [2015]. Unfortunately, it was not possible to retrieve antiretroviral therapy regimens of patients and to analyze correlation details.

In addition to clinical samples, external quality assessment panels were tested that would further challenge each assay's ability to detect HIV-1 non-B subtypes, besides B subtype. In both the QCMD and WHO-ST panels, the two assays detected all samples and agreement between them was good with difference of less than 0.30 and $0.40\log_{10}$ copies/ml, respectively. However, Aptima HIV measured slightly higher VL in samples harboring subtype C and subtype D that were closer to the target values still demonstrating to be more accurate in the quantification of these non-B subtypes than RealTime.

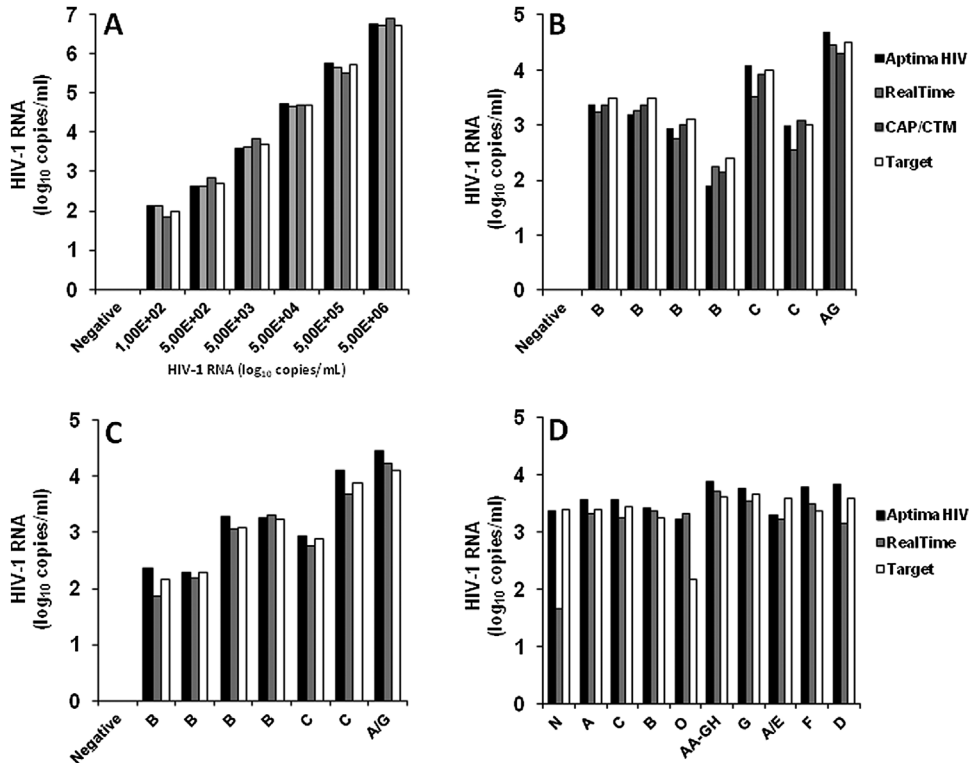


Fig. 4. Comparison of Aptima HIV and RealTime for the ability to quantitate different HIV-1 subtypes. External quality assessment panels were tested with both assays. (A) Acrometrix copies/ml panel tested in Aptima HIV (black bars), RealTime (light gray bars), and CAP/CTM (dark gray bars). White bars represent the consensus target values. (B) Qnostics panel tested in Aptima HIV (black bars), RealTime (light gray bars), and CAP/CTM (dark

gray bars). The consensus target value is indicated as a white bar. (C) QCMD panel tested in Aptima HIV (black bars), RealTime (light gray bars). The consensus target value is indicated as a white bar. (D) WHO-ST panel tested in Aptima HIV (black bars) and RealTime (light gray bars). The target value is from the first WHO-ST panel and should be equivalent for the second panel, according to the manufacturer instructions.

Furthermore, RealTime did not quantify the Group N sample of the WHO-ST panel by $1.73 \log_{10}$ copies/ml, but additional replicates would be necessary, as the RealTime assay does not support testing Group N according to the package insert [Abbott Molecular: Real Time HIV-1].

Thorough evaluation of new technologies for monitoring HIV-1 RNA levels is necessary, as most recent treatment guidelines suggest, that virologic suppression occurs at VLs below 50 copies/ml [Thompson et al., 2012; WHO Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, 2014; Williams et al., 2014; EACS Guidelines, 2015; NIH Panel on Antiretroviral Guidelines for Adults and Adolescents, 2016]. According to these guidelines, assays should be both accurate and precise at low VL ($<1,000$ copies/ml) as treatment failure is defined as two consecutive VL measurements above a threshold ranging from 200 to 1,000 copies/ml. Using the WHO-IS preparation, we observed, within the range of HIV-1 RNA concentrations tested here (2,000–31 copies/ml or 5700–88 IU/ml), high agreement between the target value and the results with both assays. The assays were highly correlated with excellent linearity upon

dilution. Moreover, our data suggest that Aptima HIV is more sensitive compared to RealTime, being able to detect HIV RNA in 100% of replicates of the WHO-IS preparation diluted down to 7 copies/ml (or 20 IU/ml) and in a higher number of low viremic clinical samples. Further investigation using Probit analysis is warranted to confirm these potentially important findings. Recently, there is increased desire for greater sensitivity of HIV-1 RNA molecular assays, as high number of patients show persistent residual viremia (i.e., HIV-1 RNA detected below 50 copies/ml) despite effective HIV treatment [McKinnon et al., 2015], and the extent of residual viremia is predictive of subsequent virological failure [Gianotti et al., 2015]. In addition, residual viremia may be used to guide the selection of patients who may undergo safe simplification strategies [Sarmati et al., 2015]. To this respect, several ultrasensitive viral load assays for the determination of residual viremia have been established [Palmer et al., 2003; Drosten et al., 2006; Edelmann et al., 2010; Amendola et al., 2011b], but they are often cumbersome, and, more importantly, all of them are based on different procedures and results are difficult to compare [Bustin et al., 2009]. Therefore, a fully

automated and standardized commercial assay able to accurately detect/quantify very low HIV viral loads is highly desirable.

Previous studies have reported assay comparisons between CAP/CTM and RealTime using the 2nd international HIV-1 RNA WHO standard [Glaubitz et al., 2011; Amendola et al., 2014]. In these studies, the agreement between assays is often better at VLs above 200 copies/ml than at a clinically relevant threshold below 50 copies/ml [Amendola et al., 2014; Swenson et al., 2014]. For both Aptima HIV and RealTime, the measurements at low VL (60 copies/ml) were both accurate and precise, in agreement with the manufacturers' LOQ claims. With clinical samples, the comparison here indicates that the degree of inter-assay concordance between Aptima HIV and RealTime is 91.8% at the 40 copies/ml level. This is slightly higher than the concordance seen at 50 copies/ml between CAP/CTM and RealTime (89.81%) in an earlier study [Amendola et al., 2014].

Though these data indicate that the performance of Aptima HIV on clinical samples and panels is equivalent to the reference assay presented here (RealTime), most guidelines suggest consecutive measurements of viral load with a single assay for therapeutic monitoring of individual patients to confirm clinically relevant changes in viral load [Thompson et al., 2012; WHO Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection, 2014; Williams et al., 2014; EACS Guidelines, 2015; NIH Panel on Antiretroviral Guidelines for Adults and Adolescents, 2016]. Furthermore, recent data support not changing ART in HIV-1-infected individuals when VL remains <200–1,000 copies/mL as this may represent a "blip" [Grennan et al., 2012; Laprise et al., 2013]. Differences in levels between 50–1,000 copies/mL can lead to clinical consequences, including adjustment of ART, dependent on the guideline followed and clinical interpretation [Henrich et al., 2012; Gianotti et al., 2013]. It is recommended that the performance characteristics of a diagnostic assays should be carefully tested, especially when focusing on low VLs that remain a focus in clinical management, and are of great importance in the era of targeting functional cure of HIV infection, when stringent clinical evaluation of therapeutic strategies is warranted.

In summary, the performance of the Aptima HIV-1 Quant Dx assay can be considered equivalent to those of the Abbott RealTime HIV-1 assay demonstrating high efficiency for detection and quantification of all VL values and all main HIV-1 non-B subtypes and CRFs. Remarkable characteristics of the new assay are the elevated accuracy and reproducibility, even at low HIV RNA values, and apparent higher sensitivity compared to RealTime.

Along with excellent performance, it is important to highlight the full automation, ease of use, and improved workflow of the Aptima assay on the compact Panther system. This system allows random access testing of various analytes, processing up to

275 samples in an 8-hr shift and returning of results in about 2.5 hr. This enables high flexibility to adapt to low or high throughput testing. Combined with the clinical performance data, these characteristics make the Aptima HIV-1 Quant Dx Assay run on the Panther system an attractive solution for routine monitoring of HIV-1 VL in clinical laboratories.

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