Evaluation of the Abbott *m*2000 RealTime Human Immunodeficiency Virus Type 1 (HIV-1) Assay for HIV Load Monitoring in South Africa Compared to the Roche Cobas AmpliPrep-Cobas Amplicor, Roche Cobas AmpliPrep-Cobas TaqMan HIV-1, and BioMérieux NucliSENS EasyQ HIV-1 Assays[▽]

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The implementation of antiretroviral therapy demands the need for increased access to viral load (VL) monitoring. Newer real-time VL testing technologies are faster and have larger dynamic ranges and fully automated extraction to benefit higher throughputs in resource-poor environments. The Abbott RealTime human immunodeficiency virus type 1 (HIV-1) assay was evaluated as a new option for testing for HIV-1 subtype C in South Africa, and its performance was compared to the performance of existing assays (the Cobas AmpliPrep-Cobas TaqMan HIV-1, version 1, assay; the AmpliPrep-Cobas Monitor standard HIV-1 assay; and the NucliSENS EasyQ-EasyMag HIV-1 assay) in a high-throughput laboratory. The total precision of the RealTime HIV-1 assay was acceptable over all viral load ranges. This assay compared most favorably with the Cobas AmpliPrep-Cobas TaqMan HIV-1 assay ($R^2 = 0.904$), with a low standard deviation of difference being detected (0.323 copies/ml). The bias against comparator assays ranged from -0.001 copies/ml to -0.228 copies/ml. Variability in the reporting of VLs for a 20-member subtype panel compared to the variability of other assays was noted with subtypes G and CRF02-AG. The RealTime HIV-1 assay can test 93 samples per day with minimal manual preparation, less staff, and the minimization of contamination through automation. This assay is suitable for HIV-1 subtype C VL quantification in South Africa.

South Africa bears a huge burden of human immunodeficiency virus (HIV) infection and has an estimated 5.5 million infected individuals (9). In September 2007, approximately 329,000 individuals received antiretroviral (ARV) treatment as part of the national program (24a, 40). Globally, viral load (VL) testing has been used to monitor treatment, determine the prognosis and the risk of disease progression, and identify treatment failures (47). South African treatment guidelines use VL results to determine the time for the switch from a first-line to a second-line drug regimen (24). Work from the region has demonstrated that the use of VL monitoring together with targeted adherence approaches results in significant conservation of the first-line drug regimen (2). In addition, it has been demonstrated that the switching of therapy on the basis of clinical and immunological criteria alone results in the development of more complex resistance profiles (42), thus reinforcing the value of VL monitoring. Initial WHO guidelines for ARV treatment in resource-poor settings referred to the use of VL monitoring as optional (45). There has been a recent shift in thinking, with the revised guidelines referring to VL testing

as being desirable in resource-limited settings (11) and with greater international efforts being focused on improving access to virological testing.

Nucleic acid amplification technologies are used for VL testing in most settings and are based on either signal amplification (27) or target amplification (20) methodological approaches. The trend now is toward the use of the real-time detection of amplicons (43), which simply means that detection is done as the product accumulates during the exponential phase of the reaction. Newer real-time technology options are faster and have higher throughputs, larger dynamic ranges, and fully automated extraction steps (43). To ensure that the South African National Health Laboratory Service can cope with the high volumes of samples anticipated as the treatment program matures, several automated, real-time monitoring assays have been explored in country.

In the study described here, we investigated and compared the performance of the Abbott Diagnostics (Abbott Park, IL) RealTime HIV type 1 (HIV-1) assay (abbreviated RealTime HIV-1) on the *m*2000sp/*m*2000rt automated extraction platform (Abbott Molecular Inc., Des Plaines, IL) to the performance of the Cobas AmpliPrep-Cobas TaqMan HIV-1, version 1, assay (abbreviated CAP/CTM HIV-1; F. Hoffmann-La Roche, Diagnostics Division, Basel, Switzerland); the NucliSENS EasyQ-EasyMag HIV-1, version 1.1, assay (abbreviated NucliSENS HIV-1; bioMérieux, Boxtel, The Nether-

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lands); and the AmpliPrep-Cobas Monitor standard HIV-1 assay (abbreviated CAP/CA HIV-1), assays which are already established within the South African laboratory setting (32, 33).

The first three assays mentioned above use real-time platforms with fluorescence detection systems, and CAP/CA HIV-1 is based on endpoint detection. Previous publications reported on the performance of CAP/CTM HIV-1 (12, 17, 25, 26, 28) and NucliSENS HIV-1 (8, 13, 32, 33). RealTime HIV-1 has previously been validated against the Roche HIV-1 Monitor assay (which uses a manual RNA extraction step) and CAP/CTM HIV-1 with the *m1000* system, which is less automated than the *m2000sp* system used in this study (6, 15). Other studies have compared RealTime HIV-1 to the Roche Cobas Monitor assay for VL testing for HIV-1 and hepatitis C virus (29, 37, 38). Swanson and colleagues (2006) compared RealTime HIV-1 with the fully automated *m2000* system, CAP/CA HIV-1 (version 1.5), the Versant HIV-1 RNA (version 3.0) system, and the Abbott LCx HIV-1 RNA quantitative system (38). A recent study has also compared RealTime HIV-1 to NucliSENS HIV-1 for the detection of the HIV-1 clades prevalent in China (46). Although those reports described the performance of the Abbott VL assay alone or in comparison to that of several existing VL assays for the detection of different subtypes, the study presented here is the first evaluation of RealTime HIV-1, NucliSENS HIV-1, CAP/CTM HIV-1, and CAP/CA HIV-1 predominantly for the detection of HIV-1 subtype C in South Africa.

MATERIALS AND METHODS

Sample collection and storage. Whole-blood samples were collected from 150 consenting patients presenting for a routine HIV VL test at the ARV clinic of the Johannesburg Hospital, South Africa, and placed in K₃EDTA. The plasma was extracted within 4 h of collection and was stored in aliquots at -80°C until further processing. The samples were then thawed and centrifuged at 3,000 × g for 5 min, and specified volumes were added to each assay according to the manufacturers' instructions. This study was conducted with ethics approval (M061105) from the Human Ethics Committee of the University of the Witwatersrand (Johannesburg, South Africa).

Methodologies for VL determination. All assays were performed by dedicated, trained operators in good laboratory practice-compliant laboratories, according to the manufacturers' instructions. The *m2000* system is based on the real-time PCR technology and consists of two instruments: the Abbott *m2000sp* instrument (automated extraction) and the *m2000rt* instrument (real-time PCR), which amplifies and detects HIV-1 amplicons. The system automates a variety of manual processing steps, such as pipetting, which helps to reduce the hands-on time required to prepare patient samples. The detection of the PCR product in real time uses a probe of a relatively new design: partially double-stranded probes. The probe consists of two DNA fragments of different lengths: the longer fragment is complementary to the target DNA and is bound to a fluorescent marker, while the shorter fragment holds the quencher molecule. When the target DNA is not present, the long probe binds to the quencher probe and no fluorescence is detected; when the target DNA is present, the long probe preferentially binds to the target DNA and is able to fluoresce to give a quantifiable signal. The advantage of the use of these probes is their increased tolerance of mismatches (16, 19), which is particularly useful for viruses, such as HIV, which are highly mutable. Table 1 summarizes the salient characteristics of each assay.

Statistical analysis. To accommodate the differences in the reportable ranges between the endpoint and real-time assays, as outlined in Table 1, and to prevent the skewing of the data, all the values were modified according to the CAP/CA HIV-1 limits. Any continuous value of <400 copies/ml was changed to read 400 copies/ml, and any value of >750,000 copies/ml was changed to read 750,000 copies/ml (6). These adjusted values were then log₁₀ transformed. The data were sorted according to qualitative and quantitative values. Samples in which HIV-1 was not detected by one or more assays were excluded from the continuous data

TABLE 1. Characteristics of Abbott RealTime HIV-1, NucliSENS HIV-1, CAP/CTM HIV-1, and CAP/CA HIV-1

Abbreviation	Full name	Target in HIV-1	Extraction	Amplification and detection	Amplification and detection instrument	Actual plasma vol (ml) used for extraction	Assay sample input vol (ml)	Reported result	Linear range	Conversion	95% detection
RealTime HIV-1	Abbott RealTime HIV-1 assay	<i>pol</i> IN	Automated <i>m2000sp</i> RNA	Real-time PCR, noncompetitive fluorescent detection (16, 19)	<i>m2000rt</i>	0.8	0.6	No. of copies/ml or IU/ml; detectable quantifiable, detectable beyond upper quantifiable limit	40–1 × 10 ⁷ copies/ml	1 copy = 1.74 IU	39 copies/ml (68 IU/ml)
NucliSENS HIV-1	NucliSENS EasyQ-EasyMag HIV-1 assay	<i>gag</i>	Automated EasyMag total nucleic acid	Real-time nucleic acid sequence-based PCR with fluorescent detection (molecular beacons with reporter and quencher molecule) (4, 39)	NucliSENS analyzer	1.0	1.0	(<40 copies/ml), undetectable IU/ml or no. of copies/ml; detectable within linear range, not detectable, or below detectable limit	100–3 × 10 ⁶ IU/ml	1 copy = 1 IU ^a	250 IU/ml
CAP/CTM HIV-1	Cobas AmpliPrep-Cobas TaqMan HIV-1 assay	<i>gag</i>	Automated Cobas AmpliPrep RNA	Real-time PCR: fluorescent detection with TaqMan probes (5'-nuclease probe breakage) with fluorophore and quencher (18)	Cobas TaqMan	1.0	0.85	No. of copies/ml or IU/ml; detectable quantifiable, detectable beyond upper quantifiable limit (<40 copies/ml), undetectable	40–1 × 10 ⁷ copies/ml	1 copy = 1.7 IU	40 copies/ml (68 IU/ml)
CAP/CA HIV-1	Cobas AmpliPrep-Cobas Amplicor HIV-1 Monitor (version 1.5) (standard) assay	<i>gag</i>	Automated Cobas AmpliPrep RNA	Manual loading, PCR with endpoint detection: photometric quantification of an enzyme-linked color reaction (1)	Cobas Amplicor	0.35	0.20	No. of copies/ml or IU/ml	400–750,000 copies/ml	1 copy = 1.96 IU	400 copies/ml

^a Confirmed by supplier's memorandum.

analysis but were used for the qualitative analysis. All assay results are reported in copy numbers/ml.

Precision and accuracy of RealTime HIV-1. The intravariability and intervariability for only RealTime HIV-1 were confirmed in this study of local specimens and compared to the manufacturer's claims. Three samples were diluted in HIV-negative human plasma (to concentrations of 100,000 copies/ml, 1,000 copies/ml, and 100 copies/ml) and stored in 25 aliquots of 1 ml each. Intravariability was measured from five aliquots tested in one run. Intervariability was measured by analyzing a different run each day for 5 days (five runs). Variability was assessed by using the standard deviation (SD) and the coefficient of variation (CV) on both the absolute and the log-transformed data, which are reported as a range (lowest to highest) for the five runs. These values were compared to the manufacturer's claims. As a guide, an SD value of ≤ 0.15 for the log-transformed values and a CV of $\leq 35\%$ for the absolute values is generally acceptable (5).

Accuracy was determined for continuous values by using the Bland-Altman difference plot (3) and the percent similarity (30), which measure the amount of agreement between assays. Linear regression is also reported with the equation of the line, the R^2 value, and the P value for the intercept. The Bland-Altman plot records the bias (with the limits of agreement) between two methods. The distance between the two limits of agreement, which is the value of both limits of agreement added (ignoring their signs) and divided by 2, is also reported and shows the overall difference for 95% of the data. The bias of all the assays converted to IU/ml with confidence intervals is also reported. The percentage of outliers of $>\log 0.5$ and $>\log 1.0$ copy/ml were also calculated for each comparison. The percent similarity (30) determines the percent accuracy between two or more methods by using the mean percent similarity and the precision between two methods by using the percent similarity SDs. Both these values are reported as overall agreement between methods as a percent similarity CV.

Qualitative analysis. The assays were also evaluated with respect to the following qualitative parameters: sample handling, the time required to obtain a reportable result, capacity (number of patient samples and number of controls per run, extraction, and detection), and the robustness of the tests. All analyses were performed with Microsoft Excel software and SAS (version 9.1) software with the Enterprise guide (version 3.1).

Subtype panel. A 20-member HIV-1 subtype panel derived from HIV-1 infected blood donors in Cameroon, South Africa, Thailand, and Uganda was obtained from the Abbott Diagnostics Global Surveillance Program and consisted of five subtype CRF02_AG, three subtype CRF01_AE, three subtype G, three subtype A, three subtype D, and three subtype F isolates. Subtype assignments were based on sequence analysis of *gag* p24, *pol* IN, and *env* gp41 IDR, as described previously (36), thus including the target regions of all the assays being evaluated. The panel was designed to represent common group M, non-subtype B subtypes and circulating recombinant forms. The panel samples were stored at -80°C prior to testing, and testing was performed only by the three real-time assays and not on the Cobas Amplicor platform. The panel had not previously been tested by CAP/CTM HIV-1 or NucliSENS HIV-1.

RESULTS

RealTime HIV-1 precision analysis. The intravariability for the RealTime HIV-1 was as follows: the SD was 0.04 to 0.07, the CV was 0.91 to 1.4%, and the absolute CV was 11.3 to 17.9% for $\log_5$ copies/ml; the SD was 0.05 to 0.09, the CV was 1.6 to 2.9%, and the absolute CV was 11.7 to 18.9% for $\log_3$ copies/ml; and the SD was 0.04 to 0.16, the CV was 1.7 to 7.9%, and the absolute CV was 9.4 to 34.0% for $\log_2$ copies/ml. The intervariability was as follows: the SD was 0.07 to 0.11, the CV was 1.5 to 2.2%, and the absolute CV was 18.6 to 28.8% for $\log_5$ copies/ml; the SD was 0.09 to 0.15, the CV was 2.5 to 4.7%, and the absolute CV was 18.7 to 34.7% for $\log_3$ copies/ml; and the SD was 0.13 to 0.25, the CV was 7.7 to 10.7%, and the absolute CV was 32.5 to 52.1% for $\log_2$ copies/ml. The intra-assay variability appeared to be stable throughout the runs for the entire range of log values, although as expected, the assay appeared to be less precise in the lower VL range ($\log 2.0$), but with only one run producing a variability (CV) of $>35\%$ on the absolute result. This increased variability appeared in run 5, which contributed to the increased intervari-

TABLE 2. Bland-Altman plots and percent similarity statistical parameters for measurement of agreement between RealTime HIV-1 and NucliSENS HIV-1, CAP/CTM HIV-1, and CAP/CA HIV-1^a

Assay	Linear regression			Bland-Altman difference plots							% Similarity		
	Equation	<i>P</i> value on intercept	<i>R</i> ²	Bias ^b		SD	Limits of agreement	Distance over limits	No. (%) outliers of:		Mean	SD	CV
				No. of copies/ml	IU/ml				>Log 0.5 copy/ml	>Log 1.0 copy/ml			
NucliSENS HIV-1	<i>y</i> = 0.939 <i>x</i> + 0.286	0.195	0.868	−0.001 (−0.098, 0.094)	0.233 (0.136, 0.329)	0.395	0.789, −0.791	0.79	14 (20.9)	2 (2.98)	100.20	4.73	4.72
CAP/CTM HIV-1	<i>y</i> = 0.899 <i>x</i> + 0.348	0.046	0.908	0.125 (0.045, 0.20)	0.136 (0.057, 0.125)	0.323	0.771, −0.521	0.646	7 (10.4)	2 (2.98)	99	3.66	3.70
CAP/CA HIV-1	<i>y</i> = 0.942 <i>x</i> + 0.5	0.017	0.883	−0.228 (−0.318, −0.137)	0.30 (0.209, 0.391)	0.371	0.514, −0.97	0.742	22 (32.8)	0	102.80	4.70	4.57

^a All results are reported as numbers of copies/ml unless stated otherwise for $n = 67$ samples.
^b Values in parentheses are 95% confidence intervals.

ability, and may reflect a loss of VL on storage, which becomes more noticeable at the lower limit. Total assay variability, according to the Abbott manufacturer's claim (<0.3 SD copy/ml) however, is acceptable at all VL ranges.

RealTime HIV-1 accuracy analysis by description of data as continuous and discrete variables. The 150 samples yielded 67 continuous values and 83 samples yielded results that registered lower than the detectable limit or target not detected. The latter results were obtained for patients attending the clinic for monitoring of VLs while they were receiving ARV treatment. These discrete results yielded 10 continuous values (12%) for NucliSENS HIV-1 (2 values were greater than the reported linear limits of 120 IU/ml to 750 IU/ml), 5 continuous values (6%) for CAP/CTM HIV-1 (51 copies/ml, 53 copies/ml, 68 copies/ml, 146 copies/ml, 155 copies/ml), 3 continuous values (3.6%) for RealTime HIV-1 (45 copies/ml, 50 copies/ml, 167 copies/ml), and no continuous values (0%) for CAP/CA HIV-1.

The remaining continuous values were capped according to the limits for CAP/CA HIV-1 (400 to 750,000 copies/ml). The mean and median values for the respective assays were as follows: log 4.72 copies/ml for NucliSENS HIV-1, log 4.95 copies/ml for CAP/CTM HIV-1, log 4.72 copies/ml for RealTime HIV-1, and log 4.94 for CAP/CA HIV-1. The medians for the four assays ranged from 4.94 to 5.44 copies/ml, showing that the values for the majority of samples in this data set were in the upper range.

The comparative statistics are detailed in Tables 2 to Table 5, in which the results of each assay are compared to those of all the other assays. The Bland-Altman difference plots for these assay combinations is also represented in Fig. 1. The results of RealTime HIV-1 compared to those of all assays showed that the no-intercept model could be applied when the results of RealTime HIV-1 are compared to those of NucliSENS HIV-1. Comparison of the results of RealTime HIV-1 to those of CAP/CTM HIV-1 and CAP/CA HIV-1 had a significant intercept ($P < 0.05$). However, the coefficient of determination for CAP/CTM HIV-1 was the highest, showing that the equation of the line between RealTime HIV-1 and CAP/CTM HIV-1 represents 90.8% of the data. The smallest bias in copies/ml was with NucliSENS HIV-1, and the smallest bias in IU/ml was with CAP/CTM HIV-1. The latter comparison also had the smallest variability (the lowest SD) and also the smallest distance over the 95% limits of agreement. The lowest number of outliers of $<\log 0.5$ copy/ml was from the comparison of RealTime HIV-1 with CAP/CTM HIV-1, but the comparison of RealTime HIV-1 with CAP/CA HIV-1 showed no clinically relevant outliers ($>\log 1.0$ copy/ml). The comparison of RealTime HIV-1 and CAP/CTM HIV-1 has the lowest percent similarity CV (overall accuracy and precision).

The comparison of the results of NucliSENS HIV-1 and those of all other assays showed that the no-intercept model could be applied to the comparison of the results of NucliSENS HIV-1 with those of RealTime HIV-1, and the highest R^2 value occurred with CAP/CA HIV-1. The smallest bias was with RealTime HIV-1 when the results were reported in copies/ml but was with CAP/CA HIV-1 when the results were reported in IU/ml. CAP/CA HIV-1 also had the lowest variability (SD) and the smallest distance over the limits of agreement. The fewest outliers $<\log 0.5$ copy/ml was with RealTime HIV-1 and

TABLE 3. Bland-Altman plots and percent similarity statistical parameters for measurement of agreement between NucliSENS HIV-1 and CAP/CTM HIV-1, RealTime HIV-1, and CAP/CA HIV-1^a

Assay	Linear regression			Bland-Altman difference plots					% Similarity		
	Equation	P value on intercept	R ²	Bias ^b		Limits of agreement	Distance over limits	No. (%) outliers of: $>\log 0.5$ copy/ml	$>\log 1.0$ copy/ml	Mean	SD
				No. of copies/ml	IU/ml						
CAP/CTM HIV-1	$y = 0.867x + 0.5$	0.021	0.857	0.126 (0.027, 0.225)	-0.096 (-0.195, 0.002)	0.406 0.938, -0.686	0.812	18 (26.9)	0	99	4.92
RealTime HIV-1	$y = 0.92x + 0.356$	0.104	0.868	0.002 (-0.095, 0.098)	-0.23 (-0.329, -0.137)	0.395 0.792, -0.788	0.79	14 (20.9)	2 (2.98)	100.20	4.75
CAP/CA HIV-1	$y = 0.936x + 0.526$	0.011	0.886	-0.226 (-0.316, -0.137)	0.067 (-0.022, 0.157)	0.366 0.958, -0.506	0.732	14 (20.9)	3 (4.47)	102.80	5.22
											5.08

^a All results are reported as numbers of copies/ml, unless stated otherwise, for $n = 67$ samples.

^b Values in parentheses are 95% confidence intervals.

CAP/CA HIV-1, with no clinically relevant outliers ($>\log 1.0$ copy/ml) being detected compared to the results of CAP/CTM HIV-1. The comparison of NucliSENS HIV-1 and RealTime HIV-1 had the lowest percent similarity CV (overall accuracy and precision).

The comparison of the results of CAP/CTM HIV-1 and those of all other assays showed that the no-intercept model could be applied between all three other assays ($P > 0.05$), with the highest coefficient of determination being achieved with RealTime HIV-1. The smallest bias was between NucliSENS HIV-1 and RealTime HIV-1 for results reported in copies/ml and NucliSENS HIV-1 for results reported in IU/ml. The least variability was with RealTime HIV-1, and the smallest differences for 95% of the data pairs were obtained with RealTime HIV-1. RealTime HIV-1 had the fewest outliers of $<\log 0.5$ copy/ml, but there was no clinically relevant difference ($>\log 1.0$ copy/ml) with NucliSENS HIV-1. The comparison of CAP/CTM HIV-1 and RealTime HIV-1 had the lowest percent similarity CV (overall accuracy and precision).

The comparison of the results of CAP/CA HIV-1 and those of all other assays showed that the no-intercept model could be applied to all assays, with the highest coefficient of determination being achieved with CAP/CTM HIV-1. The smallest bias was with NucliSENS HIV-1 and RealTime HIV-1 for results reported in copies/ml and with NucliSENS HIV-1 for results reported in IU/ml. The lowest variability was with CAP/CTM HIV-1, which also had the smallest distance over the limits of agreement. The fewest outliers of $<\log 0.5$ copy/ml was with NucliSENS HIV-1, and no clinically relevant outliers ($>\log 1.0$ copy/ml) were achieved with RealTime HIV-1. The comparison of the results of CAP/CA HIV-1 and CAP/CTM HIV-1 had the lowest percent similarity CV (overall accuracy and precision).

Overall, the assay combinations with the highest coefficients of determination were RealTime HIV-1 and CAP/CTM HIV-1 ($R^2 = 0.908$). The smallest bias was between RealTime HIV-1 and NucliSENS HIV-1 (-0.001 copy/ml) and between NucliSENS HIV-1 and CAP/CA HIV-1 (0.067 IU/ml). The least variability in differences was between RealTime HIV-1 and CAP/CTM HIV-1 (SD, 0.323 copy/ml), which also had the smallest distance between limits of agreement (0.646 copy/ml). The fewest total number of outliers (both $>\log 0.5$ copy/ml and $>\log 1.0$ copy/ml) occurred between RealTime HIV-1 and CAP/CTM HIV-1, whereas the comparisons of CAP/CTM HIV-1 and NucliSENS HIV-1 and of RealTime HIV-1 and CAP/CA HIV-1 showed no clinically relevant outliers ($>\log 1.0$ copy/ml). In terms of overall accuracy and precision (measured by the percent similarity), the assay combinations with the lowest percent similarity CV were CAP/CTM HIV-1 and CAP/CA HIV-1 (3.7%) and RealTime HIV-1 and CAP/CTM HIV-1 (3.7%).

Subtype panel analysis. All three real-time assays quantified the 20 members of the subtype panel. NucliSENS HIV-1 reported one subtype F specimen as invalid, but on repeat testing yielded 3,300 IU/ml. NucliSENS HIV-1 generated lower values than the other assays for many of the specimens, and RealTime HIV-1 tended to generate higher values than the other assays. Only four samples (two of subtype G and two of subtype CRF02-AG) produced differences of $>\log 1.0$ copy/ml between assays. Three were

TABLE 4. Bland-Altman plots and percent similarity statistical parameters for measurement of agreement between CAP/CTM HIV-1 and NucliSENS HIV-1, RealTime HIV-1, and CAP/CA HIV-1^a

Assay	Linear regression			Bland-Altman difference plots							% Similarity		
	Equation	P value on intercept	R ²	Bias ^b		SD	Limits of agreement	Distance over limits	No. (%) outliers of:		Mean	SD	CV
				No. of copies/ml	IU/ml				>Log 0.5 copy/ml	>Log 1.0 copy/ml			
NucliSENS HIV-1	$y = 0.988x + 0.178$	0.452	0.857	-0.126 (-0.225, -0.027)	0.096 (-0.002, 0.195)	0.406	0.686, -0.938	0.812	18 (26.9)	0	101.50	4.86	4.75
RealTime HIV-1	$y = 1.009x + 0.08$	0.662	0.908	-0.124 (-0.208, -0.046)	-0.136 (-0.215, -0.057)	0.323	0.522, -0.77	0.646	7 (10.5)	2 (2.98)	101.40	3.86	3.81
CAP/CA HIV-1	$y = 1.004x + 0.335$	0.102	0.893	-0.352 (-0.438, -0.267)	0.16 (0.078, 0.248)	0.349	0.346, -1.05	0.698	25 (37.3)	3 (4.47)	104	4.23	4.07

^a All results are reported as numbers of copies/ml, unless stated otherwise, for *n* = 67 samples.

^b Values in parentheses are 95% confidence intervals.

^a All results are reported as numbers of copies/ml, unless stated otherwise, for $n = 67$ samples.

^b Values in parentheses are 95% confidence intervals.

TABLE 5. Bland-Altman plots and percent similarity statistical parameters for measurement of agreement between CAP/CA HIV-1 and NucliSENS HIV-1 and RealTime HIV-1^a

Assay	Linear regression				Bland-Altman difference plots				% Similarity		
	Equation	P value on intercept	R ²	Bias ^b	IU/ml	SD	Limits of agreement	Distance over limits	No. (%) outliers of:		CV
									>Log 0.5 copy/ml	>Log 1.0 copy/ml	
NucliSENS HIV-1	$y = 0.946x + 0.038$	0.858	0.858	-0.067 (0.137, 0.316)	-0.067 (-0.157, 0.022)	0.366	0.958, -0.506	0.732	14 (20.9)	3 (4.47)	97.80 4.02 4.11
CAP/CTM HIV-1	$y = 0.889x + 0.191$	0.323	0.893	-0.16 (0.267, 0.437)	-0.16 (-0.249, -0.079)	0.349	1.048, -0.348	0.698	25 (37.3)	3 (4.47)	96.60 3.57 3.70
RealTime HIV-1	$y = 0.937x + 0.084$	0.697	0.883	0.228 (0.137, 0.318)	-0.30 (-0.39, -0.21)	0.371	0.97, -0.514	0.742	22 (32.8)	0	97.80 4.35 4.45

^a All results are reported as numbers of copies/ml, unless stated otherwise, for $n = 67$ samples.^b Values in parentheses are 95% confidence intervals.

between RealTime HIV-1 and NucliSENS HIV-1 and one was between RealTime HIV-1 and CAP/CTM HIV-1.

Qualitative assay analysis for determination of robustness of assays. All assays gave valid results for all except one of the specimens when they were used to test the patient specimens. One invalid result was reported by CAP/CTM HIV-1 (retesting yielded a target not similar to the targets detected by the other assays). Table 6 outlines the throughput of samples for all four assays that can be performed in the laboratory in Johannesburg, South Africa, together with the number of staff required for each method. The throughput was the highest for NucliSENS HIV-1, followed by that for RealTime HIV-1. Although the TaqMan 48 instrument was used in this study, a TaqMan 96 instrument is available on the market, and that instrument would generate 84 patient results per run (including 12 controls per batch for a total of 96 samples). Staff requirements are greater for NucliSENS HIV-1. RealTime HIV-1 and CAP/CTM HIV-1 can also be performed in a laboratory area without separating the extraction and amplification components. Both the NucliSENS HIV-1 and the RealTime HIV-1 instruments have small footprints compared to the footprints of the Roche instruments, and an added advantage of RealTime HIV-1 is the utilization of the *m2000rt* instrument for additional real-time assay expansion.

DISCUSSION

The South African ARV program is unique for a program in a resource-limited setting for the following reasons: VL testing is currently used to determine the need for a switch of a failing drug regimen, the result is required within 72 h of collection, the volumes of samples that need to be processed for VL determination are enormous, and the epidemic predominantly involves a single subtype, HIV-1 subtype C. National routine VL testing laboratories, as in many resource-constrained countries, are characterized by a shortage of skilled technical staff and limited laboratory space. The requirements of the VL testing program are thus (i) an accurate, reliable, and robust assay; (ii) a high-throughput platform with which to facilitate a rapid turnaround time; (iii) small instrument footprints; (iv) the ability to use less skilled staff; (v) simpler transport requirements; and (vi) an affordable cost per reportable result. Many VL evaluations have been conducted locally (10, 21, 31, 32–35), but the present study is the first to investigate the newly available RealTime HIV-1, an automated (*m2000sp* and *m2000rt*) assay, in a high-throughput laboratory in the South African environment of HIV-1 subtype C in a side-by-side comparison with three existing platforms.

The variability of RealTime HIV-1 appears to be acceptable across the VL ranges investigated, which concurs with the findings of other studies (13, 23, 29). There is increased variability in the lower VL range ($\log_2$), which is typical of most VL assays (5). RealTime HIV-1 showed the least fluctuation ($n = 3$ [3.6%]) at the lower limit of detection compared to the fluctuations shown by the other real-time assays. The statistical models concur that RealTime HIV-1 compares favorably with all the existing assays tested in this laboratory and overall performs the best in comparison with the performance of CAP/CTM HIV-1. Similar comparisons of these two assays have been reported in the literature (6, 44). The overall comparison

FIG. 1. Bland-Altman analysis scatter plots of the comparison of the results of all assays to each other. The vertical axis is the difference between the assays. The horizontal axis is the average log-transformed values of all assays, represented as copies/ml (c/ml). The assay names are given on each plot.

between RealTime HIV-1 and NucliSENS HIV-1 was the least favorable, which was similarly noted in a Chinese study (46). Our study also highlights the changes that can occur in the bias between assays when a conversion between copy number and IU is made and that caution is required when one changes between assays or reporting units. One such difference was noted between CAP/CTM HIV-1 and CAP/CA HIV-1, in which the bias changed from -0.35 copy/ml to 0.16 IU/ml. The genetic diversity of HIV-1 presents a significant challenge to the development of assays capable of reliably quantifying all strains of the virus (7, 15, 34). In the present study, we utilized a 20-member panel comprising subtype A, D, F, G,

CRF01-AE, and CRF02-AG strains over and above the local subtype C samples to evaluate the comparative performance characteristics of the three real-time assays. All three real-time assays were able to quantify all panel members. All three systems adequately detected subtypes B, D, and F. The subtypes for which $>\log 1.0$ variance was achieved were subtypes G ($n = 2$) and CRF02-AG ($n = 2$) with NucliSENS HIV-1 and RealTime HIV-1 and subtype CRF02-AG ($n = 1$) with CAP/CTM HIV-1 and RealTime HIV-1. These differences for NucliSENS HIV-1 and CAP/CTM HIV-1 have been reported previously (7, 8, 13–15, 31). The qualitative characteristics of RealTime HIV-1 compare

TABLE 6. Assay characteristics for a one-shift result reporting cycle, not including daily maintenance and consumable loading and reagent preparation

Assay	No. of controls/ no. of samples to be tested	Extraction capacity for one instrument	Amplification and detection	No. of reportable patient samples/day	No. of staff
CAP/CA HIV-1	3/21	One AmpliPrep: 1 run = 21 samples = 2 h ^b	One Amplicor instrument (manual master mixture): 1 run = 21 patient samples = 6 h	21 ^c	2
CAP/CTM HIV-1	3/21	One AmpliPrep: 1 run = 21 samples = 2 h, second run = 21 samples = 1 h	One TaqMan 48 instrument ^a : 1 run = 42 patient samples = 4 h	45	1
NucliSENS HIV-1	3/21 ^a	One EasyMag: 6 runs (21 samples/run) = 4 h (40mins/run) = 126 samples	One EasyQ analyzer: 3 runs, 42 patient samples each run = 126 patient samples = 3 h	126	2
Real Time HIV-1	3/93	One m2000sp: 1 run = 93 samples = 3.5 h	One m2000rt (automated master mixture): 1 run = 93 samples = 3.5 h	93	1

^a This is laboratory specific. This value is per the Johannesburg protocol.
^b Additional runs (second run of 21 samples in 1 h and third run of 21 samples in 1 h) can be prepared during the same shift but cannot be amplified and detected during the same day.
^c After 1-h result reporting.

favorably with those of existing assays, in that it performs test of 93 samples in one shift (results are reportable in one 8-h day). Although this is less time than that required for the current NucliSENS HIV-1 technology, RealTime HIV-1 requires less manual preparation (38) and therefore fewer staff. In addition, the amplification procedure can be left overnight, which increases the number of tests in a cycle, although not in a single shift. In this study the RealTime HIV-1 methodology was found to be easy to perform, and no repeat tests were required for any sample. Controls are also supplied with each batch, and the number of controls is also the same if 48 or 96 tests are performed. This assay showed overall good performance and potential for use with samples from populations predominantly infected with HIV-1 subtype C. It is reliable, minimizes contamination through the use of automation, and has a rapid turnaround time (with few requirements for time-consuming manual steps); and the assay has the potential to be expanded for use for the detection of other organisms: hepatitis C virus (43), *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* (22).

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