



Protocols

m-PIMA™ HIV1/2 VL: A suitable tool for HIV-1 and HIV-2 viral load quantification in West Africa

Halimatou Diop-Ndiaye^{a,*}, Pauline Yacine Sène^b, Khadidiatou Coulibaly^c, Marième Diallo^d, Sada Diallo^b, Karim Diop^c, Aissatou Sow-Ndoye^e, Mengue Fall^b, Anna Julienne Selbe Ndiaye^e, Evans Mathebula^f, Adjratou Aissatou Ba^b, Charlotte Lejeune^d, Ndeye Marie Pascaline Manga^b, Makhtar Camara^a, Cheikh Tidiane Ndour^c, Coumba Toure Kane^e

^a Cheikh Anta Diop University and Bacteriology-Virology UTH Aristide le Dantec, Dakar, Senegal

^b Bacteriology-Virology UTH Aristide le Dantec, Dakar, Senegal

^c Division de Lutte contre le SIDA/IST, Dakar, Senegal

^d Clinton Health Access Initiative, Dakar, Senegal

^e Institut de Recherche en Santé, de surveillance épidémiologique et de Formation, Dakar, Senegal

^f School of Health Systems and Public Health, Faculty of Health Science, University of Pretoria, Pretoria, South Africa



ARTICLE INFO

Keywords:

HIV-1
HIV-2
Plasma Viral load
POC
Senegal

ABSTRACT

Point-of-Care for HIV viral RNA quantification seems to be a complementary strategy to the existing conventional systems. This study evaluated the performance of the m-PIMA™ HIV1/2 Viral Load for the quantification of both HIV-1 and HIV-2 RNA viral load. A total of 555 HIV-1 and 90 HIV-2 samples previously tested by Abbott RealTime HIV-1 (Abbott, Chicago, USA) and Generic HIV-2® Charge virale (Biocentric, France) were tested using the m-PIMA™ HIV1/2 Viral Load at the HIV National Reference lab in Senegal. For HIV-1, Pearson correlation and Bland-Altman plots showed a coefficient $r = 0.97$ and a bias of $-0.11 \log_{10}$ copies/ml (95% confidence interval [CI]: -0.086 to $-0.133 \log_{10}$ copies/ml) for the m-PIMA™ HIV1/2 Viral Load, respectively. Sensitivity and specificity at $3 \log_{10}$ copies/ml (threshold of virological failure) were 93.6% (95%[CI]: 91.5% to 95.6%) and 99.1% (95%[CI]: 98.3% to 99.9%), respectively. For HIV-2, a correlation of $r = 0.95$ was also noted with a bias of $-0.229 \log_{10}$ copies/ml (95%[CI]: -0.161 to $-0.297 \log_{10}$ copies/ml). Sensitivity and specificity at $3 \log_{10}$ copies/ml were 97.6% (95%[CI]: 94.3% to 100%) and 93.9% (95%[CI]: 88.9% to 98.8%), respectively. These results confirmed that m-PIMA™ HIV1/2 VL could be a good alternative for HIV-1 and HIV-2 viral load testing in decentralized settings in Senegal.

1. Introduction

Viral load testing allows early identification of treatment failure in patients on antiretroviral therapy (Rodger et al., 2016). In countries with limited resources, such as Senegal, access to viral load testing remains a major challenge (Liégeois et al., 2019). Indeed, the centralization of viral load platforms in national or regional laboratories makes it difficult for sample transportation, which leads to delays in testing. These long delays in sample processing and return of results leads to loss of follow-up of a large number of patients (Meloni et al., 2019). Point-of-Care (POC) technologies offer an innovative approach for viral load monitoring, allowing real access to molecular biology in decentralized settings in resource-limited countries (Villa et al., 2020) by

getting around the challenges associated with centralized testing (Murray et al., 2017; Meggi et al., 1999). The use of POC for HIV-1 monitoring has improved patient management in most of the countries where it was implemented (Swathirajan et al., 2017; Moyo et al., 2016; Gueudin et al., 2016; Ndlovu et al., 2018), and will certainly play a big role in achieving the goal of HIV elimination by 2030 and the 95–95–95 objectives for 2025.

In addition, Senegal and neighboring countries such as the Republic of Guinea, Bissau Guinea and Ivory Coast face another unique challenge related to the management of HIV-2 infected patients due to few HIV-2 techniques available for viral load testing (Bertine et al., 2017; Desclaux et al., 2003). Therefore, there is a real need for a highly sensitive and specific nucleic acid test for the detection and/or quantification of HIV-2

* Corresponding author.

E-mail addresses: halimatoudiop@yahoo.fr, halimatou.ndiaye@ucad.edu.sn (H. Diop-Ndiaye).

<https://doi.org/10.1016/j.jviromet.2023.114872>

Received 14 September 2023; Received in revised form 5 December 2023; Accepted 15 December 2023

Available online 19 December 2023

0166-0934/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

RNA in plasma in molecular labs as well as in the field.

The m-PIMA™ HIV1/2 VL (Abbott, Chicago, USA), based on fully automated real-time nucleic acid amplification and detection technology, is the only POC that has been pre-qualified by the World Health Organization (WHO) and approved for use for the quantification of both HIV-1 and HIV-2 viral RNA (Phillips et al., 2016; Manoto et al., 2018). Moreover, m-PIMA™ HIV1/2 VL is a device that can perform quantitative viral load testing in the field supported by a power drum (Gueguen et al., 2021). Therefore, the m-PIMA™ HIV1/2 VL test offers an innovative approach for HIV-1 and HIV-2 viral monitoring in an area with co-circulation of the 2 types of HIV viruses.

This study evaluated the performance of the m-PIMA™ HIV1/2 VL test in comparison with the Abbott RealTime HIV-1 (Abbott, Chicago, USA) and with GENERIC HIV-2® Charge virale (Biocentric, France) for HIV-1 and HIV-2 viral load determination.

2. Methodology

2.1. Study design

2.1.1. HIV-1 sampling

The first part of the evaluation included a panel of 455 HIV-1 plasmas stored in the -80°C Freezer at the National Reference Laboratory (HNRL) biobank of Bacteriology-Virology at Aristide le Dantec Hospital (Dakar, Senegal) for a maximum 2 years. Samples were included in the study if they satisfied the following criteria, which included that patients were under antiretroviral therapy (ART) for at least 6 months, having a viral load determined by Abbott RealTime HIV-1 (Abbott, Chicago, USA) with a minimum of 40% of samples having viral above 1000 copies/ml. An additional 100 whole blood samples collected from HIV-1 positive patients who were 18 years and above and on ART for at least 6 months were prospectively collected in Ethylene diamine tetra acetic acid (EDTA) tubes in Dakar as part of their follow-up visit. Plasma samples were obtained by centrifugation of whole blood tubes at 2500 rpm for 10 min and stored at -80°C until tested.

2.1.2. HIV-2 sampling

The second part of the evaluation included 90 plasma HIV-2 positive samples collected prospectively in EDTA tubes from patients naive to antiretroviral treatment. All patients consented to participate in this study and were over 18 years of age. Given the high prevalence of HIV-2 in southern regions in Senegal, samples were obtained mainly from this part of the country, but HIV-2 patients were also included in other sites depending on the positive numbers of HIV-2 patients in the sites. Plasma samples obtained by centrifugation of whole blood tubes were stored in the freezer at -20°C in the regions for a maximum of 1 month before transporting to the HNRL in dry ice and stored at -80°C until tested.

2.2. Viral load quantification

Viral load quantification was performed for each platform according to the manufacturer's instructions for use.

2.2.1. Abbott RealTime HIV-1

The Abbott RealTime HIV-1 (Abbott, Chicago, USA) assay performed on the m2000sp/rt automated platform is an in vitro qRT-PCR targeting the integrase region of the highly conserved *pol* gene. The test detects HIV-1 groups M, N, O and P and several CRFs by combining fully automated extraction on Abbott m2000sp and amplification coupled with real-time detection on Abbott m2000rt. The quantification range of the assay is 1.60 to 7.01 \log_{10} copies/ml for an input volume of 600 μL of plasma (Tang et al., 2007). This test is WHO prequalified (RealTi, 2020) and enrolled in the NIAID/NIH Virology Quality Assurance Program (VQA) since 2005 in the National Reference Laboratory.

2.2.2. GENERIC HIV-2® Charge virale

The GENERIC HIV-2® Charge virale (Biocentric, France) test is an in vitro test for quantifying HIV-2 RNA in human plasma samples targeting the *LTR* region and *gag* gene. The test was performed using 500 μL of plasma with a limit of detection of 22 copies/ml (1.34 \log_{10} copies/ml) and the quantification range of the assay is 1.10 to 10.7 \log_{10} copies/ml. This test is intended to detect and quantify HIV-2 group A and B. Briefly, after automated extraction using Nordiag Arrow (Promega, USA), a real time RT-PCR was performed on an open real-time PCR platforms Amplex NG48 (Biosynex, France) using the GENERIC HIV2® Charge virale kit (Biocentric, France) (GENERIC HIV-2, n.d.). This test is CE approved and is used as the gold standard for HIV-2 viral load monitoring in Senegal.

2.2.3. m-PIMA™ HIV 1/2 Viral Load Test

The m-PIMA™ HIV-1/2 (Abbott, Chicago, USA), quantification system is a nucleic acid amplification test that uses the principle of the multiplexed PCR method allowing amplification and detection of more than one target at the same time. The assay is designed to identify and distinguish at the same time HIV-1 subgroup (M/N), the HIV-1 subgroup (O) and HIV-2 and incorporates a series of controls for validation.

The m-PIMA™ HIV1/2 VL test was performed using 50 μL of plasma with a limit of detection of 800 copies/ml (2.9 \log_{10} copies/ml) with a quantification range of less than 2.9 \log_{10} copies/ml to more than 6 \log_{10} copies/ml. All reagents are contained in a fully self-contained, disposable single-use cartridge and once covered, cannot be re-opened; it remains completely sealed. The sample or reagent was not in contact with the analyzer, thus greatly reducing any possibility of cross-contamination. No pre-treatment (or other manipulation) of the sample is required. PCR amplification and real-time fluorescence detection are performed using the m-PIMA™ analyzer with a turnaround time of approximately 1 h (m-PIMA™, nd). This test is WHO prequalified for both HIV-1 and HIV-2 viral load (Anon). The tests were performed by 2 biologists of HNRL who were trained by the technical support of Abbott.

2.3. HIV-2 viral load quality control

2.3.1. External quality control at Max Von Pettenkofer institute (Munich, Germany)

As GENERIC HIV-2® Charge virale was not WHO prequalified, we used an external quality control for the HIV-2 samples. All HIV-2 samples were sent to Max Von Pettenkofer institute (Munich, Germany) and tested on a home-made real-time PCR technique for HIV-2 viral load quantification using 500 μL of plasma with 5 copies/ml of limit of detection (Max von Pettenkofer-Institut, n.d.).

2.3.2. Quality control using a HIV-2 standard panel

An internal quality control was also carried out using serially diluted HIV-2 standard of group A panel (NHZ, lot: 10269296) (Material No: 0400-0094) from the LGC Clinical Diagnostic, Inc (Milford, USA). Briefly, a 10X serial dilution of the standard was performed by adding 1 vol of HIV-2 standard (VL: 8.01 \log_{10} copies/ml) to 9 volumes of negative human plasma; then added 1 vol of the first dilution to 9 volumes of negative plasma and successively to finally have a panel with estimated viral load ranging from 7.01 \log_{10} copies/ml to 2.01 \log_{10} copies/ml. HIV-2 viral load for each dilution was performed side-by-side using the GENERIC HIV-2® Charge virale kit and m-PIMA™ HIV1/2 VL on two different m-PIMA™ analyzers (NAT-04000533 and NAT-04000510). Repeatability assays were also performed using quantified dilution from 5.01 \log_{10} copies/ml to 3.01 \log_{10} copies/ml by testing each dilution in triplicate on the 2 assays. For reproducibility assays, 2 dilutions (5.0 and 3.0 \log_{10} copies/ml) were tested once a day by the same operator during 3 days in the two devices and results recorded.

2.4. Statistical analysis

To facilitate the statistical comparison, viral load (VL) value of each

sample was transformed into \log_{10} copies/ml and all VL below the detection limit of the m-PIMATM HIV1/2 VL were assigned the value of 2.9 \log_{10} copies/ml (799 copies/ml). The differences between the VL values of reference and field method (D-Log) were calculated and considered significant when above 0.5 \log_{10} copies/ml in absolute value.

To assess agreement between techniques correlation coefficient of Pearson and Bland-Altman plots were determined for VL values obtained by m-PIMATM HIV1/2 and the GENERIC HIV2[®] Charge virale (reference method) using MethVal software (Method Validator Software 1.1.9.0, Philippe Marquis, Metz, France). Briefly, the software makes direct calculation of the mean, standard deviation (SD), r coefficient, slope and intercept with their 95% confidence interval (95% CI) and the correlation curve. MethVal also draw the Bland Altman diagram with the mean difference (95% CI), the upper and lower limit represent the mean plus or minus 1.96 SD, and 95% of the values are generally fitting between these limits. The total number of the samples in the dataset is also displayed.

Sensitivity and specificity of m-PIMATM HIV1/2 VL were also estimated at detection limit (2.9 \log_{10} copies/ml) and at the threshold of virological failure (3 \log_{10} copies/ml) using R software version 3.5.0 (2018-04-23). The 95% confidence interval was used for these analyses. Repeatability and reproducibility of the m-PIMATM HIV1/2 VL was assessed by calculating the coefficient of variation compared to manufacturer's data.

2.5. Ethical and regulatory considerations

The purpose of the study and protocol specific procedures to be followed or interventions were explained to the participants before written consent was obtained and sample collection. The study protocol and relevant supporting documents were approved by the National Study Committee for Health Research in Senegal (Reference: Protocol SEN 18/48) and administrative authorization was issued by the Senegalese Ministry of Health and Social Action.

3. Results

3.1. m-PIMATM HIV1/2 VL versus Abbott RealTime HIV-1

This evaluation included a total of 555 HIV-1 plasma samples including 455 from the laboratory biobank and 100 from infected patients recruited prospectively for the purpose of this evaluation. The Table 1 is describing the VL values of the HIV-1 samples tested.

The agreement between the tests was 95.9% with 23 discordant samples at 2.9 \log_{10} copies/ml, and 97.1% with 16 discordant samples at 3 \log_{10} copies/ml (Table 2). m-PIMATM HIV1/2 VL sensitivity and specificity were 91% (95%[CI]: 88.5% to 93.3%) and 98.8% (95%[CI]: 97.9% to 99%) at 2.9 \log_{10} copies/ml, and 93.6% (95%[CI]: 91.5% to 95.6%) and 99.1% (95%[CI]: 98.3% to 99.9%) at 3 \log_{10} copies/ml, respectively.

The mean VL were 3.6 \log_{10} copies/ml with a SD of 1.1 \log_{10} copies/ml for the Abbott RealTime HIV-1 assay and 3.5 \log_{10} copies/ml with a SD of 0.9 \log_{10} copies/ml for the m-PIMATM HIV1/2 VL.

With D-log determination, overall agreement between the two assays

was 91.2% (506/555). The remaining 8.8% samples were considered discordant as their D-log $> \pm 0.5 \log_{10}$ copies/ml (Table 3). Out of these discrepancies, five samples were overestimated by the m-PIMATM HIV1/2 VL (including 3 below the limit of 2.9 \log_{10} copies/ml) and 44 were overestimated by the Abbott Real Time HIV-1 assay (including 6 not quantified by m-PIMATM HIV1/2 VL).

The Passing-Bablok regression curve showed a significant correlation ($r = 0.97$) between the HIV-1 RNA levels obtained by the two assays, with an intercept of 0.45 (95%[CI]: 0.37 to 0.52) and a slope of 0.846 (95%[CI]: 0.822 to 0.872). Similarity between the two assays assessed by the Bland-Altman plot method showed a mean difference of $-0.11 \log_{10}$ copies/ml (95% [CI]: -0.086 to $-0.133 \log_{10}$ copies/ml) between the m-PIMATM HIV1/2 VL assay and Abbott RealTime HIV-1 assay (Fig. 1).

3.2. m-PIMATM HIV 1/2 VL versus GENERIC HIV-2[®] Charge virale

The comparison between the two assays was made on 90 HIV-2 positive samples collected. The VL values of the HIV-2 samples tested were described Table 1. The agreement between tests was 97.7% (with 02 discordant samples) at the limit of m-PIMATM detection, and 95.5% at 3 \log_{10} copies/ml (with 04 discordant samples) (Table 4).

The m-PIMATM HIV 1/2 VL sensitivity and specificity of m-PIMA HIV-1 VL were 100% and 96.8% (95%[CI]: 91.7% to 99.9%) at 2.9 \log_{10} copies/ml, and 97.6% (95%[CI]: 94.3% to 100%) and 93.9% (95 [CI]: 88.9% to 98.8%) at 3 \log_{10} copies/ml, respectively.

The mean viral load was 3.3 \log_{10} copies/ml with a SD of 0.6 \log_{10} copies/ml, for the Generic HIV-2[®] Charge virale and 3.5 \log_{10} copies/ml with a SD of 0.8 \log_{10} copies/ml for the m-PIMATM HIV1/2 VL.

With D-log determination, overall agreement between the two assays was 84.4% (76 samples), and 15.6% (14 samples) had a D-Log $> \pm 0.5 \log_{10}$ copies/ml; an overestimation of viral load values was observed by the m-PIMATM HIV1/2 VL with D-Log ranging from -0.6 to $-1.3 \log$ copies/ml (Table 5).

A good correlation ($r = 0.95$) was observed between the two techniques with an intercept of -0.82 (95% CI, -0.51 to -1.13) and a slope of 1.316 (95%[CI]: 1.224 to 1.409).

Similarity between the two assays assessed by the Bland-Altman diagram method showed a mean difference of 0.229 \log_{10} copies/ml (95% [CI]: 0.161 to 0.297 \log_{10} copies/ml) in VL measurement between m-PIMATM HIV1/2 VL and Generic HIV-2[®] Charge virale test (Fig. 2).

3.3. Quality control

3.3.1. External quality control: m-PIMATM HIV 1/2 VL and Myp (Munich RT-PCR technique, NIBSC)

The comparison between the techniques was carried out on the 90 HIV-2 samples with an overall agreement between tests 98.8% (with 01 discordant sample) and 97.7% (with 02 samples discordant) at 2.9 \log_{10} copies/ml and 3 \log_{10} copies/ml, respectively (Table 6).

The m-PIMATM HIV 1/2 VL sensitivity was the same at 2.9 and 3 \log_{10} copies/ml with 97.7% (95% [CI]: 94.6% to 100%). However, the specificity was 100% (95% [CI]: 96.8% to 100%) at 2.9 \log_{10} copies/ml and 97.9% (95% [CI]: 94.7% to 100%) at 3 \log_{10} copies/ml.

Table 1

Viral load values for HIV-1 and HIV-2 samples.

HIV-1 samples (N = 555)		HIV-2 samples (N = 90)	
Viral load using Abbott m2000 (copies/ml)	n (%)	Viral load using Biocentric (copies/ml)	n (%)
<40	45 (8.1)	<22	44 (48.9)
41<VL* <800	301 (54.2)	41< VL <800	9 (10.0)
800< VL <1000	6 (1.1)	800< VL <1000	2 (2.2)
VL >1000	203 (36.6)	VL >1000	35 (38.9)

* VL: Viral Load.

Table 2
Agreement between m-PIMA™ HIV 1/2 VL and Abbott RealTime HIV-1 at detectability level (2.9 log₁₀ copies/ml) and virological failure threshold (3 log₁₀ copies/ml).

m-PIMA™ HIV1/2 viral load	Detectable* Undetectable**	Abbott RealTime HIV-1			
		2.9log ₁₀ copies/ml		3log ₁₀ copies/ml	
		>=2.9log ₁₀ copies/ml	<2.9log ₁₀ copies/ml	>= 3log ₁₀ copies/ml	<3log ₁₀ copies/ml
		191	4	190	3
		19	341	13	349

* Detectable: means VL quantified above the threshold.
** Undetectable: means with VL values not detected, or detected under the threshold.

Table 3
Discrepancies between m-PIMA™ HIV1/2 VL and Abbott RealTime HIV-1 viral load with D-log calculation.

Log copies/ml																
Abbott RealTime HIV-1	3.78	2.9	4.91	2.9	2.9	5.98	4.06	5.3	3.77	5.44	3.45	6.25	4.65	5.99	5.15	5.75
m-Pima™ HIV1/2 viral load	5.16	4.13	5.9	3.83	3.58	5.47	3.53	4.77	3.23	4.9	2.9	5.71	4.09	5.44	4.58	5.18
D-Log	-1.38	-1.23	-0.99	-0.93	-0.68	0.51	0.53	0.53	0.54	0.54	0.55	0.54	0.56	0.55	0.57	0.57

Log copies/ml																
Abbott RealTime HIV-1	4.76	4.49	4.06	4.66	4.03	5.81	4.13	4.1	5.97	3.88	6.15	4.63	5.57	4.79	3.62	5.6
m-Pima™ HIV1/2 viral load	4.18	3.91	3.47	4.08	3.44	5.21	3.51	3.47	5.31	3.21	5.48	3.94	4.87	4.08	2.9	4.88
D-Log	0.58	0.58	0.59	0.58	0.59	0.6	0.62	0.63	0.66	0.67	0.67	0.69	0.7	0.71	0.72	0.72

Log copies/ml																
Abbott RealTime HIV-1	4.68	6.22	5.06	4.83	3.72	5.5	5.85	5.01	5.27	3.96	3.77	3.9	5.68	4.26	5.61	5.82
m-Pima™ HIV1/2 viral load	3.96	5.49	4.32	4.04	2.9	4.62	4.96	4.12	4.42	3.09	2.9	2.9	4.56	2.9	4.17	4.19
D-Log	0.72	0.73	0.74	0.79	0.82	0.88	0.89	0.89	0.85	0.87	0.87	1	1.12	1.36	1.44	1.63

In light grey, samples over-quantified by m-PIMA and below the limit of 800 copies/ml (2.9 log₁₀ copies/ml)
In dark grey, samples over-quantified by Abbott RealTime HIV-1 and not quantified by m-PIMA (VL less than 800 copies/ml or 2.9 log₁₀ copies/ml)

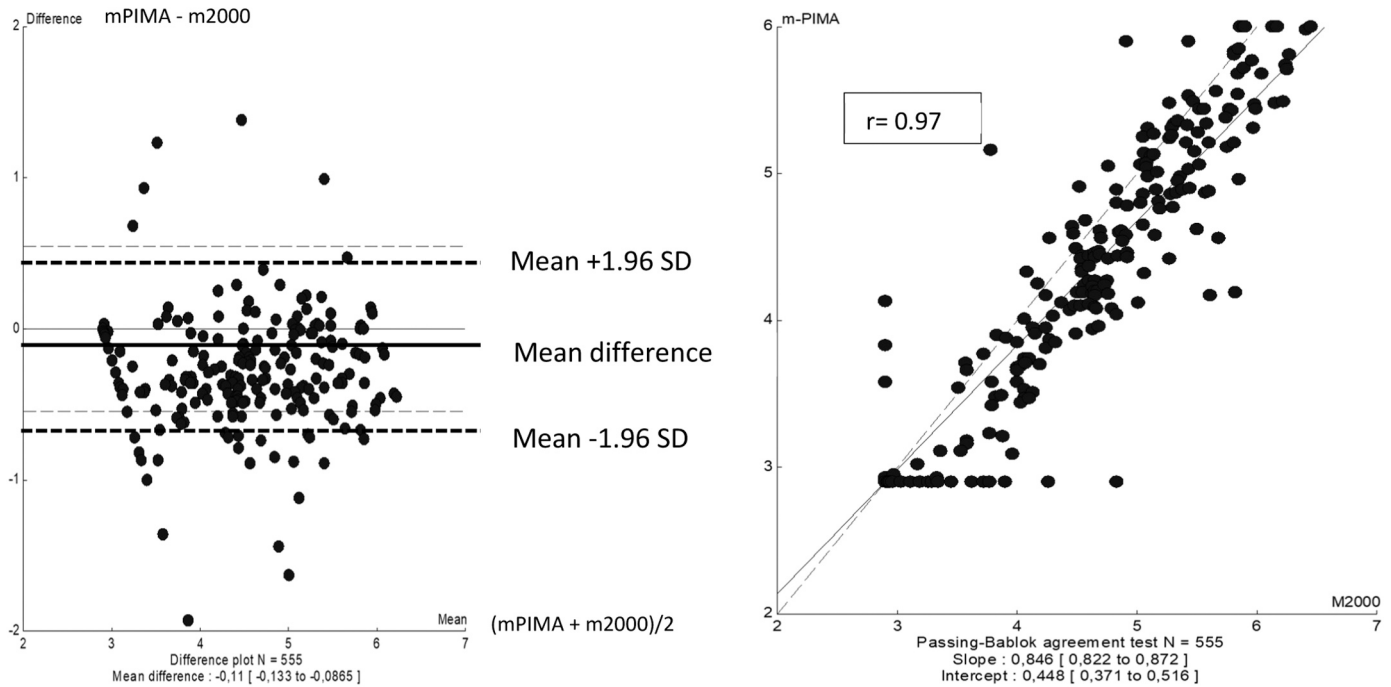


Fig. 1. Bland-Altman plots and Passing Bablok regression between m-PIMA™ HIV1/2 VL to Abbott RealTime HIV-1.

The mean VL was 3.4 log₁₀ copies/ml with a SD of 0.7 log₁₀ copies/ml for the Mvp VL and 3.5 log₁₀ copies/ml with a SD of 0.8 log₁₀ copies/ml for the m-PIMA™ HIV1/2 VL.

With D-log determination, overall concordance between the tests was 93.3%, and 7.7% (04 samples) had a D-Log > ± 0.5 log₁₀ copies/ml; a slight overestimation of viral load values was observed with the m-PIMA™ HIV1/2 VL with D-Log ranging from −0.6 to −0.8 log copies/ml (Table 7).

A good correlation (r = 0.98) was found using the linear regression line with an intercept of −0.42 (95%[CI]: −0.60 to −0.23) and a slope of

Table 4

Agreement between m-PIMA HIV 1/2 VL and Generic HIV-2 Charge virale test at detectability level ($2.9 \log_{10}$ copies/ml) and virological failure threshold ($3 \log_{10}$ copies/ml).

		Generic HIV-2® Charge virale (Biocentric)			
		$2.9 \log_{10}$ copies/ml		$3 \log_{10}$ copies/ml	
		$\geq 2.9 \log_{10}$ copies/ml	$< 2.9 \log_{10}$ copies/ml	$\geq 3 \log_{10}$ copies/ml	$< 3 \log_{10}$ copies/ml
m-PIMA™ HIV1/2 viral load	Detectable*	42	2	40	3
	Undetectable**	0	46	1	46

* Detectable: means VL quantified above the threshold.

** Undetectable: means with VL values not detected or detected under the threshold.

Table 5

Discrepancies between m-PIMA™ HIV1/2 viral load and Generic HIV-2 Charge virale with D-log calculation.

Generic HIV-2® Charge virale	Viral load values (Log copies/ml)													
	3.7	4.6	3.6	5	3.1	4.6	3.7	3.2	4.6	4	3	4.6	3.2	4.1
m-PIMA™ HIV1/2 viral load	5	5.5	4.6	5.9	3.9	5.4	4.4	3.9	5.3	4.7	3.7	5.3	3.8	4.7
D-Log	-1.3	0.9	-1	-0.9	-0.8	-0.8	-0.7	-0.7	-0.7	-0.7	-0.7	-0.7	-0.6	-0.6

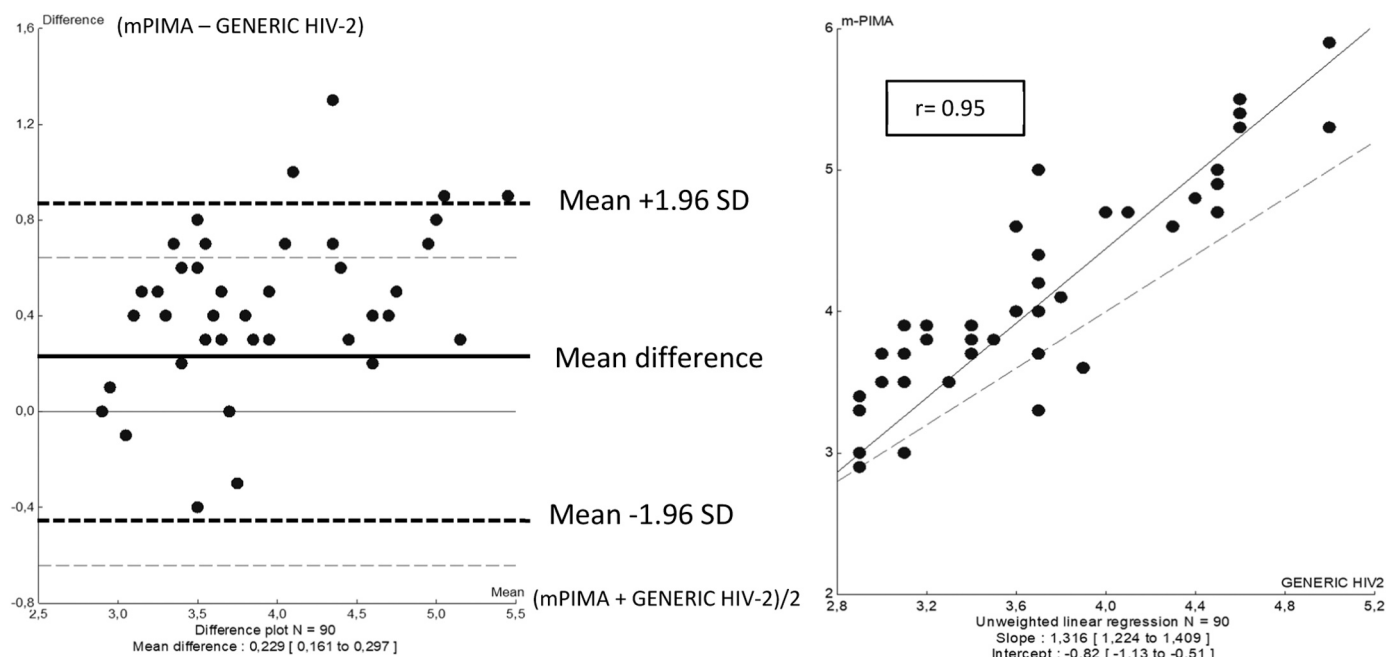


Fig. 2. Bland-Altman plots and Linear regression line between m-PIMA™ HIV 1/2 VL test and Generic HIV-2 VL (Biocentric).

Table 6

Agreement between m-PIMA™ HIV 1/2 VL and Mvp (RT-PCR Munich, NIBSC) at detectability level ($2.9 \log_{10}$ copies/ml) and virological failure threshold ($3 \log_{10}$ copies/ml).

		Mvp (RT-PCR Munich, NIBSC)			
		$2.9 \log_{10}$ copies/ml		$3 \log_{10}$ copies/ml	
		$\geq 2.9 \log_{10}$ copies/ml	$< 2.9 \log_{10}$ copies/ml	$\geq 3 \log_{10}$ copies/ml	$< 3 \log_{10}$ copies/ml
m-Pima HIV 1/2 VL	Detectable*	43	0	42	1
	Undetectable**	1	46	1	46

* Detectable: means VL quantified above the threshold.

** Undetectable: means with VL values not detected or detected under the threshold.

1.158 (95%[CI]: 1.105 to 1.211).

Similarity between the two assays assessed by the Bland-Altman plots showed a mean difference of $0.121 \log_{10}$ copies/ml (95%[CI]: $0.077 \log_{10}$ copies/ml to $0.165 \log_{10}$ copies/ml) in VL measurement (Fig. 3).

3.3.2. External quality control: GENERIC HIV-2® Charge virale versus Mvp (Munich RT-PCR technique, NIBSC)

The comparison between the techniques was also carried out on the 90 HIV-2 samples with an overall agreement between tests 98.8% (with 01 discordant sample) and 97.7% (with 02 discordant samples) at

Table 7

Discrepancies between m-PIMA™ HIV1/2 VL and Mvp (RT-PCR Munich, NIBSC) with D-log calculation.

Viral load values (Log copies/ml)				
Mvp (RT-PCR NIBSC)	3.9	3.1	4.8	4.7
m-PIMA™ HIV 1/2 VL	4.7	3.7	5.4	5.4
D-Log	-0.8	-0.6	-0.6	-0.7

2.9 log₁₀ copies/ml and 3 log₁₀ copies/ml, respectively (Table 8).

For the GENERIC HIV-2® Charge virale, sensitivity and specificity were 97.7% (95%[CI]: 94.6% to 100%) and 100% at 2.9 log₁₀ copies/ml, and 95.3% (95%[CI]: 90.9% to 99.6%) and 100% at 3 log₁₀ copies/ml, respectively.

The mean viral load was 3.4 log₁₀ copies/ml with a SD of 0.6 log₁₀ copies/ml for the Mvp Viral Load and 3.3 log₁₀ copies/ml with a SD of 0.7 log₁₀ copies/ml for the GENERIC HIV-2® Charge virale, respectively.

With D-log determination, overall concordance between the tests was 93.3%, and 7.7% (04 samples) had a D-Log > ± 0.5 log₁₀ copies/ml; a slight overestimation of viral load values was observed with the Munich RT-PCR (Table 9) with D-Log ranging from 0.6 to 0.9 log copies/ml.

A good correlation (r = 0.96) was found using the linear regression line with an intercept of 0.51 (95%[CI]: -0.69 to 0.33) and a slope of 0.819 (95%[CI]: 0.708 to 0.871). Similarity between the two assays

assessed by the Bland-Altman plots showed a mean difference of -0.108 log₁₀ copies/ml (95% CI: -0.063 to -0.152 log₁₀ copies/ml) in VL measurement (Fig. 4).

3.3.3. Quality control using HIV-2 standard panel

The performance of the m-PIMA™ HIV-1/2 VL test was also assessed in comparison to the Generic HIV-2® Charge virale tests using the commercial panels from the HIV-2 standard of group A (NHZ, lot: 10269296).

For the 02 members of the panel with high titer (7.0 and 6.0 log₁₀ copies/ml) m-PIMA™ HIV 1/2 reported the result "> 1000,000 copies/ml" while the results obtained by the Generic HIV-2 test were 6.90 and 5.91 log₁₀ copies/ml, respectively (Table 10). Quantitative values of viral load were obtained between 5 and 3 log₁₀ copies/ml. However below 3 log₁₀ copies/ml, the panel titrated at 2.01 log₁₀ copies/ml was quantified only by the Generic HIV-2® test with 2.52 log₁₀ copies/ml, while it remains not detected by the m-PIMA™ HIV 1/2 VL test

Table 9

Discrepancies between Mvp (RT-PCR Munich, NIBSC) and Generic HIV-2 Viral Load (Biocentric) with D-log calculation.

Viral load values (Log ₁₀ copies/ml)				
Mvp (RT-PCR Munich, NIBSC)	5.6	4.2	4.4	4.6
Generic HIV-2® Charge virale	5.0	3.6	3.7	3.7
D-Log	0.6	0.6	0.7	0.9

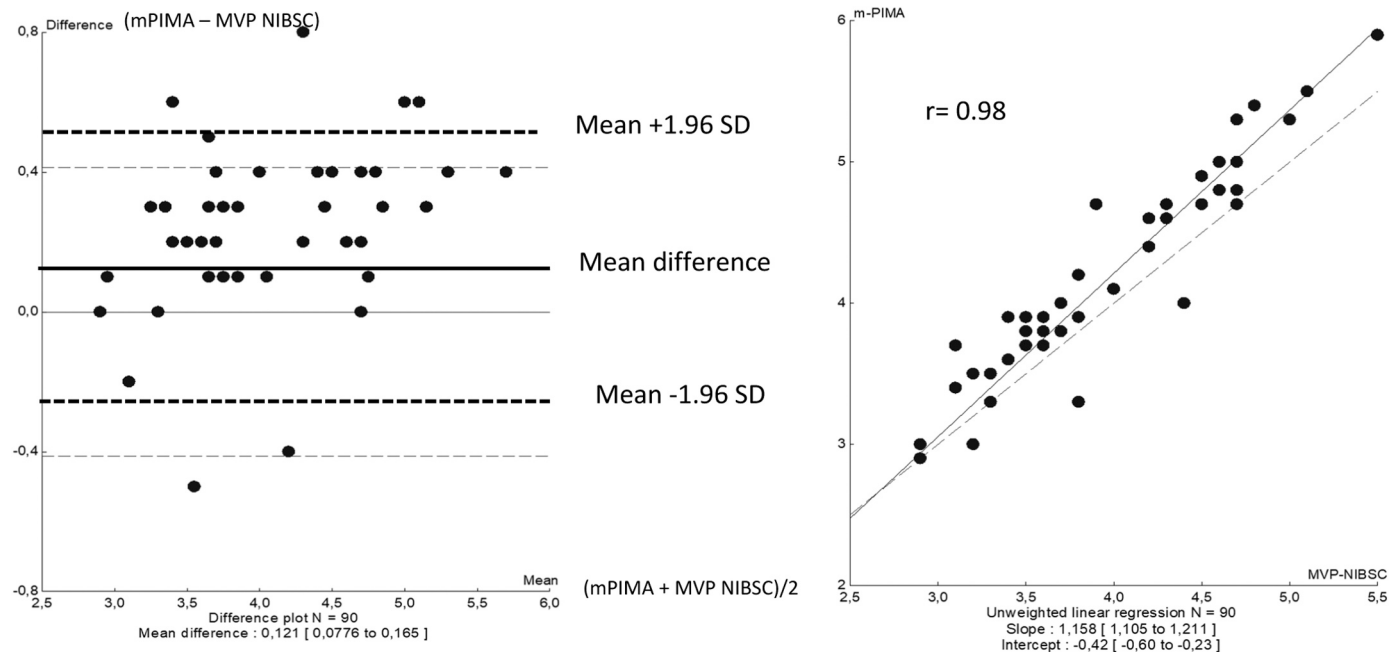


Fig. 3. Bland-Altman plots and Linear regression line between m-PIMA™ HIV 1/2 VL test and Mvp (Munich RT-PCR technique, NIBSC).

Table 8

Agreement between Mvp (RT-PCR Munich, NIBSC) and Generic HIV-2 Viral Load (Biocentric) at detectability level (2.9 log₁₀ copies/ml) and virological failure threshold (3 log₁₀ copies/ml).

		Mvp (RT-PCR Munich, NIBSC)			
		2.9log ₁₀ copies/ml		3log ₁₀ copies/ml	
		>=2,9log ₁₀ copies/ml	<2,9log ₁₀ copies/ml	>= 3log ₁₀ copies/ml	<3log ₁₀ copies/ml
Generic HIV-2® Charge virale (Biocentric)	Detectable*	42	0	41	0
	Undetectable**	1	47	2	47

*Detectable: means VL quantified above the threshold

** Undetectable: means with VL values not detected or detected under the threshold

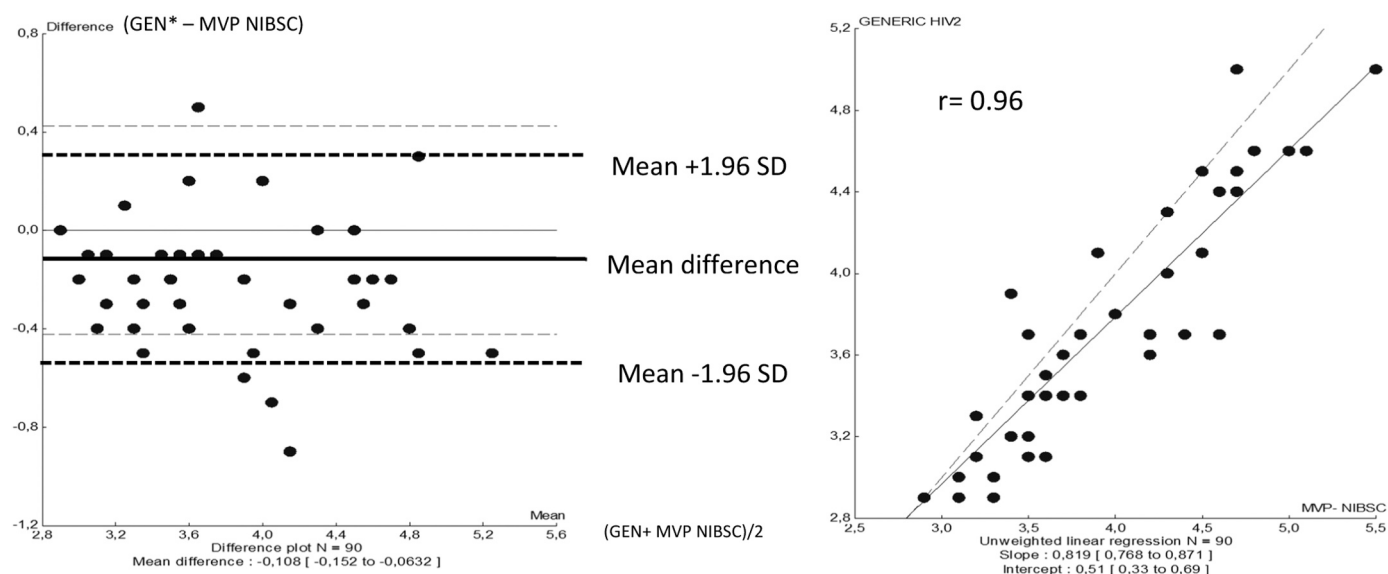


Fig. 4. Bland-Altman plots and Linear regression line between Mvp (Munich RT-PCR technique, NIBSC) and Generic HIV-2 VL (Biocentric). *GEN= GENERIC HIV-2.

Table 10

Viral load measurements of HIV-2 Standard group A by m-PIMATM HIV1/2 VL Generic HIV-2 Charge virale.

Panel Log ₁₀ copies/ml	Mean viral load value GENERIC HIV-2® (Log ₁₀ copies/ml)	Mean viral load value m-PIMA TM HIV 1/2 VL (Log ₁₀ copies/ml)
7.01	6.91	>10 ⁶
6.01	5.91	>10 ⁶
5.01	5.07	5.37
4.01	4.12	4.3
3.01	3.32	3.40
2.01	2.52	Not detected

Table 11

Quality control panel results using the GENERIC HIV-2® Charge virale (Biocentric).

	GENERIC HIV-2® Charge virale (Biocentric)					
Panel Value	7.01	6.01	5.01	4.01	3.01	2.01
Replicate 1	7	5.93	5.1	4.15	3.27	2.65
Replicate 2	6.81	5.89	5.04	4.08	3.36	2.38
Mean	6.91	5.91	5.07	4.12	3.32	2.52
D-log	0.11	0.10	0.06	0.11	0.31	0.51

Table 12

Quality control panel results using the m-PIMATM HIV 1/2 VL test and repeatability assays.

	m-Pima HIV 1/2 VL					
	NAT-04000533 Device			NAT-04000510 Device		
Panel value	5.01	4.01	3.01	5.01	4.01	3.01
Replicate 1	5.25	4.19	3.36	5.41	4.33	3.35
Replicate 2	5.38	4.39	3.50	5.41	4.30	3.36
Replicate 3	5.48	4.42	3.35	5.08	4.31	3.11
Mean	5.37	4.33	3.40	5.30	4.31	3.27
Standard deviation	0.12	0.12	0.08	0.19	0.01	0.14
Coefficient of variation	0.02	0.03	0.02	0.04	0.00	0.04
D-Log	0.36	0.32	0.39	0.29	0.30	0.26

(Table 10).

With D-log determination, neither m-PIMATM HIV-1/2 VL nor the Generic HIV-2® Charge virale showed a significant difference with the panel viral load values (Table 11 and Table 12).

Replicates analysis of m-PIMATM HIV-1/2 VL values (repeatability) on two m-PIMATM devices (NAT-04000533 and NAT-04000510) showed very low coefficients of variation below 0.05 (Table 12).

Analysis of reproducibility values for m-PIMATM HIV-1/2 VL showed at 5,01 log₁₀ copies/ml and 3,01 log₁₀ copies/ml coefficients of variation of 0.00 and 0.02 for NAT-04000533, and 0.01 and 0.06 for NAT-04000510. (Table 13).

4. Discussion

POC and near-POC are recommended by the latest WHO clinical guidelines for viral load monitoring and early detection of virological failure (WHO, n.d.). The use of POC tests can improve the clinical management of people living with HIV by significantly reducing the turnaround time for health care workers to receive their patient's results and take clinical action (Reid et al., 2013; Nicholas et al., 2019).

This study evaluated the performance of the m-PIMATM HIV1/2 VL test for the quantification of HIV-1 and HIV-2 plasma viral load monitoring in Senegal to achieve the goal for HIV elimination by 2030. Moreover, HIV-2 virological monitoring represents a major challenge in achieving this goal due to lack of WHO approved and pre-qualified viral load tests.

In this study, 97.1% overall agreement was found between the m-PIMATM HIV 1/2 VL and Abbott RealTime HIV-1%, and 97.7% between m-PIMATM HIV 1/2 VL and Generic HIV-2® VL test at the clinical failure threshold of 1000 copies/ml (World Health Organization, 2016). These

Table 13

Quality control panel using the m-PIMATM HIV 1/2 VL and reproducibility assays.

	NAT-04000533		NAT-04000510	
Panel Value	5.01	3.01	5.01	3.01
Day 1	5.60	3.73	5.47	3.52
Day 2	5.60	3.92	5.51	3.70
Day 3	5.59	3.86	5.58	3.93
Mean	5.60	3.84	5.52	3.72
Standard deviation	0.01	0.10	0.05	0.21
Coefficient of variation	0.00	0.02	0.01	0.06

results corroborates the WHO prequalification report and previous studies in Kenya and Brazil, which showed 95% of overall agreement (Bwana et al., 2019; Mariani et al., 2020).

When the m-PIMA™ HIV 1/2 VL was compared to Abbott Real Time HIV-1, a correlation of 0.97 and a bias - 0.11 log₁₀ copies/ml were found. Similar results were also reported in several studies that compared m-PIMA™ HIV-1/2 VL to Abbott RealTime HIV-1 in centralized laboratory as well as in multisite studies (Meggi et al., 1999; Bwana et al., 2019; Mariani et al., 2020). Indeed, Mariani and colleagues had reported a correlation of 0.93 and a bias of -0.20 log₁₀ copies/ml when comparing m-PIMA™ HIV -1/2 VL and Abbott RealTime HIV-1 on 413 frozen plasmas (Mariani et al., 2020). Moreover, a multisite study performed in Kenya using 566 plasma samples tested by laboratory technicians on m-PIMA™ HIV-1/2 and Abbott RealTime HIV-1 showed a correlation of 0.92 and a bias of 0.16 log₁₀ copies/ml (Bwana et al., 2019). In addition, another multisite study in Mozambique compared 686 samples tested by nurses with m-PIMA™ HIV-1/2 VL versus Cobas AmpliPrep/Cobas TaqMan 96 HIV-1 v2 (Roche Diagnostic) and reported a correlation of 0.92 and a bias of 0.20 log₁₀ copies/ml (Meggi et al., 1999). The WHO prequalification report found a bias of -0.21 log₁₀ copies/ml compared to the COBAS AmpliPrep/COBAS TaqMan HIV-1, v2.0 test, with 95.1% sensitivity and 99% specificity at 1000 copies/ml (Anon).

Regarding the capacity of HIV-1 detection, m-PIMA™ HIV1/2 VL showed good performances at 3 log₁₀ copies/ml with a sensitivity and specificity of 93.6% and 99.1%, respectively. These results are in line with data from WHO m-PIMA™ HIV1/2 VL prequalification with a sensitivity and specificity of 95.1% and 99.4%, respectively at the threshold of 3 log₁₀ copies/ml.

Few studies have reported on HIV-2 viral load quantification on the m-PIMA™ HIV-1/2 VL, albeit with small sample sizes. Apart from the WHO prequalification studies, only Mariani and colleagues have reported m-PIMA™ HIV-1/2 VL evaluation in order to detect the ability to quantify HIV-2 RNA on 16 HIV-2 samples (Mariani et al., 2020). Indeed, HIV-2 viral load techniques are rare and m-PIMA™ HIV-1/2 VL is the only one that reported a WHO prequalification related to HIV-2 viral load. (Ferns and Garson, 2006). Generic HIV-2® VL(Biocentric) was the only commercial RUO (research use only) technique available at the time of the study. However, the comparison showed a correlation of 0.95 and a bias 0.229 log₁₀ copies/ml. A better correlation ($r = 0.98$) and lowest bias of 0.121 log₁₀ copies/ml with the m-PIMA™ were observed when the external quality control was tested using the laboratory based in-house RT-PCR HIV-2 assay as MvP in Munich, Germany. However, all these results were aligned with WHO prequalification results with $r = 0.92$ (Anon). Replicates analysis of m-PIMA™ HIV-1/2 VL values (repeatability and reproducibility) on two devices (NAT-04000533 and NAT-04000510) showed very low coefficients of variation below 0.06 and also confirmed the performances of m-PIMA™ HIV-1/2 VL.

If m-PIMA™ appears to be suitable to detect viral failure for patients under ART, the main limitation could be its limit of detection related to the input volume (50 microliter). Indeed, m-PIMA™ is not able to quantify viral loads under 800 copies/ml and therefore will not allow to assess low level viremia (Chang et al., 2012; Avettand-Fenoel et al., 2014; Ekouevi et al., 2013).

Currently, the threshold of 50 copies/ml defined as the threshold for virus undetectability is used for ART success and determine whether a patient is eligible for referral to streamlined ART programs. Moreover, patients with an undetectable viral load using any WHO prequalified combination of sample and testing platform, including dried blood spot samples, and continue taking medication as prescribed have zero risk of transmitting HIV to their sexual partner(s) (Dorward et al., 2021; Une charge virale indétectable, 2022).

The threshold of 1000 copies/ml is defined as viral load suppression, and a recent review provides evidence of almost zero or negligible risk of sexual transmission of HIV for patients who have a suppressed but detectable viral load and who are taking medication as prescribed

(<https://apps.who.int/iris/bitstream/handle/10665/360860/9789240055179-eng.pdf>).

Therefore, POC such as m-PIMA™ HIV1/2 VL and alternative sample types such as dried blood spot samples, can support the goals of treatment program to accurately measure and report viral load results as unsuppressed, suppressed and undetectable (Broyles et al., 2023).

As a POC, the benefit of using m-PIMA™ is to provide same-day HIV-1 and/or HIV-2 viral load and get back results to accelerate clinical decision-making and reduce clinic visits and patient travel costs (Msimango et al., 2020). The simple interface, low volume of plasma used, and shorter execution time give an advantage for suitable implementation in clinical settings, especially in settings with poor access to viral load testing. Access to this test will allow the prioritization of patients for adherence counselling, and will reduce loss to follow-up by improving early detection of virological failure (Meloni et al., 2019; Mariani et al., 2020; Vojnov et al., 2016). The achievement of the UNAIDS 95–95–95 targets depends on HIV surveillance and the prevention tools of a future epidemic of ART-resistant HIV strains that could delay these targets in sub-Saharan Africa (Guichet et al., 2016).

The strength of the study included a relatively large samples collected prospectively to assess HIV-2 quantification. Although there was a difference in the regulatory statuses of the two assays (WHO PQ vs CE marking), sending the samples to be tested in Munich as a form of QC and testing of standardizes commercial panels assisted in confirming the results.

The main limitation of this study was the number of samples with VL less than 1000 copies/ml (64% observed vs 60% expected). Indeed, despite the prospective recruitment, only 36% of patients had a VL above 1000 copies/ml and on ART for at least 6 months. Therefore, due to the m-PIMA inability to quantify under 800 copies/ml, analysis was conducted at the limit of detection of m-PIMA and also at the threshold of virological failure, 1000 copies/ml, which is most important in clinical practice according to WHO recommendations.

Also, the study was conducted in centralized settings, when all tests were leaded by two trained technologists at the HIV National Reference Laboratory in Senegal, which meant the data generated did not reflects the performance and reliability of the tests in the intended POC setting. Moreover, the analysis of the testing error rate for m-PIMA™ HIV1/2 VL was not performed properly, so it was not possible to extrapolate the challenges regarding its use in decentralized settings.

5. Conclusion

Because of the simplicity, rapid results and its good performance, m-PIMA™ HIV1/2 VL could help in viral load scaling up and antiretroviral treatment monitoring in Senegal for HIV-1 as well as HIV-2 patients in health care centers, as part of their routine clinical care.

This would help to achieve the ambitious targets of the third "95" throughout the country. However, the implementation of these technology in decentralized health care centers should be monitored in order to better optimize their clinical and operational utility.

CRedit authorship contribution statement

Ndiaye Anna J S: Writing – review & editing, Conceptualization. **Fall Mengue:** Investigation, Formal analysis. **Lejeune Charlotte:** Supervision, Funding acquisition, Conceptualization. **Diop-Ndiaye Halimatou:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Ba Adjratou A:** Methodology, Formal analysis. **Camara Makhtar:** Writing – review & editing, Validation. **Coulibaly Khadydiatou:** Supervision, Project administration. **Manga Ndeye M P:** Investigation, Formal analysis. **Sène Pauline Yacine:** Writing – original draft, Software, Project administration, Formal analysis, Data curation. **Tourekane Coumba:** Writing – review & editing, Validation, Funding acquisition, Conceptualization. **Matheubula Evans:** Writing – review &

editing, Methodology, Conceptualization. **Ndour Cheikh T:** Writing – review & editing, Validation, Conceptualization. **Diallo Marième:** Supervision, Project administration. **Diop Karim:** Validation, Conceptualization. **Diallo Sada:** Investigation, Formal analysis. **Sow-Ndoye Aissatou:** Investigation, Formal analysis.

Declaration of Competing Interest

The authors do not declare any conflict of interest.

Data availability

Data will be made available on request.

Acknowledgment

We thank the study participants, the molecular biology unit of the laboratory of bacteriology and virology of the Aristide Le Dantec hospital in Dakar-Senegal, the national TB program and the division for fight against AIDS. This study was supported by the Clinton Health Access Initiative (CHAI) and the UNICEF Senegal and Abbott Diagnostics.

References

- (https://extranet.who.int/pqweb/sites/default/files/PQDx_0226-032-00_m-PIMA-HI_V12-Detect_v6.pdf) (accessed October 28, 2021).
- Avettand-Fenoel, V., Damond, F., Gueudin, M., Matheron, S., Méléard, A., Collin, G., et al., 2014. New sensitive one-step real-time duplex PCR method for group A and B HIV-2 RNA load. *J. Clin. Microbiol.* 52, 3017–3022. <https://doi.org/10.1128/JCM.00724-14>.
- Bertine, M., Gueudin, M., Méléard, A., Damond, F., Descamps, D., Matheron, S., et al., 2017. New highly sensitive real-time PCR assay for HIV-2 Group A and Group B DNA quantification. *J. Clin. Microbiol.* 55, 2850–2857. <https://doi.org/10.1128/JCM.00755-17>.
- Broyles, L.N., Luo, R., Boeras, D., Vojnov, L., 2023. The risk of sexual transmission of HIV in individuals with low-level HIV viraemia: a systematic review. *Lancet Lond. Engl.* [https://doi.org/10.1016/S0140-6736\(23\)00877-2](https://doi.org/10.1016/S0140-6736(23)00877-2).
- Bwana, P., Ageng'o, J., Danda, J., Mbugua, J., Handa, A., Mwau, M., 2019. Performance and usability of mPIMA™ HIV 1/2 viral load test in point of care settings in Kenya. *J. Clin. Virol. Publ. Pan Am. Soc. Clin. Virol.* 121, 104202 <https://doi.org/10.1016/j.jcv.2019.104202>.
- Chang, M., Gottlieb, G.S., Dragavon, J.A., Cherne, S.L., Kenney, D.L., Hawes, S.E., et al., 2012. Validation for clinical use of a novel HIV-2 plasma RNA viral load assay using the Abbott m2000 platform. *J. Clin. Virol.* 55, 128–133. <https://doi.org/10.1016/j.jcv.2012.06.024>.
- Desclaux, A., Ciss, M., Taverne, B., Sow, P.S., Egrot, M., Faye, M.A., et al., 2003. Access to antiretroviral drugs and AIDS management in Senegal. *AIDS Lond. Engl.* 17 (Suppl 3), S95–S101. <https://doi.org/10.1097/00002030-200317003-00013>.
- Dorward, J., Sookraj, Y., Ngobese, H., Lessells, R., Sayed, F., Bulo, E., et al., 2021. Protocol for a randomised feasibility study of Point-Of-care HIV viral load testing to Enhance Re-suppression in South Africa: the POWER study. *BMJ Open* 11, e045373. <https://doi.org/10.1136/bmjopen-2020-045373>.
- Ekouevi, D.K., Balestre, E., Coffie, P.A., Minta, D., Messou, E., Sawadogo, A., et al., 2013. Characteristics of HIV-2 and HIV-1/HIV-2 Dually Seropositive Adults in West Africa Presenting for Care and Antiretroviral Therapy: The IeDEA-West Africa HIV-2 Cohort Study. *PLOS ONE* 8, e66135. <https://doi.org/10.1371/journal.pone.0066135>.
- Ferns, R.B., Garson, J.A., 2006. Development and evaluation of a real-time RT-PCR assay for quantification of cell-free human immunodeficiency virus type 2 using a Brome Mosaic Virus internal control. *J. Virol. Methods* 135, 102–108. <https://doi.org/10.1016/j.jviromet.2006.02.005>.
- GENERIC HIV-2 Charge Virale. biocentric n.d. (<https://www.biocentric.com/generic-hiv-2-charge-virale>) (accessed November 11, 2022).
- Gueguen, M., Nicholas, S., Poulet, E., Schramm, B., Szumilin, E., Wolters, L., et al., 2021. Implementation and operational feasibility of SAMBA I HIV-1 semi-quantitative viral load testing at the point-of-care in rural settings in Malawi and Uganda. *Trop. Med. Int. Health TM IH* 26, 184–194. <https://doi.org/10.1111/tmi.13519>.
- Gueudin, M., Baron, A., Alessandri-Gradt, E., Lemée, V., Mourez, T., Etienne, M., et al., 2016. Performance evaluation of the new HIV-1 quantification assay, xpert HIV-1 viral load, on a wide panel of HIV-1 variants. *J. Acquir Immune Defic. Syndr.* 72, 521–526. <https://doi.org/10.1097/QAI.0000000000001003>.
- Guichet, E., Aghokeng, A., Serrano, L., Bado, G., Toure-Kane, C., Eymard-Duvernay, S., et al., 2016. Short communication: high viral load and multidrug resistance due to late switch to second-line regimens could be a major obstacle to reach the 90-90-90 UNAIDS objectives in Sub-Saharan Africa. *AIDS Res. Hum. Retrovir.* 32, 1159–1162. <https://doi.org/10.1089/AID.2016.0010>.
- Liégeois, F., Eymard-Duvernay, S., Boyer, S., Maradan, G., Kouanfack, C., Domyeum, J., et al., 2019. Heterogeneity of virological suppression in the national antiretroviral programme of Cameroon (ANRS 12288 EVOLCAM). *HIV Med.* 20, 38–46. <https://doi.org/10.1111/hiv.12681>.
- Manoto, S.L., Lugongolo, M., Govender, U., Mthunzi-Kufa, P., 2018. Point of care diagnostics for HIV in resource limited settings: an overview. *Med. Kaunas. Lith.* 54, E3 <https://doi.org/10.3390/medicina54010003>.
- Mariani, D., de Azevedo, M.C.V.M., Vasconcellos, I., Ribeiro, L., Alves, C., Ferreira, O.C., et al., 2020. The performance of a new point-of-care HIV virus load technology to identify patients failing antiretroviral treatment. *J. Clin. Virol. Publ. Pan Am. Soc. Clin. Virol.* 122, 104212 <https://doi.org/10.1016/j.jcv.2019.104212>.
- Max von Pettenkofer-Institut. <https://www.mvp.uni-muenchen.de/> (accessed July 6, 2023).
- Meggi, B., Bollinger, T., Zitha, A., Mudenyanga, C., Vubil, A., Mutsaka, D., et al., 1999. Performance of a true point-of-care assay for HIV-1/2 viral load measurement at antenatal and postpartum services. *J. Acquir Immune Defic. Syndr.* 87, 693–699. <https://doi.org/10.1097/QAI.00000000000002621>.
- Meloni, S.T., Agbaji, O., Chang, C.A., Agaba, P., Imade, G., Oguiche, S., et al., 2019. The role of point-of-care viral load monitoring in achieving the target of 90% suppression in HIV-infected patients in Nigeria: study protocol for a randomized controlled trial. *BMC Infect. Dis.* 19, 368 <https://doi.org/10.1186/s12879-019-3983-6>.
- Moyo, S., Mohammed, T., Wirth, K.E., Pragma, M., Bennett, K., Holme, M.P., et al., 2016. Point-of-care cepheid xpert HIV-1 viral load test in rural African communities is feasible and reliable. *J. Clin. Microbiol.* 54, 3050–3055. <https://doi.org/10.1128/JCM.01594-16>.
- m-PIMA™ HIV-1/2 Viral Load Test n.d. (<https://www.globalpointofcare.abbott/en/product-details/m-pima-hiv-1-2-viral-load.html>) (accessed October 28, 2021).
- Msimango, L., Gibbs, A., Shoji, H., Ngobese, H., Humphries, H., Drain, P.K., et al., 2020. Acceptability of point-of-care viral load testing to facilitate differentiated care: a qualitative assessment of people living with HIV and nurses in South Africa. *BMC Health Serv. Res.* 20, 1081 <https://doi.org/10.1186/s12913-020-05940-w>.
- Murray, T.Y., Sherman, G.G., Nakwa, F., MacLeod, W.B., Sipambo, N., Velaphi, S., et al., 2017. Field evaluation of performance of alere and cepheid qualitative HIV assays for pediatric point-of-care testing in an academic hospital in Soweto, South Africa. *J. Clin. Microbiol.* 55, 3227–3235. <https://doi.org/10.1128/JCM.01021-17>.
- Ndlovu, Z., Fajardo, E., Mbofana, E., Maparo, T., Garone, D., Metcalf, C., et al., 2018. Multidisease testing for HIV and TB using the GeneXpert platform: a feasibility study in rural Zimbabwe. *PLoS One* 13, e0193577. <https://doi.org/10.1371/journal.pone.0193577>.
- Nicholas, S., Poulet, E., Wolters, L., Wapling, J., Rakesh, A., Amoros, I., et al., 2019. Point-of-care viral load monitoring: outcomes from a decentralized HIV programme in Malawi. *J. Int AIDS Soc.* 22, e25387 <https://doi.org/10.1002/jia2.25387>.
- Phillips, A.N., Cambiano, V., Nakagawa, F., Ford, D., Apollo, T., Murungu, J., et al., 2016. Point-of-care viral load testing for Sub-Saharan Africa: informing a target product profile. *Open Forum Infect. Dis.* 3, ofw161 <https://doi.org/10.1093/ofid/ofw161>.
- RealTi A. WHO Prequalification of Diagnostics Programme 2020.
- Reid, S.D., Fidler, S.J., Cooke, G.S., 2013. Tracking the progress of HIV: the impact of point-of-care tests on antiretroviral therapy. *Clin. Epidemiol.* 5, 387–396. <https://doi.org/10.2147/CLEP.S37069>.
- Rodger, A.J., Cambiano, V., Bruun, T., Vernazza, P., Collins, S., van Lunzen, J., et al., 2016. Sexual activity without condoms and risk of HIV transmission in serodifferent couples when the HIV-positive partner is using suppressive antiretroviral therapy. *JAMA* 316, 171–181. <https://doi.org/10.1001/jama.2016.5148>.
- Swathirajan, C.R., Vignesh, R., Boobalan, J., Solomon, S.S., Saravanan, S., Balakrishnan, P., 2017. Performance of point-of-care Xpert HIV-1 plasma viral load assay at a tertiary HIV care centre in Southern India. *J. Med. Microbiol.* 66, 1379–1382. <https://doi.org/10.1099/jmm.0.000514>.
- Tang, N., Huang, S., Salituro, J., Mak, W.-B., Cloherty, G., Johanson, J., et al., 2007. A RealTime HIV-1 viral load assay for automated quantitation of HIV-1 RNA in genetically diverse group M subtypes A-H, group O and group N samples. *J. Virol. Methods* 146, 236–245. <https://doi.org/10.1016/j.jviromet.2007.07.003>.
- Une charge virale indétectable, une meilleure santé des personnes vivant avec le VIH. OMS Bur Régional Pour Afr 2022. (<https://www.afro.who.int/fr/countries/chad/news/une-charge-virale-indetectable-une-meilleure-sante-des-personnes-vivant-avec-le-vih>) (accessed July 10, 2023).
- Villa, G., Abdullahi, A., Owusu, D., Smith, C., Azumah, M., Sayeed, L., et al., 2020. Determining virological suppression and resuppression by point-of-care viral load testing in a HIV care setting in sub-Saharan Africa. *EClinicalMedicine* 18, 100231. <https://doi.org/10.1016/j.eclinm.2019.12.001>.
- Vojnov, L., Markby, J., Boeke, C., Harris, L., Ford, N., Peter, T., 2016. POC CD4 testing improves linkage to HIV care and timeliness of ART initiation in a public health approach: a systematic review and meta-analysis. *PLoS One* 11, e0155256. <https://doi.org/10.1371/journal.pone.0155256>.
- WHO publishes new clinical and service delivery recommendations for HIV prevention, treatment and care n.d. (<https://www.who.int/news/item/17-03-2021-who-publishes-new-clinical-recommendations-on-hiv-prevention-infant-diagnosis-antiretroviral-therapy-initiation-and-monitoring>) (accessed August 5, 2021).
- World Health Organization, 2016. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach, 2nd ed., World Health Organization, Geneva.