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Multicenter clinical comparative evaluation of Alinity m HIV-1 assay performance

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ABSTRACT

Background: Accurate, rapid detection of HIV-1 RNA is critical for early diagnosis, treatment decision making, and long-term management of HIV-1 infection.

Objective: We evaluated the diagnostic performance of the Alinity m HIV-1 assay, which uses a dual target/dual probe design against highly conserved target regions of the HIV-1 genome and is run on the fully automated Alinity m platform.

Study design: This was an international, multisite study that compared the diagnostic performance of the Alinity m HIV-1 assay to four commercially available HIV-1 assays routinely used in nine independent clinical laboratories. Alinity m HIV-1 assay precision, detectability, and reproducibility was compared across four study sites.

Results: The Alinity m HIV-1 assay produced comparable results to currently available HIV-1 assays (correlation coefficient > 0.995), with an overall bias of -0.1 to 0.10 Log_{10} copies/mL. The Alinity m HIV-1 assay and its predecessor *m*2000 HIV-1 assay demonstrated comparable detection of 16 different HIV-1 subtypes ($R^2 = 0.956$). A high level of agreement (> 88 %) between all HIV-1 assays was seen near clinical decision points of 1.7 Log_{10} copies/mL (50 copies/mL) and 2.0 Log_{10} copies/mL (200 copies/mL). Alinity m HIV-1 assay precision was 0.08 and 0.21 Log_{10} copies/mL at VLs of 1000 and 50 copies/mL, respectively, with a high level of detectability (≥ 97 % hit rate) and reproducibility across sites.

Conclusions: The Alinity m HIV-1 assay provides comparable diagnostic accuracy to current HIV-1 assays, and when run on the Alinity m system, has the capacity to shorten the time between diagnosis and treatment.

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Table 1
Characteristics of the HIV-1 Assays Compared in the Study.

Manufacturer	Assay Platform	Assay Target Region	Quantifiable Range (copies/mL)	HIV-1 Diagnostic Confirmation	Sample Process Volume (mL)
Abbott	Alinity m HIV-1 assay	Integrase & LTR	20-10,000,000	Yes	0.60
Abbott	*m*2000sp/rt RealTi*m*e HIV-1 assay	Integrase	40-10,000,000	No	0.60
Siemens	Versant kPCR HIV-1 assay	Integrase	37-11,000,000	No	0.50
Hologic	Panther Aptima HIV-1 assay	Integrase & LTR	30-10,000,000	Yes	0.50
Roche	CAP/CTM HIV-1 assay v 2.0	LTR & GAG	20-10,000,000	No	0.85

Table 2
Comparison of HIV-1 Levels Using the Alinity m and RealTi*m*e HIV-1 Assays[a].

		RealTi*m*e HIV-1 (n)		
		Not detected	< 50[b] copies/mL	> 40 copies/mL
Alinity m HIV-1 (n)	Not detected	37	452	3[c]
	< 20 copies/mL	21	268	17[d]
	> 20 copies/mL	4[e]	138[f]	494[g]

[a] Four additional samples were reported as > Upper Limit of Quantitation (ULQ) by both methods; 2 additional samples were reported as > ULQ by RealTi*m*e HIV-1 assay and quantitated by Alinity m HIV-1 assay.
[b] Category contains pulled results from sites which utilize < 40 copies/mL and one study site which utilizes < 50 copies/mL (without differentiation between detected and not detected) for reporting of clinical results rather than the assay-specific LOQ.
[c] *m*2000 results ranged from 1.61 to 1.72 l Log_{10} copies/mL.
[d] *m*2000 results ranged from 1.73 to 2.02 Log_{10} copies/mL.
[e] Alinity m results ranged from 1.32 to 1.38 Log_{10} copies/mL.
[f] Alinity m results ranged from 1.27 to 1.43 Log_{10} copies/mL.
[g] Alinity m quantitated two samples at 6.79 and 6.87 Log_{10} copies/mL.

1. Introduction

An estimated 37.9 million individuals are living with HIV-1 worldwide; of these, only 24.5 million are receiving antiretroviral treatment [1]. The Joint United Nations Program on HIV/AIDS (UNAIDS) organization announced an ambitious program to address this gap, called 90 − 90-90 [2], which has the following goals: by 2020, 90% of patients with HIV will know their status, 90% of patients with an HIV diagnosis will be receiving sustained antiretroviral treatment, and 90% of patients on antiretroviral treatment will have achieved viral suppression. To reach these goals, early diagnosis and accurate HIV-1 RNA viral load (VL) monitoring are critical for guiding treatment decisions and successful long-term disease management. Current drug therapies and treatment protocols have demonstrated the ability to reduce the VL to an undetectable level, at which the virus cannot be transmitted [3]. According to current European treatment guidelines, treatment success (i.e., virological suppression) is defined as an HIV-1 RNA concentration below 50 copies/mL [4–13]. The definition of treatment failure (or virological failure) varies in global and country-specific guidelines, but is usually defined as an HIV-1 RNA concentration of 50, 200 or 1000 copies/mL [4–13].

HIV-1 molecular diagnostics are continually challenged due by the high level of viral genetic diversity, with four lineages designated as groups M, N, O, and P [14]. The highly prevalent group M is further subdivided into 10 subtypes and several circulating recombinant forms, with the most recent discovery of subtype L from Democratic Republic of Congo [15]. This high genetic diversity presents a challenge for the development of assays for initial diagnosis and subsequent monitoring of therapeutic response [16]. The ability of molecular diagnostics assays to reliably detect HIV-1 and provide accurate VL quantification depends on the design of the assay primers and probes targeting the highly conserved genomic region.

There are several commercially available HIV-1-molecular diagnostics platforms including Abbott *m*2000sp/rt RealTi*m*e HIV-1, Siemens Versant® kPCR HIV-1, Roche *cobas*® AmpliPrep/*cobas* TaqMan (CAP/CTM) HIV-1 v2.0, and Hologic Panther® Aptima HIV-1. Abbott Molecular recently developed the Alinity m HIV-1 assay, which uses a dual target/dual probe design against highly conserved target regions of the HIV-1 genome [16]. The Alinity m HIV-1 assay is run on the Alinity m system, which is a fully automated, continuous, random-access molecular diagnostic analyzer with a processing capacity of 300 samples per 8 -h shift. Here, we report the results of the first field study examining the performance of the Alinity m HIV-1 assay compared to 4 commercially available HIV-1 assays across a wide dynamic range, on diverse HIV-1 subtypes, and at specific clinically relevant decision points.

2. Materials and Methods

2.1. Clinical specimens and study sites

The performance of the Alinity m HIV-1 assay (Abbott Molecular, Des Plaines, IL, USA) was compared to 4 commercially available HIV-1 assays in a multicenter, international study performed at 9 independent International Standard Organization (ISO)-accredited clinical laboratories: Victorian Infectious Disease Reference Laboratory, Melbourne, Australia; Hôpital Universitaire Henri Mondor, Créteil/Paris, France; Medizinisches Infektiologiezentrum Berlin, Germany; PZB Aachen, Germany; Azienda Ospedaliera di Padova, Padua, Italy; Lancet Laboratories, Johannesburg, South Africa; Hospital Universitario Ramón y Cajal, Madrid, Spain; Laboratori de Referència de Catalunya, El Prat de Llobregat/Barcelona, Spain; and West of Scotland Specialist Virology Centre, Glasgow, UK.

The study was performed in accordance with the principles of Good Clinical Practice and conducted in adherence with the Declaration of Helsinki. Only surplus patient plasma samples were used for this study. All samples were anonymized before study initiation, and an identification number containing no patient identifiers was assigned to each surplus sample.

Alinity m HIV-1 assay performance was evaluated across 100 clinical samples with known HIV-1 subtypes. HIV-1 resistance and subtype testing was performed using the Vela *Sentosa* SQ HIV Genotyping Assay [17].

2.2. HIV-1 detection and quantitation

Each clinical specimen was tested using two different HIV-1 assays: the Alinity m HIV-1 assay and a commercially available HIV-1 assay routinely used at each study site (Table 1). Comparator assays were either based on real-time PCR: RealTi*m*e HIV-1 assay (Abbott Molecular, Des Plaines, IL, USA), CAP/CTM HIV-1 v2.0 test (Roche

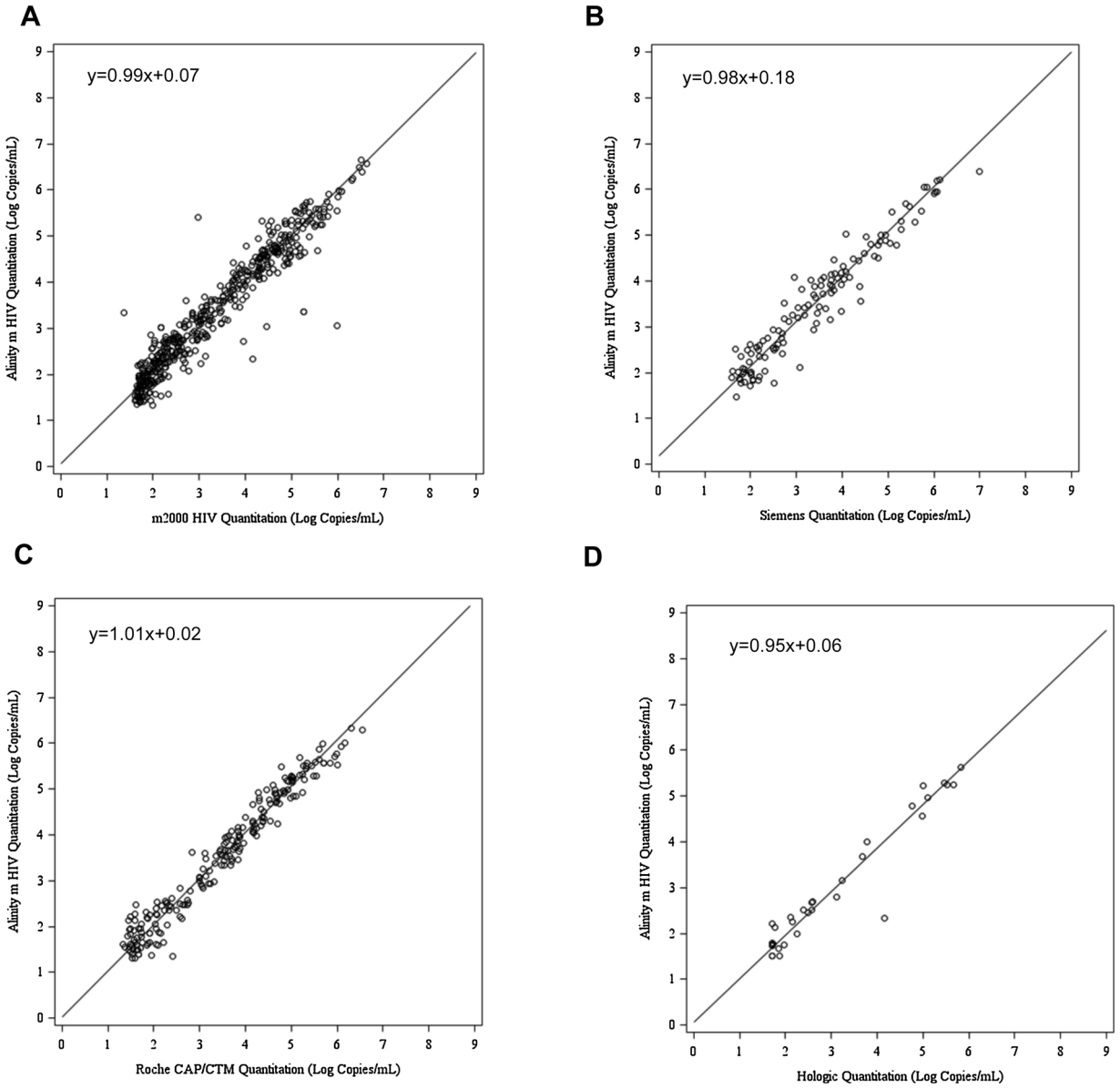

Fig. 1. Comparison of Alinity m HIV-1 assay and routine clinical HIV-1 assay performance. Deming regression of HIV-1 RNA levels showing correlation between Alinity m and (A) RealTi*m*e *m*2000 HIV-1 assay, (B) kPCR HIV-1 assay, (C) CAP/CTM HIV-1 v2.0 assay, and (D) Panther Aptima HIV-1 assay.

Table 3
Performance Comparison of Alinity m HIV-1 assay to Comparator HIV-1 Assays.

Comparator Assay	Samples (n)	Correlation Coefficient (r)	Mean of Bias	SD of Bias
Abbott *m*2000sp/rt RealTi*m*e HIV-1 assay	494	0.955	0.04	0.388
Siemens Versant kPCR HIV-1 assay	121	0.963	0.10	0.351
Roche CAP/CTM HIV-1 assay v 2.0	213	0.980	0.05	0.280
Hologic Panther Aptima HIV-1 assay	32	0.964	−0.10	0.384

Molecular Systems, Pleasanton, CA, USA), Versant kPCR 1.5 (Siemens, Germany) or transcription-mediated amplification (TMA): Aptima HIV-1 Quant assay (Hologic Inc., San Diego, CA, USA).

Alinity m HIV-1 assay: HIV-1 was isolated from 600 µL of sample per the manufacturer's instructions. The data were analyzed by the system software, version 1.3.1 or later. The dynamic range of quantification of the Alinity m HIV-1 assay is 20 to 10^7 copies/mL (1.3–7.0 Log_{10} copies/mL). The Alinity m HIV-1 assay primers and probes target the integrase and LTR regions of the viral genome.

RealTi*m*e HIV-1 assay: HIV-1 was processed from 600 µL of sample using the *m*2000*sp*™ and the *m*2000*rt* analyzers for automated sample extraction and real-time PCR amplification and detection, according to the manufacturer's instructions. The data were analyzed with the Abbott RealTi*m*e *m*2000*rt* software, version 8. The dynamic range of quantification of the RealTi*m*e HIV-1 assay is 40 to 10^7 copies/mL (1.6–7.0 Log_{10} copies/mL). The RealTi*m*e HIV-1 assay primers and probes target the viral integrase gene.

CAP/CTM HIV-1 v2.0 assay: HIV-1 was processed from 850 µL of sample using the cobas AmpliPrep and cobas TaqMan 96 analyzer for automated sample extraction and real-time PCR amplification and

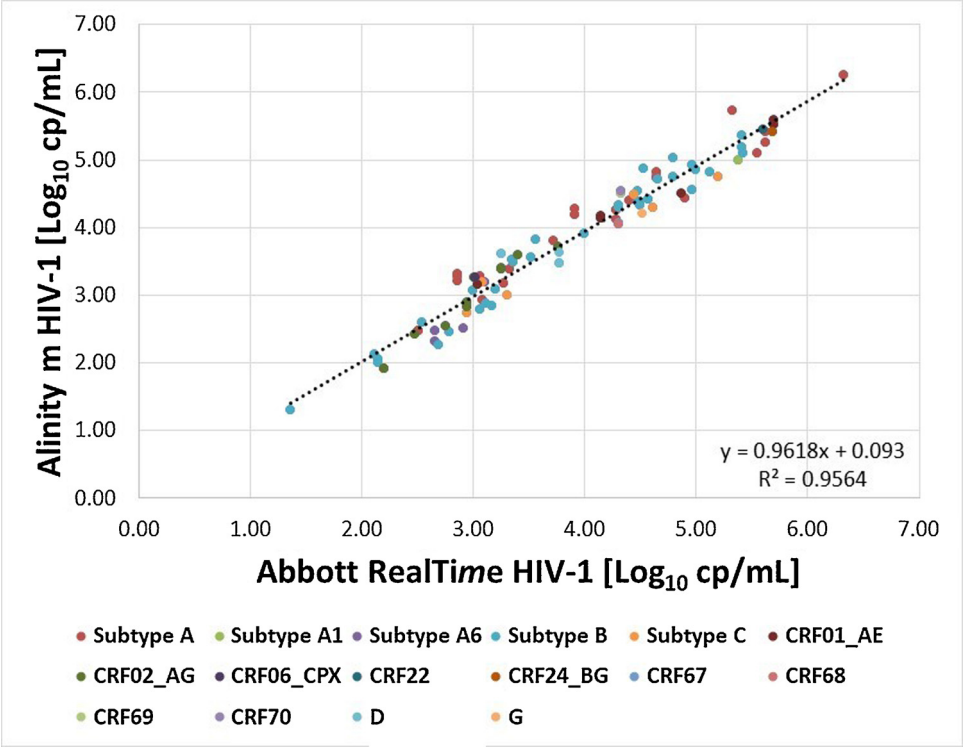

Fig. 2. Alinity m and *m*2000 HIV-1 assay performance across different HIV-1 subtypes. Linear regression of HIV-1 levels showing correlation between Alinity m and RealTi*m*e HIV-1 assays.

Table 4
Comparison of HIV-1 Levels Using the Alinity m and kPCR HIV-1 Assays[a].

		Versant kPCR 1.5 (n)		
		Not detected	< 37 copies/mL	> 37 copies/mL
Alinity m HIV-1 (n)	**Not detected**	0	1	0
	< 20 copies/mL	0	10	13[b]
	> 20 copies/mL	0	7[c]	121

[a] One additional sample was reported as > ULQ by kPCR 1.5 HIV-1 assay and quantitated by Alinity m HIV-1 assay.
[b] 13 samples were diluted with Alinity m Dilution Kit. 12/13 samples were diluted 1:2.5 and 1/13 samples was diluted 1:50. Versant kPCR viral load ranged between1.57 – 3.77 Log_{10} copies/mL.
[c] Alinity m results ranged from 1.60 to 2.10 Log_{10} copies/mL.

detection, according to the manufacturer's instructions. The dynamic range of quantification of HIV-1 for the CAP/CTM HIV-1 v2.0 assay is 20 to10^7 copies/mL (1.3–7 Log_{10} copies/mL). The LTR and GAG regions of the viral genome are targeted by the CAP/CTM HIV-1 v2.0 assay primers and probes.

Versant kPCR 1.5 assay: HIV-1 was isolated from 500 µL of sample according to the manufacturer's instructions. The dynamic range of quantification of the Versant kPCR 1.5 assay is 37 to 10^7 copies/mL (1.47–7.04 Log_{10} copies/mL). The Versant kPCR 1.5 assay targets the integrase region of the viral genome.

Aptima HIV-1 Quant assay: On the Panther analyzer system, HIV-1 was isolated from 500 µL of sample according to the manufacturer's instructions. The dynamic range of quantification of Aptima HIV-1 Quant assay is 30 to 10^7 copies/mL (1.47–7.0 Log_{10} copies/mL). The integrase and LTR regions of the viral genome are targeted by the Aptima HIV-1 Quant assay.

Table 5
Comparison of HIV-1 Levels Using the Alinity m HIV-1 and CAP/CTM HIV-1 v2.0 Assays[a].

		CAP/CTM HIV-1 v2.0 (n)		
		Not detected	< 20 copies/mL	> 20 copies/mL
Alinity m HIV-1 (n)	Not detected	35	33	3[b]
	< 20 copies/mL	20	20	11[c]
	> 20 copies/mL	5	13[d]	213

[a] One additional sample was reported as > Upper Limit of Quantitation (ULQ) by both methods; 4 samples were reported as > ULQ by CAP/CTM HIV-1 v.20 assay and quantitated by Alinity m HIV-1 assay.
[b] CAP/CTM results ranged from 1.32 to 1.89 Log_{10} copies/mL.
[c] CAP/CTM results ranged from 1.32 to 2.01 Log_{10} copies/mL.
[d] Alinity m results ranged from 1.40 to 1.73 Log_{10} copies/mL.

Table 6
Comparison of HIV-1 Levels Using the Alinity m HIV-1 and Aptima HIV-1 Quant Assays.

		Aptima HIV-1 (n)		
		Not detected	< 50[a] copies/mL	> 50 copies/mL
Alinity m HIV-1 (n)	Not detected	0	149	0
	< 20 copies/mL	1	76	2[b]
	> 20 copies/mL	0	27[c]	32

[a] Study site utilizes < 50 copies/mL for reporting of clinical results rather than the assay-specific LOQ.
[b] Aptima HIV-1 Quant assay results quantitated at 1.75 Log_{10} copies/mL.
[c] Alinity m HIV-1 assay results ranged from 1.34 to 2.43 Log_{10} copies/mL.

Table 7
Comparison of Viral Load Range and Agreement at the Clinical Decision Points of 1.7 Log_{10} copies/mL (50 copies/mL) and 2.0 Log_{10} copies/mL (200 copies/mL) Between Alinity m HIV-1 and Comparator Assays.

Comparator Assay	Viral Load Range 1.2–2.2 Log_{10} copies/mL Mean Bias[a] (# of samples)	% Samples with < 0.5 Log_{10} copies/mL difference within range of ± 0.5 Log_{10} around at 1.7 Log_{10} copies/mL	Viral Load Range 1.8–2.8 Log_{10} copies/mL Mean Bias* (# of samples)	% Samples with < 0.5 Log_{10} copies/mL within range of ± 0.5 Log_{10} around at 2.3 Log_{10} copies/mL
*m*2000sp/rt RealTime HIV -1 assay	0.07 (135)	88	0.08 (169)	89
CAP/CTM HIV-1 assay v 2.0	0.09 (57)	88	−0.05 (42)	90
Versant kPCR HIV-1 assay	0.16 (32)	97	0.107 (41)	90
Panther Aptima HIV-1 assay	−0.0007 (8)	93	−0.04 (14)	100

HIV, human immunodeficiency virus.
[a] Bias plot = Alinity m – comparator assay.

2.3. Analytical analysis – precision and detectability

Assay precision and detectability were evaluated across 4 testing sites by using a panel consisting of different HIV-1 RNA concentrations obtained by dilution of clinical samples in normal human plasma (Exact Diagnostics, Fort Worth, TX, USA). HIV-1 RNA in each panel sample was value assigned based on the RealTi*m*e HIV-1 assay. The 12 panel members contained 1×10^3 copies/mL (3.0 Log_{10} copies/mL), 1×10^2 copies/mL (2.30 Log_{10} copies/mL), 50 copies/mL (1.70 Log_{10} copies/mL), and 20 copies/mL (1.3 Log_{10} copies/mL) of subtypes B, C, and CRF02_AG, respectively. Precision was evaluated at concentrations of > 20 copies/mL and detectability was evaluated at 20 copies/mL.

2.4. Analytical analysis - reproducibility

Reproducibility was assessed by evaluating assay controls from multiple reagent lots across testing sites. Mean, SD, and % CV of each assay's quality controls (QC) were evaluated at each participating study site along with the overall QC mean and SD from all sites.

2.5. Statistical analysis

Descriptive statistics are reported as means ± SD. Relationships between quantitative variables were studied by means of Deming regression. Bland Altman analysis was performed to evaluate the differences in quantification between the assays. All statistical analyses were performed using PC SAS version 9.3 (SAS, Cary, NC, USA). Additionally, the percentage of samples that differed by $\leq / > 0.5$ Log_{10} copies/mL and $\leq / > 1.0$ Log_{10} copies/mL were evaluated between methods at or near the clinical decision points of 200 and 50 copies/mL.

3. Results

A total of 2238 samples were tested with the Alinity m HIV-1 assay and the results were compared against those from one of the 4 comparator HIV-1 assays used at each study site: *m*2000sp/rt RealTi*m*e HIV-1 assay (1440 samples); CAP/CTM HIV-1 assay v2.0 (358 samples); Versant kPCR HIV-1 assay (153 samples); and Panther Aptima HIV-1 assay (287 samples).

Of the 1440 samples tested by Alinity m and RealTi*m*e HIV-1 assays, 494 samples fell within the dynamic range of both assays (Table 2). The correlation coefficient was 0.955 (Deming regression equation, $y = 0.99x + 0.07$; Fig. 1A; Table 3) with a mean observed bias of 0.04 ± 0.388 Log_{10} copies/mL (Table 3). Alinity m and *m*2000 HIV-1 assay performance was also compared across 16 different HIV-1 subtypes. Correlation between the Alinity m and *m*2000 HIV-1 assays was high, with an R^2 of 0.956 (n = 100, Fig. 2) and a mean observed bias of +0.06 Log_{10} copies/mL (data not shown).

Of 153 samples tested by Alinity m HIV-1 and kPCR HIV-1 assays, 121 samples fell within the dynamic range of both assays (Table 4). A strong correlation between the two assays was found (r = 0.963; Deming regression equation, $y = 0.98x + 0.18$; Fig. 1B; Table 3) along with a mean bias of 0.1 ± 0.351 Log_{10} copies/mL (Table 3).

Of the 358 samples tested by Alinity m HIV-1 assay and CAP/CTM HIV-1 v2.0 assay, 213 fell within the dynamic range of both assays (Table 5). The correlation coefficient was 0.980 (Deming regression equation, $y = 1.01x + 0.02$; Fig. 1C; Table 3) with a mean observed bias of 0.05 ± 0.280 Log_{10} copies/mL (Table 3).

Finally, of the 287 samples used to evaluate the performance of the Alinity m HIV-1 assay and Aptima HIV-1 Quant assay, a limited number of samples (n = 32) fell within the dynamic range of both assays (Table 6). Similar to other assays, strong correlation was observed (r = 0.964; Deming regression equation, $y = 0.95x + 0.06$; Fig. 1D) with a mean observed bias of -0.1 ± 0.384 Log_{10} copies/mL (Table 3).

At the clinical decision points of 200 and 50 copies/mL, a high level

Table 8
(a) Precision, (b) Detection Rate, and (c) Reproducibility of the Alinity m HIV-1 Assay.

	Panel member (Subtype)	Target concentration (Log_{10} copies/mL)	No. of replicates	Mean of measured HIV-1 RNA (Log_{10} copies/mL)	SD (Log_{10} copies/mL)	CV (%)
(8a)	C	3.0	69	3.05	0.08	2.8
Precision	C	2.3	58	2.27	0.11	4.9
	C	1.7	53	1.63	0.16	9.8
	CRF02_AG	3.0	62	3.02	0.11	3.6
	CRF02_AG	2.3	66	2.28	0.18	7.7
	CRF02_AG	1.7	47	1.66	0.17	10.3
	B	3.0	58	2.97	0.09	3.2
	B	2.3	60	2.22	0.12	5.5
	B	1.7	67	1.79	0.21	11.7

	Panel member (Subtype)	Target concentration (Log_{10} copies/mL)	No. of replicates	No of replicates detected	Detection rate (%)
(8b)	C	1.3	60	58	97
Detection Rate	CRF02_AG	1.3	61	61	100
	B	1.3	58	58	100

	Control	Target HIV-1 RNA (Log_{10} copies/mL)[a]	No. of replicates	Mean of measured HIV-1 RNA (Log_{10} copies/mL)	SD (Log_{10} copies/mL)	CV (%)
(8c)	LPC	3.04 – 3.16	346	3.07	0.14	4.56
Reproducibility	HPC	4.94 – 5.03	346	4.95	0.09	1.82

CV: coefficient of variation; LPC: low positive control; HPC: high positive control; SD standard deviation.
[a] Target concentration for LPC and HPC obtained.

of agreement (> 88 %) was observed between the Alinity m HIV-1 assay and the comparator HIV-1 assays (Table 7).

Precision of the Alinity m HIV-1 assay was evaluated across 4 study sites by testing dilutions of clinical samples (Table 8a–c). As shown in Table 8a, the coefficients of variation ranged from 2.8–11.7%. At a concentration near the lower limit of detection, the Alinity m HIV-1 assay exhibited a high level of detectability (≥ 97 % hit rate; Table 8b). Reproducibility was characterized by a coefficient of variation of 4.56 % for the low positive control (LPC) and of 1.82 % for the high positive control (HPC; Table 8c).

4. Discussion

This multicenter, international study was the first field evaluation of the Alinity m HIV-1 assay, which demonstrated excellent correlation to currently commercially available HIV-1 assays (correlation coefficient > 0.955). The overall observed bias ranged from -0.1 to 0.10 Log_{10} copies/mL. In 98.5 % of quantifiable clinical sample results, the differences between the comparator HIV-1 assay and the Alinity m HIV-1 assay were ≤1 Log_{10} copies/mL; and in 90.7 % of quantifiable clinical sample results, the differences were ≤0.5 Log_{10} copies/mL (data not shown). A high level of agreement was observed between the Alinity m HIV assay and comparator HIV-1 assays at the clinically relevant concentrations of 50 copies/mL and 200 copies/mL.

The Alinity m HIV-1 assay demonstrated comparable quantitation to its current predecessor, the RealTi*m*e HIV-1 assay. Discordant quantitation between any two methodologies could be due to sample storage length or temperature between the testing times of the two assays and intrinsic differences in the assay design features (e.g., calibration strategy, target regions). Although the current study included several variables that could impact assay precision, our analysis across 4 independent sites demonstrated that the Alinity m HIV-1 assay's precision was 0.08 and 0.21 Log_{10} copies/mL at the nominal level of 1000 and 50 copies/mL, respectively. Alinity m HIV-1 assay QC quantitation demonstrated excellent reproducibility across the different sites. The overall mean and SD for the low and high positive QC was 3.07 ± 0.14 Log_{10} copies/mL and 4.95 ± 0.09 Log_{10} copies/mL, respectively.

Limitations of our study include the use of surplus samples for the assay comparison, which in many cases did not provide sufficient sample volume to resolve discordant assay results. The study was performed at multiple study sites, each using different clinical routine sample sets. The precision study utilized multiple (4–7 lots) different lots of amplification and detection, lysis buffer, and Sample Prep, which may have introduced further variability. Finally, only a limited number of quantifiable samples were run using the Panther Aptima HIV-1 assay.

Despite these limitations, our study found comparable performance of the Alinity m HIV-1 assay versus several currently commercially available HIV-1 assays across a wide range of HIV-1 subtypes and multiple independent study sites. The overall observed bias (-0.10 to 0.10 Log_{10} copies/mL) was not clinically meaningful. Importantly, for samples with VL results near clinical decision points, we found a high agreement between the Alinity m HIV-1 assay and the various routinely used comparator assays (> 88 %).

Disclosure statement

AV, AG, EG, FA, FO, GN, MK, LM, RE, RG, SB, KJ and MP have no conflicts of interest.

HK has received speaker honorariums and/or travel grants from AbbVie, GILEAD, Hexal, Hormosan, Janssen, MSD, Roche, and ViiV.

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SC has acted as advisor for Abbott and Cepheid.

BR, JD, and KP are employees of Abbott GmbH.

AJ, DL, MJP, and NM are employees of Abbott Molecular Inc.

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CRediT authorship contribution statement

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