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Detection and quantification of HIV-1 RNA with a fully automated transcription-mediated-amplification assay

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ABSTRACT

Background: Nucleic acid testing is the major method used to monitor HIV viral load. Commercial systems based on real-time PCR assays are available for high-volume centralized laboratory testing, but they are not fully automated.
Objectives and study design: We have compared the diagnostic performance of the Hologic Aptima HIV-1 Quant Dx assay (Aptima) (based on real-time TMA) on the Panther instrument, a fully-automated random access platform, to that of, the Roche Cobas Ampliprep Cobas TaqMan (CAP/CTM) HIV-1 version 2.0 (based on real-time PCR).
Results: Probit analysis of replicate dilutions of NIBSC WHO International HIV-1 Standard, gave LODs of 8.6 c/ml for Aptima and 15.2 c/ml for CAP/CTM. The agreement between the assays was excellent when measuring HIV RNA in a calibrated reference ($\kappa = 0.90$, $p < 0.001$) and good when measuring clinical samples ($\kappa = 0.62$, $p < 0.001$). The correlation among the samples quantified by the two methods was very good ($r = 0.95$, $p < 0.001$) and the mean difference between the values obtained with the two assays was 0.02 log c/ml for B and non-B subtypes. The vast majority of results showed <0.5 log variance between the two assays (89%); only one sample showed results that differed by over 1.0 log c/ml.
Conclusion: The performance of the new fully automated Aptima assay is adequate for clinical monitoring of HIV-1 RNA during infections and treatment. The Aptima assay is well suited for routine laboratory use.

1. Background

The HIV-1 viral load (VL) is a key marker for monitoring response to antiretroviral therapy (ART). Clinical trials and cohort studies have shown that obtaining a VL < 50 copies/ml predicts long-term virological suppression and immunological and clinical benefits [1–3]. Optimal viral suppression is generally defined as a viral load persistently below the level of detection of a routine assay, which is <20–75 copies/ml depending on the assay used.

Several commercial viral load assays are presently available, most of which employ real-time PCR [4–6]. Their performance and degree of automation vary but they are widely used in clinical practice throughout the world. Real-time PCR-based assays generally provide a broad linear dynamic range, and good sensitivity, specificity and reproducibility. The past decade has seen a convergence in the results of commercial systems due to calibration with an international standard, but individual patients should still be monitored with a single assay system.

The Aptima HIV assay (Hologic, Inc.,San Diego,Calif.) is a real-Time assay that targets the long terminal repeat (LTR) and the pol gene of HIV-1 groups M, N, and O. RNA is extracted from plasma using the automated target capture technology, followed by amplification and detection using fluorescent probes on the panther instrument, a fully-automated random access platform [7]. We have compared the performance of the Aptima on the Panther platform with that of the Cobas Ampliprep Cobas TaqMan (CAP/CTM) v2.0 assay using HIV-1 panels for specificity, cross contamination risk, sensitivity, reproducibility, linearity and ease of use. We also assessed clinical performance by testing clinical plasma samples tested either retrospectively or prospectively in both assays.

2. Study design

2.1. Evaluation of analytical performance

Sensitivity, specificity, reproducibility and linearity were all assessed by the same operator using the following test panels: 20

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negative plasmas; 20 replicates of each RNA concentration prepared from a commercially available HIV-1 standard (HIV-1 RNA, 3rd International Standard, 10/152 NIBSC) diluted (800, 400, 200, 100, 50, 25, 12.5, 6.25 and 3.125 IU/ml) with negative plasma collected from blood donors into EDTA tubes. These samples were assayed with both the Aptima and CAP/CTM assays. Quality control samples containing 3–5 log c/ml HIV-1 RNA were also tested in triplicate on 15 consecutive days.

2.2. Evaluation of assay performance with clinical samples

The study was designed in two parts. First, the methods were compared by quantifying the HIV RNA in 291 prospective consecutive clinical samples received by the Toulouse University Hospital Laboratory of Virology for routine assessment of HIV viral load. Second, we assayed the HIV RNA in 123 retrospective plasma samples from HIV-positive patients attending the Toulouse University Hospital, infected with different HIV-1 subtypes. The samples were stored at −80 °C, thawed out, diluted to give a volume big enough to measure RNA with both techniques.

2.3. Plasma HIV RNA assays

2.3.1. Aptima HIV-1 Quant Dx assay Aptima

The Aptima system targets two highly conserved regions of HIV-1 RNA *(pol and LTR).* These regions are amplified using specific primers designed to amplify HIV-1 groups M, N and O. The LOD is 12 copies/ml, the lower limit of quantification (LLOQ) is 30 copies/ml, and the quantitative dynamic range is 30–10,000,000 c/ml. It is designed to run on the fully automated Panther platform,with random access capability. Each test requires 500 μl of specimen plus an additional 200 μl for the dead volume.

2.4. Cobas Ampliprep Cobas TaqMan HIV-1 v2.0 (CAP/CTM)

This fully automated real-time PCR assay uses two specific dual-labeled probes targeting the *gag* and LTR regions and is designed to quantify HIV-1 groups M and O. The LOD and LLOQ are both 20 copies/ml and the dynamic range is 20–10,000,000 c/ml. Nucleic acid extraction and real-time PCR steps are automatically performed in the "docked" configuration of the Cobas TaqMan 96 instrument, using an input volume of 1040 μl of specimen (850 μl per test and 190 μl dead volume).

2.5. Data analysis

For data analysis, data obtained in International Units (IU/ml) were first calculated in copies/ml (c/ml) according to the conversion factors given by the manufacturers (1 IU = 0.35 c for Aptima; 1 IU = 0.59 c for CAP/CTM) and then they were log transformed (Log c/ml). The LODs of both assays, the lowest concentration of HIV RNA detected in 95% of cases, was determined by Probit analysis. Qualitative results were compared with MacNemar chi-squared test, and the agreement between paired results was assessed by calculating the kappa value (κ). The low-concentration precisions of the two assays were compared using a single panel of samples. Reproducibility was assessed for each concentration by calculating the standard deviation. Passing Bablok and Bland-Altman analyses were performed on the quantitative results and the Spearman correlation was calculated to assess the strength of the correlation. P values less than 0.05 were considered significant. All analyses were conducted using XLStat 14 and Stata 14.0 software.

Table 1
n°1 Detection and quantification of replicates (n = 20) of NIBSC diluted to low-level HIV RNA.

RNA level (IU/ml)	Analytical parameters	APTIMA HIV	CAPCTM HIV
800	Detected (n)/Quantified (n)	20/20	20/20
	Mean (log IU/ml)	2.87	3.06
	SD (log IU/ml)	0.11	0.17
400	Detected (n)/Quantified (n)	20/20	20/20
	Mean (log IU/ml)	2.49	2.72
	SD (log IU/ml)	0.14	0.09
200	Detected (n)/Quantified (n)	20/19	20/20
	Mean (log IU/ml)	2.15	2.38
	SD (log IU/ml)	0.16	0.13
100	Detected (n)/Quantified (n)	20/3	20/20
	Mean (log IU/ml)	1.99	2.09
	SD (log IU/ml)	0.08	0.14
50	Detected (n)/Quantified (n)	20/1	19/14
	Mean (log IU/ml)	na	1.88
	SD (log IU/ml)	na	0.16
25	Detected (n)/Quantified (n)	20/0	19/7
	Mean (log IU/ml)	na	1.77
	SD (log IU/ml)	na	0.23
12.5	Detected (n)/Quantified (n)	10/0	10/1
6.25	Detected (n)/Quantified (n)	8/0	8/1
3.125	Detected (n)/Quantified (n)	7/0	3/0

na: not applicable.

3. Results

3.1. Limit of detection

HIV RNA was detected in 80.6% of samples with the Aptima assay and in 77.2% using the CAP/CTM assay (Table 1). The LODs of the two assays, calculated using 20 replicates of 9 concentrations of NIBSC standard (800, 400, 200, 100, 50, 25, 12.5, 6.25 and 3.125 IU/ml), were 24.6 IU/ml (95% confidence interval: 19.0–39.2) for the Aptima assay and 25.9 IU/ml (95% confidence interval: 20.6–38.0) for the CAP/CTM assay. The corresponding LOD values in copies/ml unit were 8.6 [CI 95%: 6.7–13.7] c/ml for the Aptima assay and 15.2 [CI 95%: 12.1–22.3] c/ml for the CAP/CTM assay.

3.2. Specificity

The specificity of the Aptima assay was assessed by testing plasma samples from 20 seronegative blood donors. Positive samples were interspersed between each negative sample. All the seronegative samples were negative for HIV RNA, and there was no cross-contamination.

3.3. Reproducibility

The within-run (repeatability) and between-run (reproducibility) precision of the Aptima assay were estimated by a panel composed of three members, with three different HIV-1 target concentrations (low, medium and high) in triplicate each day for 15 days. The mean within-run precision for the low control (mean = 3.42 log c/ml), for the medium control (mean = 4.88 log c/ml), and for the high control (mean = 5.32 log c/ml) was 0.09 log c/ml (0.05–0.16 log c/ml), 0.06 log c/ml (0.01–0.17 log c/ml), and 0.04 log c/ml (0.02–0.09 log c/ml), respectively. The reproducibility was 0.12 log c/ml, 0.10 log c/ml, and 0.07 log c/ml, respectively.

The reproducibility of the Aptima and CAP/CTM assays were further assessed by serial dilutions of the 3rd International HIV RNA WHO Standard, prepared to determine their LODs. With the different LLOQs of the two assays, HIV RNA was quantified in 63% of