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Multicenter evaluation of the cobas® HIV-1 quantitative nucleic acid test for use on the cobas® 4800 system for the quantification of HIV-1 plasma viral load

Phillip Adams[a,1], Ellen Vancutsem[b,1], Cyrielle Nicolaizeau[a], Jean-Yves Servais[a], Denis Piérard[b], Jean-Hugues François[c], Tanja Schneider[d], Ellen E. Paxinos[e], Ed G. Marins[e], Jesse A. Canchola[e], Carole Seguin-Devaux[a,*]

[a] Department of Infection and Immunity, Luxembourg Institute of Health, Luxembourg
[b] AIDS Reference Laboratory, Dept. Microbiology, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium
[c] Molecular Biology Laboratory, Centre Hospitalier de Luxembourg, Luxembourg
[d] Roche Diagnostics Ltd, Rotkreuz, Switzerland
[e] Roche Molecular Systems, Pleasanton, CA, USA

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ABSTRACT

Background and objectives: Measurement of HIV-1 viral load (VL) is necessary to monitor treatment efficacy in patients receiving antiretroviral therapy. We evaluated the performance of the cobas® HIV-1 quantitative nucleic acid test for use on the cobas® 4800 system ("cobas 4800 HIV-1").

Methods: Limit of detection, linearity, accuracy, precision, and specificity of cobas 4800 HIV-1, COBAS® AmpliPrep/COBAS® Taqman® HIV-1 version 2.0 (CAP/CTM HIV-1 v2) and Abbott RealTime HIV-1 were determined in one or two out of three sites.

Results: The limit of detection of the cobas 4800 HIV-1 for 400 µL and 200 µL input volumes was 14.2 copies/mL (95% CI: 12.5–16.6 copies/mL) and 43.9 copies/mL (37.7–52.7 copies/mL), respectively. Cobas 4800 HIV-1 demonstrated 100% specificity, and results were linear for all analyzed group M HIV-1 subtypes. Precision was high (SD < 0.19 $\log_{10}$) across all measured ranges, reagent lots and input volumes. Correlation between cobas 4800 HIV-1 and CAP/CTM HIV-1 v2 or RealTime HIV-1 was high ($R^2 \geq 0.95$). Agreement between cobas 4800 HIV-1 and CAP/CTM HIV-1 v2 was 96.5% (95.0%–97.7%) at a threshold of 50 copies/mL, and 97.2% (95.8%–98.3%) at 200 copies/mL. Agreement between cobas 4800 HIV-1 and RealTime HIV-1 was 96.6% (93.4%–98.5%) at 50 copies/mL, and 97.0% (94.0%–98.8%) at 200 copies/mL. The mean difference between cobas 4800 HIV-1 and CAP/CTM HIV-1 v2 or RealTime HIV-1 was -0.10 $\log_{10}$ or 0.01 $\log_{10}$, respectively.

Conclusions: The cobas 4800 HIV-1 test is highly sensitive, accurate and correlated well with other assays, including agreement around clinically relevant thresholds, indicating minimal overall VL quantification differences between tested platforms.

1. Background

Suppression of HIV-1 replication by antiretroviral therapy (ART) has sharply reduced HIV-related mortality and rendered HIV infection manageable for those with access to treatment [1]. Approximately 21.7 million people living with HIV-1 are receiving ART [2]. The UNAIDS 90-90-90 target encourages all countries to optimize the continuum of care until 73% of people living with HIV are virally suppressed [3]. Based on the results of the START study [4] the WHO recommended ART for all HIV-positive persons, independent of the CD4 T-cell count [5]. Since monitoring of plasma viral load (VL) is necessary to detect treatment failure, there is growing demand for access to VL measurement on a global scale [6–8].

Automated real-time PCR-based technologies, which display an increased dynamic range and are less prone to contamination, have replaced endpoint-based methods for VL measurement [9]. As the lower limits of detection (LOD) and quantification (LLOQ) of these platforms dropped [10], transient low-level viremia (viral blips of 50 to 500

* Corresponding author at: Luxembourg Institute of Health, Department of Infection and Immunity, 29, rue Henri Koch, L-4354, Esch-sur-Alzette, Luxembourg.
E-mail address: carole.devaux@lih.lu (C. Seguin-Devaux).
[1] Shared first author.

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copies/mL) and persistent low-level viremia have been more frequently detected in patients on ART. Although the clinical relevance of low-level viremia is still debated, some studies implicate it in predicting treatment failure and early occurrence of drug resistance [9,11–17]. Therefore, standardization and development of assays with a reliable readout near the LLOQ and thresholds that define treatment failure is important for clinical decision making.

Two US FDA-approved assays are commonly used for VL monitoring: the COBAS AmpliPrep/COBAS® Taqman® HIV-1 version 2.0 ("CAP/CTM HIV-1 v2"; Roche Molecular Systems) [18], and the RealTime HIV-1 assay ("RealTime HIV-1", Abbott Molecular) [19]. Several studies have shown good correlation between the assays [17,20,21] with lower agreement around the LLOQ [22,23]. The cobas® HIV-1 quantitative nucleic acid test for use on the cobas® 4800 system ("cobas 4800 HIV-1", Roche Molecular Systems) is a fully automated real-time PCR-based assay, which uses a dual-target design for subtype coverage, including groups M, N and O.

2. Objective

To evaluate the technical performance of cobas 4800 HIV-1 for HIV-1 group M (B and non-B subtypes) and groups N and O, and compare results from cobas 4800 HIV-1 to those from 2 other commerically available assays.

3. Study design

Technical performance (LOD, specificity, precision, accuracy and linearity) was assessed at Roche Diagnostics (Rotkreuz, Switzerland). Method comparison to CAP/CTM HIV-1 v2 and RealTime HIV-1 using clinical samples was conducted at Luxembourg Institute of Health, Luxembourg and Universitair Ziekenhuis Brussel, Belgium.

The frequency of invalid results on the different systems ranged between 5 and 10% in most cases. This study was not designed to compare invalid test result frequency between systems.

3.1. Limit of detection (LOD)

The WHO 2nd International Standard for HIV RNA (NIBSC code: 97/650, HIV-1 Group M Subtype B) was serially diluted in HIV-negative EDTA plasma in 3 independent series, to generate 6 HIV-1 concentrations. Eighty-four replicates per concentration, using both 400 µL and 200 µL sample input volumes, were evaluated using 3 reagent lots on 3 cobas 4800 systems. The HIV-1 LOD was estimated by probit analysis as the lowest concentration where ≥95% of samples were positive. To confirm the LOD in multiple HIV-1 groups and subtypes, cell culture supernatant virus stocks representing HIV-1 group M subtypes A, C, D, F, G, H, CRF01_AE and CRF02_AG, HIV-1 group O and N were diluted in negative plasma to a target concentration of 5000 copies/mL. Titer assignments were confirmed as previously described [24]. On each day of testing, 5000 copies/mL stocks were serially diluted to the approximate LOD (20 copies/mL). A total of 42 replicates (400 µL input volume) per subtype/group tested, distributed across 2 dilution series, 1 reagent lot and 2 cobas 4800 systems.

3.2. Linearity, accuracy, precision, and specificity

The linearity of cobas 4800 HIV-1 was evaluated using subtype B HIV-1 cell culture supernatant virus stocks diluted into negative plasma. Each panel was tested in 56 replicates per concentration using 2 reagent lots, 2 cobas 4800 systems and 2 different operators over 4 days using the 400 µL and 200 µL input volumes. Determination of mean $\log_{10}$ titer and pooled standard deviation (SD) for each assigned concentration was calculated. Linearity was assessed according to Clinical and Laboratory Standards Institute EP6-A (https://clsi.org/standards/products/method-evaluation/documents/ep06/). Accuracy was calculated by subtracting the $\log_{10}$ assigned titer from the mean $\log_{10}$ observed titer for all concentration levels within the linear range. Linearity with many HIV-1 subtypes was confirmed using the 400 µL input volume and 7 different HIV-1 concentrations spanning the linear range of the assay (20 to 10^7) with cell culture supernatant virus stocks. Each panel was tested in 12 replicates using 1 reagent lot.

Precision was evaluated over the expected linear ranges of the two input volumes (400 µL and 200 µL) using serial dilutions of cell culture supernatant virus stocks in HIV-negative EDTA plasma (8 dilution levels ranging from 23 to 1.54×10^7 copies/mL for the 400 µL input volume and 6 dilution levels ranging from 770 to 1.54×10^7 copies/mL for the 200 µL input volume). Precision was calculated using 72 replicate results generated over 15 days, 3 reagent lots and 4 operators using 3 cobas 4800 systems. Mean $\log_{10}$ titer and pooled SD were calculated for each assigned concentration/panel member and input volume.

Analytical specificity of the cobas 4800 HIV-1 test (400 µL input volume) was evaluated by testing the cross-reactivity of 27 different microorganisms (Supplementary Materials Table S1) at a concentration of 10^6 particles/mL spiked into HIV-negative or positive EDTA samples. A total of 614 HIV-1 negative EDTA plasma samples from healthy donors were tested with 1 replicate each over 5 days, using the 400 µL input volume, and using 3 different assay lots. Specificity was calculated by dividing the true negative values by the sum of the true negatives plus false positives and multiplying by 100, with the lower one-side 95% CI being calculated using Clopper-Pearson method.

3.3. Method comparison

HIV-1 RNA quantification with cobas 4800 HIV-1 was compared to the CAP/CTM HIV-1 v2 (n = 688) and RealTime HIV-1 (n = 240) using HIV-1 positive plasma samples from the Centre Hospitalier of Luxembourg and Universitair Ziekenhuis Brussel. Samples were thawed and centrifuged before evaluation to prevent false viremia in samples with undetectable VL [25]. The samples included a wide range of group M subtypes, as well as group O (Table 1), and detectable or undetectable VL. HIV-1 subtypes were assigned using the COMET HIV-1 subtyping tools (https://comet.lih.lu/) and *pol* gene (protease and reverse transcriptase) sequences from the patient's baseline sample.

All pairwise assay comparisons used $\log_{10}$ transformed results that were within the linear range for both tests and Deming regression and Bland-Altman plots. The coefficient of determination (R^2) was calculated, as well as the 95% CI for the slopes and intercept. Overall percentage agreement (OPA) at the medically relevant thresholds of 50 and 200 copies/mL between paired measurements for all samples with valid results for each test was determined.

All statistical analyses were performed using SAS® software, version 9.4 or higher (SAS Institute, Cary, North Carolina, USA).

Table 1
Subtype of samples used for cobas 4800 HIV-1 method comparisons samples with results in linear range.

HIV-1 Subtype, CRF, URF, or Group	CAP/CTM HIV-1 v2	RealTime HIV-1
A	13	14
B	100	96
C	21	24
D	8	8
F	14	13
F/B	1	1
G	29	28
H	7	6
HIV-1 Group O	2	2
CRF01_AE	12	8
CRF02_AG	23	16
Unknown (no sequence)	303	0
Total	**533**	**216**

Table 2
Limit of detection of cobas 4800 HIV-1.

Copies/mL	% positive (N positives/N valid replicates)	
	400 µL Input volume (n = 1582)	200 µL Input volume (n = 1571)
100	nd	100% (251/251)
60	100% (252/252)	99% (249/251)
30	100% (251/251)	90% (227/251)
20	98% (247/252)	nd
15	nd	69% (172/250)
10	90% (227/252)	nd
7	nd	44% (110/250)
5	64% (160/252)	nd
3.5	nd	33% (83/250)
2	34% (86/252)	nd
0	0% (0/71)	0% (0/68)
LOD via probit, 95% hit rate [95% CI], copies/mL	**14.2 [12.5 to 16.6]**	**43.9 [37.7 to 52.7]**

nd: not done.

4. Results

4.1. Limit of detection

Thirty-seven runs containing 3153 valid results were used to determine the LOD for both input volumes for cobas 4800 HIV-1. The LOD for all three reagent lots combined was 14.2 copies/mL (95% CI: 12.5–16.6 copies/mL) for the 400 µL input volume, and 43.9 copies/mL (37.7–52.7 copies/mL) for the 200 µL input volume (Table 2). No impact of HIV-1 subtype on LOD was detected (Supplementary Materials Table S2).

4.2. Linearity, accuracy, precision, and specificity

The cobas 4800 HIV-1 test was linear (i.e., 95% CI for slope includes 1) for both input volumes (400 µL and 200 µL) over 16 assay runs evaluating 1288 valid test results (Fig. 1 and data not shown). The difference between observed and assigned $\log_{10}$ titer across the linear range did not exceed ±0.15 $\log_{10}$ (Table 3). The assay was linear between 11.6–15.4 million copies/mL and 23.1–15.4 million copies/mL for the 400 µL and 200 µL input volumes, respectively. All groups and subtypes tested (HIV-1 group M subtypes A, C, D, F, G, H, CRF01_AE, CRF02_AG, HIV-1 group O, HIV-1 group N) were detected within the linear range. Each subtype showed linearity for all concentration levels tested (Supplementary Materials Table S3 and Fig. S1).

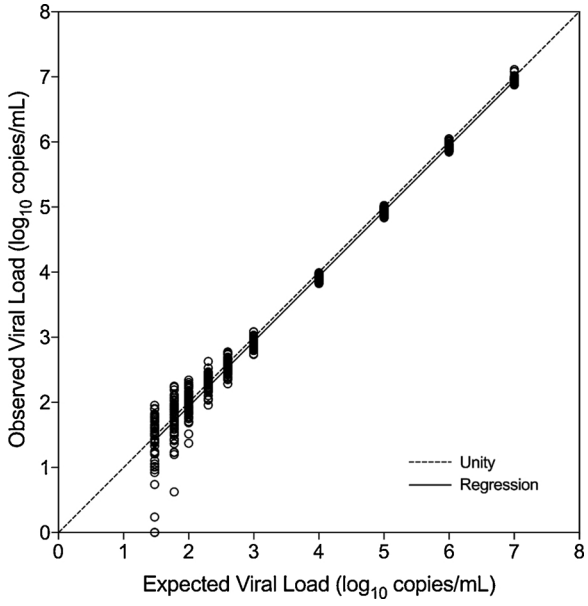

Fig. 1. Linearity of cobas 4800 HIV-1 (400 µL input volume). Observed viral load results are plotted against the expected value (see Methods). The dashed diagonal line indicates perfect agreement between observed and expected values. The slope of the regression line is 1.001 (95% CI: 0.99–1.012; R^2 = 0.984).

Precision was assessed using 999 valid test results from 28 assay runs. Precision was comparable across all reagent lots. The pooled $\log_{10}$ SD ranged from 0.05 to 0.19 for the 400 µL input volume and was 0.04 across all reagent lots for the 200 µL input volume (Supplementary Materials Table S4).

In specificity experiments, all HIV-negative plasma samples were negative (100% specificity, 95% CI: 99.4% to 100%). Results were not affected by 27 different microorganisms in HIV-1 positive or negative plasma (Supplementary Materials Table S1 and data not shown). The mean difference in $\log_{10}$ titer in the HIV-1 positive samples ranged from 0.04 to 0.31 $\log_{10}$ copies/mL.

4.3. Method comparison

4.3.1. Correlation between cobas 4800 HIV-1 and CAP/CTM HIV-1 v2

HIV-1 positive clinical samples (n = 688) were evaluated with cobas 4800 HIV-1 and CAP/CTM HIV-1 v2; 155 samples had results below the LLOQ in one or both assays. There was high concordance between 533 paired results within the overlapping linear ranges of both assays (R^2 = 0.95; Fig. 2A). Bland-Altman analysis showed a high agreement between cobas 4800 HIV-1 and CAP/CTM HIV-1 v2, with a mean difference of 0.07 $\log_{10}$ (95% CI for the mean difference: 0.04 to 0.09, Fig. 2B).

Tables 4 and 5 show the agreement between cobas 4800 HIV-1 and CAP/CTM HIV-1 v2 for clinically relevant thresholds, with an OPA of 96.2% (95% CI, 95.1%–97.1%) and 96.9% (95.9%–97.8%) for the 50 and 200 copies/mL thresholds, respectively. There were 26 discordant samples between assays at the 50 copies/mL threshold: 9 sample results were reported as <50 copies/mL and 17 sample results were reported ≥50 copies/mL, by cobas 4800 HIV-1 but not CAP/CTM HIV-1 v2 (Table 4). Of the 21 discordant results observed at the 200 copies/mL threshold, 13 results were reported as <200 copies/mL and 8 as ≥200 copies/mL by cobas 4800 HIV-1 but not CAP/CTM HIV-1 v2 (Table 5).

4.3.2. Correlation between cobas 4800 HIV-1 and RealTime HIV-1

A total of 240 HIV-1 clinical samples were evaluated with cobas 4800 HIV-1 and RealTime HIV-1; 24 samples had results below the LLOQ of one or both assays. There was high concordance among the 216 pairs with results within the linear range of both assays (R^2 = 0.96; Fig. 3A). Four samples had differences greater than 0.5 log: 3 were subtype C (2 with a higher RealTime HIV-1 result and 1 with higher cobas 4800 HIV-1 result) and 1 was subtype H. There were also 21 subtype C samples and 5 subtype H samples with ≤ 0.5 $\log_{10}$ difference. Bland-Altman analysis showed a high agreement between cobas 4800 HIV-1 and RealTime HIV-1 with a mean difference of 0.02 $\log_{10}$ (95% CI for the mean difference: −0.01 to 0.06, Fig. 3B).

Tables 6 and 7 show the agreement between cobas 4800 HIV-1 and RealTime HIV-1 for two clinical thresholds, with an OPA of 96.3% (95% CI: 93.0%–98.3%) and 97.1% (94.1%–98.8%) for the 50 and 200 copies/mL thresholds, respectively. There were 9 discordant samples at the 50 copies/mL threshold: 6 sample results with VL ≥ 50 copies/mL by cobas 4800 HIV-1 were categorized as <50 copies/mL by RealTime HIV-1. There were 3 sample results with VL < 50 copies/mL by cobas 4800 HIV-1, but categorized as ≥50 copies/mL by RealTime HIV-1 (Table 6). There were 7 discordant results observed at the 200 copies/mL threshold: 5 samples with VL ≥ 200 copies/mL by cobas 4800 HIV-1 were categorized as <200 copies/mL by RealTime HIV-1, and 2 samples with VL < 200 copies/mL by cobas 4800 HIV-1 were categorized as ≥200 copies/mL by RealTime HIV-1 (Table 7)

Table 3
Accuracy results for cobas 4800 HIV-1.

Nominal/assigned log_{10} titer, copies/mL	400 µL input			200 µL input		
	Mean observed titer, log_{10} copies/mL	Mean Difference log_{10} titer (95% CI)	%CV[a]	Mean observed titer, log_{10} copies/mL	Mean Difference log_{10} titer (95% CI)	%CV[a]
6.89	6.95	0.06 (0.05, 0.08)	11.9	6.96	0.08 (0.06, 0.10)	16.2
5.89	5.93	0.04 (0.02, 0.05)	12.6	5.93	0.04 (0.03, 0.06)	12.4
4.89	4.92	0.04 (0.02, 0.05)	10.0	4.94	0.06 (0.04, 0.07)	11.1
3.89	3.91	0.02 (0.01, 0.03)	9.0	3.92	0.03 (0.02, 0.04)	11.4
2.89	2.89	0.01 (-0.01, 0.03)	17.3	2.98	0.09 (0.07, 0.12)	20.9
2.49	2.58	0.09 (0.06, 0.11)	24.6	2.62	0.13 (0.10, 0.17)	28.9
2.19	2.30	0.11 (0.08, 0.15)	30.8	2.34	0.15 (0.10, 0.20)	45.3
1.89	2.00	0.12 (0.06, 0.17)	50.0	1.96	0.11 (0.00, 0.22)	111.9
1.66	1.75	0.09 (0.01, 0.17)	78.1	below LLOQ		
1.36	1.46	0.09 (-0.01, 0.19)	223	below LLOQ		

[a] Lognormal percent coefficient of variation = square root of {10^[SD^2 * ln(10)] – 1} * 100% where SD = standard deviation in the logarithm base 10 scale and ln (•) is the natural logarithm.

5. Discussion

Results of this study demonstrate that cobas 4800 HIV-1 has high sensitivity and specificity, and can be used for HIV-1 from all major group M subtypes, group N and O. The LOD was 14.2 copies/mL (95% CI: 12.5 to 16.6 copies/mL) for the 400 µL input volume and 43.9 copies/mL (37.7 to 52.7 copies/mL) for the 200 µL input volume. The cobas 4800 HIV-1 test showed high concordance with CAP/CTM HIV-1 v2 and RealTime HIV-1. The comparison with CAP/CTM HIV-1 v2 is similar to results obtained using the cobas 6800 HIV-1 test [26], where a small (0.11 log_{10}) difference was observed. This small difference is unlikely to be clinically significant. Test results were precise and accurate across all tested subtypes, input volumes, and VL in the linear range. The LOD of cobas 4800 HIV-1 with a low input volume of 200 µL ensures accurate quantitation near the clinically important threshold of 50 copies/mL when sample volumes are limiting, for example with pediatric samples, or when repeat testing is needed for samples with limited quantities.

We observed greater variability in the difference between the cobas 4800 HIV-1 and CAP/CTM HIV-1 v2 (Fig. 2B, upper – lower 95% CI limits 1.1 log_{10}) compared to between cobas 4800 HIV-1 and RealTime HIV-1 (Fig. 3B, upper – lower 95% CI limits 0.93 log_{10}). While the reasons for this difference are unknown, they are unlikely to be clinically significant.

The cobas 4800 HIV-1 test showed ≥96.5% agreement with the CAP/CTM HIV-1 v2 assay for categorizing VL as being above or below the 50 and 200 copies/mL thresholds. Similar levels of agreement were found between cobas 4800 HIV-1 and RealTime HIV-1. Although the use of one type of molecular platform is recommended for the follow up of VL because of the different performance characteristics of the different systems [27,28], results of this study showed improved commutability among the three assays.

Table 4
Concordance analysis of cobas 4800 HIV-1 and CAP/CTM HIV-1 v2 (50 copies/mL threshold).

cobas 4800 HIV-1	CAP/CTM HIV-1 v2		Total n (%)
	≥ 50 copies/mL	< 50 copies/mL	
≥ 50 copies/mL	506	17	523 (76)
< 50 copies/mL	9	156	165 (24.0)
Total	515	173	688 (100.0)
Overall % agreement [95% CI][a]	96.2 (662/688) [95.1 – 97.1]		

[a] Two-sided Clopper-Pearson Exact confidence interval.

Highly sensitive quantitative assays are required to monitor viral suppression in patients treated with ART. Accurate quantification near clinically relevant thresholds used to define treatment success or failure is of special importance. Current thresholds for defining treatment

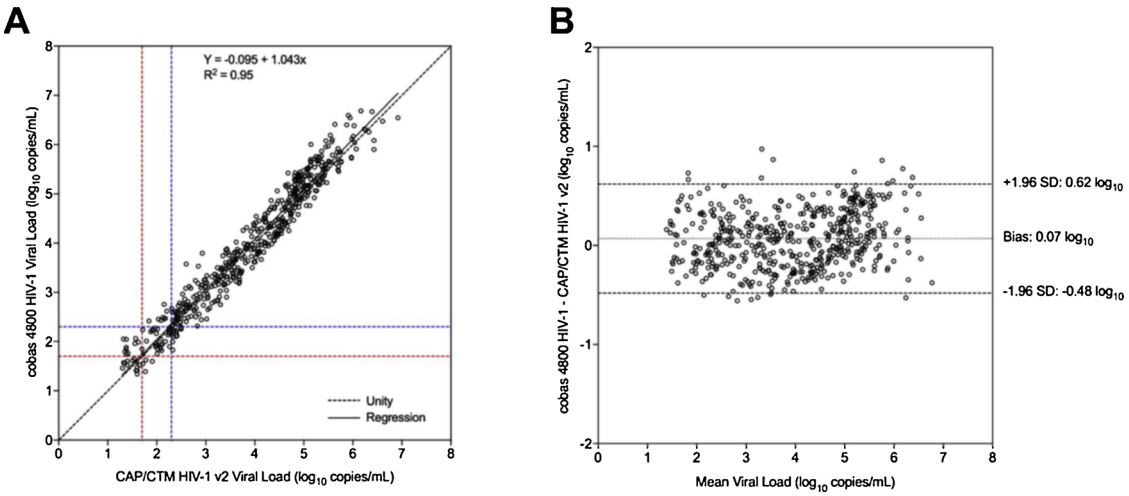

Fig. 2. Method comparison for cobas 4800 HIV-1 versus CAP/CTM HIV-1 v2 (n = 533) **A.** Deming regression analysis. Intercept: −0.095 (95% CIs: −0.179 to −0.010), Slope 1.043 (1.022–1.064). Dashed lines indicate thresholds of 50 (red) or 200 (blue) copies/mL. **B.** Bland-Altman plot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 5
Concordance analysis of cobas 4800 HIV-1 and CAP/CTM HIV-1 v2 (200 copies/mL threshold).

cobas 4800 HIV-1	CAP/CTM HIV-1 v2		Total n (%)
	≥ 200 copies/mL	< 200 copies/mL	
≥ 200 copies/mL	455	8	463 (67.3)
< 200 copies/mL	13	275	225(32.7)
Total	468	220	688(100.0)
Overall % agreement [95% CI][a]	96.9 (667/688) [95.9 – 97.8]		

[a] Two-sided Clopper-Pearson Exact confidence interval.

Table 6
Concordance analysis of cobas 4800 HIV-1 and RealTime HIV-1 (50 copies/mL threshold).

cobas 4800 HIV-1	RealTime HIV-1		Total n (%)
	≥ 50 copies/mL	< 50 copies/mL	
≥ 50 copies/mL	213	6	219 (91.2)
< 50 copies/mL	3	18	21 (8.8)
Total	216	24	240 (100.0)
Overall % agreement [95% CI][a]	96.3% (231/240) [93.0% to 98.3%]		

[a] Two-sided Clopper-Pearson Exact confidence interval.

Table 7
Concordance analysis of cobas 4800 HIV-1 and RealTime HIV-1 (200 copies/mL threshold).

cobas 4800 HIV-1	RealTime HIV-1		Total n (%)
	≥ 200 copies/mL	< 200 copies/mL	
≥ 200 copies/mL	200	5	205 (85.4)
< 200 copies/mL	2	33	35 (14.6)
Total	202	38	240 (100.0)
Overall % agreement [95% CI][a]	97.1% (233/240) [94.1% to 98.8%]		

[a] Two-sided Clopper-Pearson Exact confidence interval.

failure are 50 copies/mL according to the European AIDS Clinical Society guidelines [29], or 200 copies/mL as defined by the US Department of Health and Human Services (DHHS) guidelines [30,31]. One reason cited for the DHHS threshold is higher assay variability at low viral load [31]. A comparison between CAP/CTM HIV-1 v2 and RealTime HIV-1 in patients on ART in two AIDS Clinical Trial Group studies demonstrated a high degree of agreement between the two assays but significantly better ability to identify virological failure reliably when defined by the higher threshold [32]. In our study, although a similarly high level of agreement between the three assays at both 50 and 200 copies/ml thresholds was also shown, no significant differences were revealed between the two thresholds. This could be explained by the lower number of samples tested, especially for the correlation between cobas 4800 HIV-1 and RealTime HIV-1.

WHO recommends that national HIV programs implement VL testing for ART-treated patients biannually in the first year and annually thereafter [5]. With 21.7 million people on ART globally, meeting this goal requires significant VL testing capacity increases in laboratories in low- and middle-income countries. One part of the solution to this forecasted demand is to maximize testing throughput without a concomitant increase in the need for highly skilled laboratory workers. Medium and high-throughput automated systems such as cobas 4800 and 6800/8800 [33] may help address this need. The lower input volume of the cobas platform, acceptance of primary tubes, and the ability to test for other targets in addition to HIV-1, may be advantageous in a clinical laboratory setting [33,34].

The lower number of samples tested for the comparison between cobas 4800 HIV-1 and RealTime HIV-1, especially at or below 50 copies/mL (Table 6), could limit our ability to discern a difference in the ability of the various assays to identify patients with virological failure. However, the 95% CI around the overall percent agreement between assays are comparable.

In conclusion, we have shown that cobas 4800 HIV-1 is highly sensitive, precise and accurate. It provides VL results that are comparable to other commercially available HIV VL assays, based on minimal overall VL quantification differences between platforms within the tests' dynamic ranges, for all HIV-1 subtypes. The technical benefits of the platform, combined with the accurate measurement of low-level viremia may enable early detection of treatment failure. Detection of treatment failure at low VL may precede the emergence of drug resistance, or be a consequence of it [35,36]. Ultimately, automated platforms such as cobas 4800 HIV-1 will allow the scale-up of VL measurement that is required to achieve the "third 90" of the ambitious UNAIDS 90-90-90 targets.

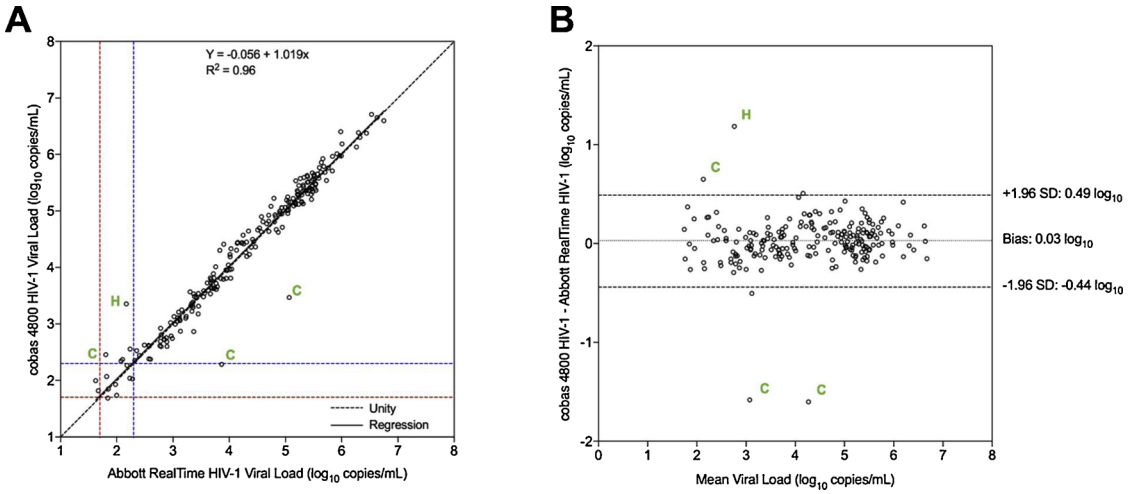

Fig. 3. Method comparison for cobas 4800 HIV-1 versus RealTime HIV-1 (n = 216) **A.** Deming regression analysis. Intercept: −0.056 (95% CIs: −0.194 to 0.082), Slope 1.019 (0.991–1.047). Dashed lines indicate thresholds of 50 (red) or 200 (blue) copies/mL. **B.** Bland-Altman plot. Green letters indicate HIV-1 subtype for samples with difference > 0.5 $\log_{10}$.

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Ethical approval

Use of anonymized residual clinical samples for evaluation of diagnostic tests was approved by the Comité National d'Ethique en Recherche of Luxembourg and the ethical committee of Universitair Ziekenhuis Brussel.

Competing interests

TS is an employee of Roche Diagnostics Ltd; MN, EEP, EGM, JAC are employees of Roche Molecular Systems, Inc. PA, EV, DP, CN, CYS, JHF and CS-D have no competing interests.

CRediT authorship contribution statement

Phillip Adams: Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Ellen Vancutsem:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing. **Cyrielle Nicolaizeau:** Investigation, Writing - review & editing. **Jean-Yves Servais:** Investigation, Writing - review & editing. **Denis Piérard:** Investigation, Writing - review & editing. **Jean-Hugues François:** Investigation, Writing - review & editing. **Tanja Schneider:** Investigation, Writing - review & editing. **Ellen E. Paxinos:** Conceptualization, Writing - original draft, Formal analysis, Funding acquisition. **Ed G. Marins:** Conceptualization, Writing - review & editing. **Jesse A. Canchola:** Data curation, Formal analysis, Visualization. **Carole Seguin-Devaux:** Conceptualization, Investigation, Supervision, Validation, Writing - review & editing.

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Appendix A. Supplementary data

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