Performance of a True Point-of-Care Assay for HIV-1/2 Viral Load Measurement at Antenatal and Postpartum Services

Bindiya Meggi, MSc, Timothy Bollinger, MSc, Alcina Zitha, MSc, Chishamiso Mudenyanga, MPH, Adolfo Vubil, PhD, Dadirayi Mutsaka, BSc, Carina Nhachigule, BSc, Nedio Mabunda, MSc, Osvaldo Loquiha, MSc, Arne Kroidl, MD, and Ilesh V. Jani, PhD

Background: Timely viral load (VL) results during pregnancy and the postpartum period are crucial for HIV disease management and for preventing mother-to-child transmission. Point-of-care (POC) VL testing could reduce turnaround times and streamline patient management. We evaluated the diagnostic performance of the novel m-PIMA HIV-1/2 VL assay (Abbott, Chicago, IL) in Mozambique.

Setting: The study was conducted in prenatal and postpartum consultation rooms in 2 primary health care clinics. Sample collection and testing on m-PIMA were performed by trained nurses.

Methods: HIV-infected pregnant and postpartum women on antiretroviral treatment (ART) or ART naive were tested using both on-site m-PIMA POC and referral laboratory-based real-time VL assays. Linear regression analysis and Bland–Altman plots were used to calculate the agreement between both.

Findings: Correlation between venous blood plasma POC and plasma laboratory-based VL was strong ($r^2 = 0.850, P < 0.01$), with good agreement between the methods [overall bias 0.202 log copies/mL (95% CI: 0.366 to 0.772 log copies/mL)]. Using the threshold of 1000 copies/mL, which is used to determine ART failure, the sensitivity and specificity of the POC VL assay were 95.0% (95% CI: 91.6% to 97.3%) and 96.5% (95% CI: 94.2% to 98.0%), respectively. The correlation coefficient between the venous and capillary sample types was 0.983 ($r^2 = 0.966$).

Conclusions: On-site, nurse-performed POC VL testing is feasible and accurate in resource-limited primary health care settings. The operational challenge of plasma separation within clinics for POC testing was successfully overcome using minicentrifuges. The use of capillary blood could simplify the execution of the assay in a clinical environment.

Key Words: point-of-care testing, HIV, viral load

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INTRODUCTION

Capacity for HIV viral load (VL) testing in sub-Saharan Africa has increased considerably in recent years, driven by updated recommendations from the World Health Organization and a collective ambition to reach the global 90-90-90 HIV targets. However, most VL testing capacity remains centralized in high-throughput laboratories or semidecentralized reference hubs, both of which are dependent on sample referral and result delivery systems that do not permit same-day results return to patients or clinicians. Indeed, long turnaround times for HIV VL results have been documented across sub-Saharan Africa, with a 2016 study reporting a range of 28–50 mean days in 5 countries with low-income or lower-middle-income status. This suboptimal turnaround time is similar to those seen for other centralized testing programs such as early HIV infant diagnosis. A true POC testing solution that allows VL-informed clinical management during the same facility visit as specimen collection could help countries address gaps in their tiered laboratory systems and improve access to VL.

Point-of-care (POC) testing for HIV diagnosis and disease monitoring has been implemented with success in many resource-limited settings, with accurate performance, patient impact, and cost-effectiveness. Yet in settings with a large HIV burden, a POC device, deployed within a public primary health care facility, will unlikely be able to process the high demand for VL testing. It is, therefore, more feasible that higher-risk populations are given priority access to same-day results using POC testing, whereas less urgent VL requests are referred to off-site hubs or centralized laboratories. Such groups prioritized for POC VL might include patients with suspected treatment failure, children and adolescents, or pregnant and breastfeeding women. Timely VL results for this latter group are essential not only for the health of HIV-positive mothers but also for preventing mother-to-child transmission (PMTCT).

Several evaluations have already established the good performance of POC and near-POC technologies for HIV VL...
assays. Consensus is emerging that decentralized POC VL testing is highly accurate and will play an important role in improving access to this important test. Operationalization, however, is a key component of bridging the gap between technical performance and actual impact in practice. An important question for a true POC VL solution, whereby specimens are not referred but rather processed at the actual point of patient care, is how to efficiently obtain plasma yet retain a one-stop, single-consultation experience for patients and health care workers. Such an assessment requires a field evaluation that determines the performance of the technology in real-world conditions using the operator cadre and service delivery approach that reflect how the technology would actually be implemented in a setting such as Mozambique’s public health care system.

We therefore evaluated the performance of the true POC m-PIMA HIV-1/2 VL test (Abbott) deployed to antenatal and postpartum consultation rooms in primary health care facilities and operated by maternal and child health (MCH) nurses. We further conducted an unpowered substudy of the performance of capillary versus venous blood collection methods for the POC assay.

**METHODS**

**Study Design and Setting**

A cross-sectional study was performed at 2 primary health care facilities in Maputo City, Mozambique: Polana Caniço and 1st de Maio Health Centers. These health facilities were selected because of the proximity to both the research center where data were managed and the reference laboratory. A total of 699 women in the PMTCT cascade (pregnant and postpartum) had an onsite HIV VL measurement on a POC device by a nurse and a referred test on a laboratory-based automated instrument. Both operators (nurse and laboratory technician) were blinded to reciprocal results.

**Participants**

Pregnant and postpartum women aged 18 years or older and who had documented HIV infection were included in the study irrespective of being on ART. Exclusion criteria comprised any serious medical conditions that could disrupt the accuracy of normal laboratory testing and its interpretation; however, no participants met exclusion criteria. Three groups were considered in the following VL ranges: undetectable (<1000 copies/mL), 1000–10,000, and >10,000 copies/mL. Patients were targeted to represent one of the 3 groups based on their duration on antiretroviral treatment (ART) and suspected treatment failure. Treatment-naïve patients were included to ensure adequate enrollment into the highest VL range group (>10,000 copies/mL). Baseline VLs are not part of routine care in Mozambique, but for this study, baseline testing was performed to target patients for inclusion in the highest VL range group. At least 100 patients in each group were needed, and recruitment was intentionally targeted to attain these groups. In addition, 93 women were included in a capillary blood substudy.

**Study Procedures**

Blood collection and testing on the POC platform were performed by nurses in their consultation rooms. For each participant, venous blood was collected into 6 mL K2 EDTA tubes (Becton Dickinson, Franklin Lakes, NJ), and for capillary substudy, additionally 5–6 drops of finger capillary blood were collected into 0.5 mL EDTA microtainer tubes. A unique study identification number was assigned to each patient to link results from paired tests. Basic demographic data, including date of birth, sex, and date of ART initiation, were collected from all patients.

**Venous Blood POC VL Testing**

For venous blood POC testing, performed by MCH nurses, 0.5 mL of whole blood was transferred into labeled 0.5 mL EDTA microtainer tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 5000 rpm for 5 minutes for plasma separation using a minicentrifuge (myFuge Mini Centrifuge; Benchmark Scientific, Sayreville, NJ). Specifications for centrifugation were based on manufacturer recommendation. Each patient sample was centrifuged individually. Testing was performed during normal consultation hours between 8 AM and 3 PM. The minicentrifuges were provided by the study team and are not part of the POC manufacturer’s product offering. Fifty microliters of plasma were transferred into the m-PIMA HIV-1/2 VL cartridge (Abbott) using a Pasteur pipette (Table 1). The cartridge with the sample was immediately introduced into the m-PIMA analyzer (Abbott) that quantifies HIV-1 groups M/N and O or HIV-2 RNA. POC VL test results were obtained after approximately 70 minutes.

The lower limit of detection (LOD) of this test is 800 copies of viral RNA per mL, and the results are available in 4 categories: not detected, detected but below LOD (<800 copies/mL), detected between 800 and 1,000,000 copies/mL, and above 1,000,000 copies/mL (maximum displayed result).

**Capillary Blood POC VL Testing**

The capillary blood (5–6 drops) was directly collected by MCH nurses into labeled 0.5 mL EDTA microtainer tubes and centrifuged at 6000 rpm for 5 minutes for plasma separation using the study minicentrifuge. Fifty microliters of plasma were transferred into the m-PIMA HIV-1/2 VL cartridge (Abbott) using a Pasteur pipette (Table 1). The cartridge with the sample was immediately introduced into the m-PIMA analyzer (Abbott).

**DBS Laboratory VL Testing**

Dry blood spots (DBS; Whatman 903; GE Healthcare Biosciences, Pittsburgh, PA) were prepared from whole blood in K2 EDTA tubes according to the routine of Mozambique’s laboratory services for VL testing. VL testing of DBS was performed per Mozambican guidelines on the Cobas AmpliPrep/Cobas TaqMan 96 HIV-1 v2 (CAP/CTM) automated instrument (Roche Diagnostics, Branchburg, NJ) or on the Abbott RealTime HIV-1 system (Abbott) depending on
1.1 mL of frozen
50 μL of whole blood was transferred to a microtube. The microtube was centrifuged for 5 minutes for plasma separation. Using a minicentrifuge with fixed rotational speed of 6000 rpm

50 μL of capillary plasma
Nurse/consultation room
m-PIMA
5–6 free-fall drops of capillary blood collected directly into a microtube that was centrifuged for 5 minutes for plasma separation using a minicentrifuge with fixed rotational speed of 6000 rpm

1 spot of DBS (80 μL prepared from whole venous blood)
Laboratory technician/centralized molecular virology laboratory at the Instituto Nacional de Saúde
Cobas AmpliPrep/Cobas TaqMan 96 HIV-1 v2 (CAP/CTM) automated instrument or Abbott Real-Time HIV-1 system
DBS card was prepared by nurse using venous whole blood and sent to centralized laboratory for processing

1.1 mL of frozen venous plasma
Laboratory technician/centralized molecular virology laboratory at the Instituto Nacional de Saúde
Cobas AmpliPrep/Cobas TaqMan 96 HIV-1 v2 (CAP/CTM) automated instrument
5 mL of venous blood collected by the nurse was sent to centralized laboratory where it was centrifuged and the plasma was stored for batch testing

availability in the laboratory. Both instruments used 80 μL of whole blood from the DBS card (one spot) (Table 1). Results from DBS testing were returned to the health facilities using standard protocols for routine follow-up of participants.

Venous Blood Laboratory VL Testing
The remaining venous whole blood specimen was transported within 4 hours to the Instituto Nacional de Saúde’s HIV reference laboratory, where it was separated by centrifugation to produce plasma within 6 hours of collection. The plasma was stored at −80°C before being tested using the Roche CAP/CTM. Around 1.1 mL of frozen plasma was used for Roche CAP/CTM VL testing by a qualified laboratory technician (Table 1). The lower LOD of the Roche CAP/CTM assay is 20 and 400 copies/mL for plasma and DBS, respectively, whereas the LOD of the Abbott assay for DBS is 837 copies/mL.

Statistical Methods
Two-way contingency tables were used to summarize the data with the performance of the m-PIMA HIV-1/2 VL assay for clinical ART patient management. The performance was assessed by determining its sensitivity, specificity, and positive and negative predictive values for VLs for ART failure threshold of 1000 copies/mL compared with laboratory-based plasma assay as the reference.

Linear regression analysis (correlation coefficient and R-squared) and Bland–Altman plots (mean bias ± 1.96 SD) were used to assess agreement between VL counts of m-PIMA and laboratory-based plasma assay. To reduce skewness, POC and laboratory test results were log_{10} transformed. Undetectable VLs were assigned values of 1 copy/mL, and results below the LOD were assigned the value of the LOD to enable log transformation. For analysis purposes only, data above the LOD were used.

Data were analyzed using RStudio 2016 (Boson, MA) and Microsoft Excel 2011 v14.1.0 (Microsoft Co., Redmond, WA).

Quality Assurance
The POC VL results were not revealed to patients. Only DBS results, which comprise the current routine care at the health facility, were used for clinical management of patients. Operators of both POC and laboratory VL tests were formally trained on the respective technologies and were blinded to reciprocal results. The training on POC VL testing consisted of 5 days of both theoretical and practical sessions. Competency was assessed based on successful sample processing, device operation, results interpretation, user error rates below 5%, and confidence in using the platform. All operators were assessed as part of the training program. The reference laboratory successfully participates in an external quality assurance provided by the US Center for Disease Control and Prevention (Atlanta).

Ethical Considerations
This study was approved by the Mozambique’s National Health Bioethics Committee with the reference number 281/CNBS/2018. Written informed consent was obtained from each participant before conducting any study procedure.
RESULTS

Patient Demographics and Clinical Characteristics

From a total of 699 participants included in the study from September 2018 to April 2019, 692 met eligibility criteria (Fig. 1). Seventy-four percent were younger than 35 years [median age (interquartile range): 29 (25–34) years], and 55% were on ART for more than 1 year [median time on ART (interquartile range): 1.64 (0.24–4.65) years]. The distribution of VL results as measured by laboratory-based plasma testing ranged from undetectable to 2,454,892 copies/mL. VL below 1000 copies/mL was present in 62.7% (427/692) of patients; of these, 58.8% (251/427) were not detected, 19.9% (85/427) were detected below 20 copies/mL, and 21.3% (91/427) were detected between 20 and 1000 copies/mL. In the remaining patients, 8.7% (60/692) had detectable VL results between 1000 and 10,000 copies/mL, and 29.2% (202/692) had VL above 10,000 copies/mL (Table 2).

The testing error rate for m-PIMA was 4.3%. Because of the availability of plasma for repeat testing, only 0.4% of the patients did not get their final result. The errors were mainly due to software and cartridge insertion.

Performance of Venous Blood Plasma m-PIMA Compared With Plasma Laboratory-Based Technology

Valid results for both POC and plasma laboratory-based testing were available for 686 patients. One hundred two (23.3%) patients with a detectable VL by laboratory plasma-based testing [58 (56.9%) were below 20 copies/mL] had an undetectable VL by the m-PIMA VL assay. Conversely, 66 (26.5%) patients with an undetectable VL by laboratory plasma-based testing had a detectable VL by the m-PIMA VL assay [53 (80.3%) were below 800 copies/mL], resulting in a sensitivity to detect nucleic acids of 76.7% (95% CI: 72.4% to 80.5%).

The correlation coefficient for VL results generated by the 2 methods was 0.922 (95% CI: 0.902 to 0.939) and r² of 0.850 with a mean bias of 0.202 log copies/mL (95% LOA: −0.366 to 0.772 log copies/mL) (Figs. 2A, B). The venous blood plasma m-PIMA VL assay had a sensitivity of 95.0% (95% CI: 91.6% to 97.3%) and a specificity of 96.5% (95% CI: 94.2% to 98.0%) for identifying treatment failure using a threshold of 1000 copies/mL when compared with plasma laboratory testing (Table 3). The POC assay had a positive predictive value of 94.3% (95% CI: 90.8% to 96.8%) and a negative predictive value of 96.9% (95% CI: 94.8% to 98.4%) for identifying virological failure at a threshold of 1000 copies/mL (Table 3).

Agreement Between Viral Loads Obtained Using Venous and Capillary Blood Plasma on the m-PIMA Assay

To investigate agreement between venous and capillary blood plasma testing of m-PIMA assay, 93 patients were tested with both venous blood and capillary blood using the POC plasma assay. The correlation coefficient between the 2 sample types was 0.983 (95% CI: 0.963 to 0.992) with r² of 0.966; the mean bias was 0.021 log copies/mL (95% LOA: −0.233 to 0.276 log copies/mL) (Figs. 2C, D).

Performance of DBS Laboratory-Based Testing Compared With Plasma Laboratory-Based Testing

Valid results for both DBS and plasma laboratory-based testing were available for 687 patients. The correlation coefficient between the 2 testing methods was 0.741 (95% CI: 0.676 to 0.795) and r² of 0.549; the mean bias was 0.627 log copies/mL (95% LOA: −0.325 to 1.479 log copies/mL) (Figs. 2E, F). The DBS laboratory-based testing had a sensitivity of 78.2% (95% CI: 72.8% to 83.1%) and a specificity of 98.6% (95% CI: 97.0% to 99.5%) for identifying treatment failure using a threshold of 1000 copies/mL when compared with plasma laboratory testing.

### TABLE 2. Patient Viral Load Results by Range and Testing Modality

| No. (%) of Patients by Test | Conventional Plasma† | POC Venous | Conventional DBS‡ | POC Capillary |
|----------------------------|-----------------------|------------|-------------------|--------------|
| Total                      | 692 (100%)            | 692 (100%) | 692 (100%)        | 93 (100%)    |
| Viral load                 |                       |            |                   |              |
| Not detected*              | 251 (36.3%)           | 285 (41.2%)| 401 (57.9%)       | 46 (49.5%)   |
| <1,000†                    | 176 (25.4%)           | 141 (20.4%)| 78 (11.3%)        | 17 (18.2%)   |
| 1000–10,000                | 60 (8.7%)             | 88 (12.7%) | 90 (13.0%)        | 14 (15.1%)   |
| >10,000                    | 202 (29.2%)           | 175 (25.3%)| 121 (17.5%)       | 16 (17.2%)   |
| Errors/not available       | 3 (0.4%)              | 3 (0.4%)   | 2 (0.3%)          | 0 (0.0%)     |

*Includes viral load results equal to zero.
†Includes results below the limit of detection of each technology: m-PIMA POC = 800 cp/mL, Roche Plasma = 20 cp/mL, Roche DBS = 400 cp/mL, and Abbott DBS = 837 cp/mL.
‡Centralized laboratory-based testing.
§Errors for POC are those whose final results were designated as error because of repeated errors or not enough sample to repeat.
DISCUSSION

This study shows that accurate HIV VL measurement using POC technology is feasible at the primary health care level in resource-limited settings. POC testing in general has been shown to increase access to essential diagnostic services, improve turnaround time for results, and positively impact clinical outcomes of patients.9,10 This study shows that nurse operators were able to reliably provide same-day results for pregnant and breastfeeding women attending routine consultation services. Provision of timely VL results with POC for this demographic may improve the prevention of vertical transmission of HIV.

The POC VL assay assessed in this study had good agreement with the conventional plasma gold standard, with a low mean bias and high sensitivity and specificity. Like our previous evaluation of a beta version of this test that used whole blood instead of plasma,18 the sensitivity of the assay improves when analyzed at higher thresholds of VL. The primary threshold for clinical management used in Mozambique, based on the current WHO guidelines, is 1000 copies/mL.19 At this level, performance was good with a sensitivity of 95.0% and a specificity of 96.5%. Such performance is in line with other commonly used VL assays on the market.16,20 POC analytical performance was in fact superior to routine DBS when compared with the gold standard conventional plasma testing, with a correlation coefficient of 0.922 between POC and conventional plasma versus a correlation coefficient of only 0.741 between conventional DBS and conventional plasma. Also, at 1000 copies/mL threshold, POC had a higher specificity than DBS (95.0% vs 78.2%) showing that DBS would have missed patients not VL-suppressed compared with POC. POC technology failed to detect HIV RNA in 23.3% of patients who had detectable VLs by the conventional laboratory-based technology. This may be due to the small sample volume used for this POC assay (50 μL of plasma instead of the 1100 μL of plasma used in conventional plasma testing).

Operational considerations are important when considering the use of POC technologies. Many challenges—both real and perceived—with POC testing at scale have been addressed, and best practices have been widely circulated based on successful CD4 and early infant diagnosis (EID) POC networks.9,10 For the m-PIMA VL assay, centrifugation is required to obtain plasma, adding an additional step that could interfere with operationalization at scale. However, nurses showed ability to successfully centrifugate, and no issues were raised in the course of the study related to the minicentrifuges and the rapid plasma separation process. In addition, our comparison of capillary and venous blood provides promising evidence that an even more streamlined specimen handling process is feasible. Using finger stick collection could make the POC VL testing more comfortable and efficient by eliminating the steps and materials required for venous collection and pipetting.

Efficient utilization of POC deployments is considered paramount to fully capitalize on investments in decentralized diagnostic networks. The m-PIMA analyzer has played an important role in Mozambique’s tiered laboratory network since 2016, deployed initially for EID. The addition of VL to the POC testing menu would not only ensure optimized use of POC instruments already placed at primary health care facilities but also fit seamlessly into the PMTCT cascade where mothers and children can access essential POC VL and EID tests in the same setting, with the same multiplexing instrument, by the same nurse operator, and during the same consultation. Other use cases may be equally instrumental in an HIV control program, such as using POC VL for management of potential treatment failure and optimizing the switch to second-line or third-line ART regimens.

This study has limitations. The decision to conduct the study within the PMTCT cascade provided valuable insights around feasibility of POC VL in primary health care facilities, but may not be readily generalized to other settings that use a different operator cadre or deployment approach. Although the benefits of POC testing have been well established,
additional research may be helpful to quantify the clinical impact of POC VL. The different biomarkers and lower limits of detection make a direct comparison challenging, as seen in the suboptimal visuals in Figure 2A, B. However, these detection limits all fall below the threshold of 1000 copies/mL currently used for clinical management in this setting. If this

TABLE 3. Results of Viral Load Testing With the Venous Blood Plasma m-PIMA HIV-1/2 Viral Load Test Compared With Reference Plasma Laboratory Testing Using the Roche CAP/CTM at Different Thresholds

| Threshold | TP    | FN    | TN    | FP    | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|-----------|-------|-------|-------|-------|-----------------|-----------------|---------|---------|
| Not detected | 335   | 102   | 183   | 66    | 76.7            | 73.5            | 83.5    | 64.2    |
| 800        | 225   | 8     | 398   | 25    | 97.0            | 94.1            | 91.1    | 98.0    |
| 1000       | 248   | 13    | 410   | 15    | 95.0            | 96.5            | 94.3    | 96.9    |
| 10,000     | 168   | 33    | 478   | 7     | 83.6            | 98.6            | 96.0    | 93.5    |

FN, false negative; FP, false positive; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.
threshold is changed, then manufacturers of this and other VL testing platforms may need to reassess their specifications around lower limits of detection.

Finally, the scope of this study is limited to a true POC solution using a device that can be deployed within consultation rooms, as opposed to near-POC technologies that are ubiquitous but are often deployed into laboratories and therefore require additional steps, personnel, time, and resources to deliver results. Other studies have looked at the performance of near-POC technologies and found similarly strong evidence around accurate performance.8,14

Our study therefore contributes to a growing body of evidence that justifies further research and the scale-up of POC VL in resource-limited settings, whereas adding an important piece: a true POC solution that can be operated by nurses with either capillary or venous blood collection and achieve a result in 70 minutes. Innovations such as these are required to turn the needle on progress toward the 90-90-90 UNAIDS target.

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