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Accuracy and Operational Feasibility of Point-of-Care (PoC) Viral Load Monitoring of HIV-positives mothers at delivery in the context of HIV vertical transmission reduction in Mozambique

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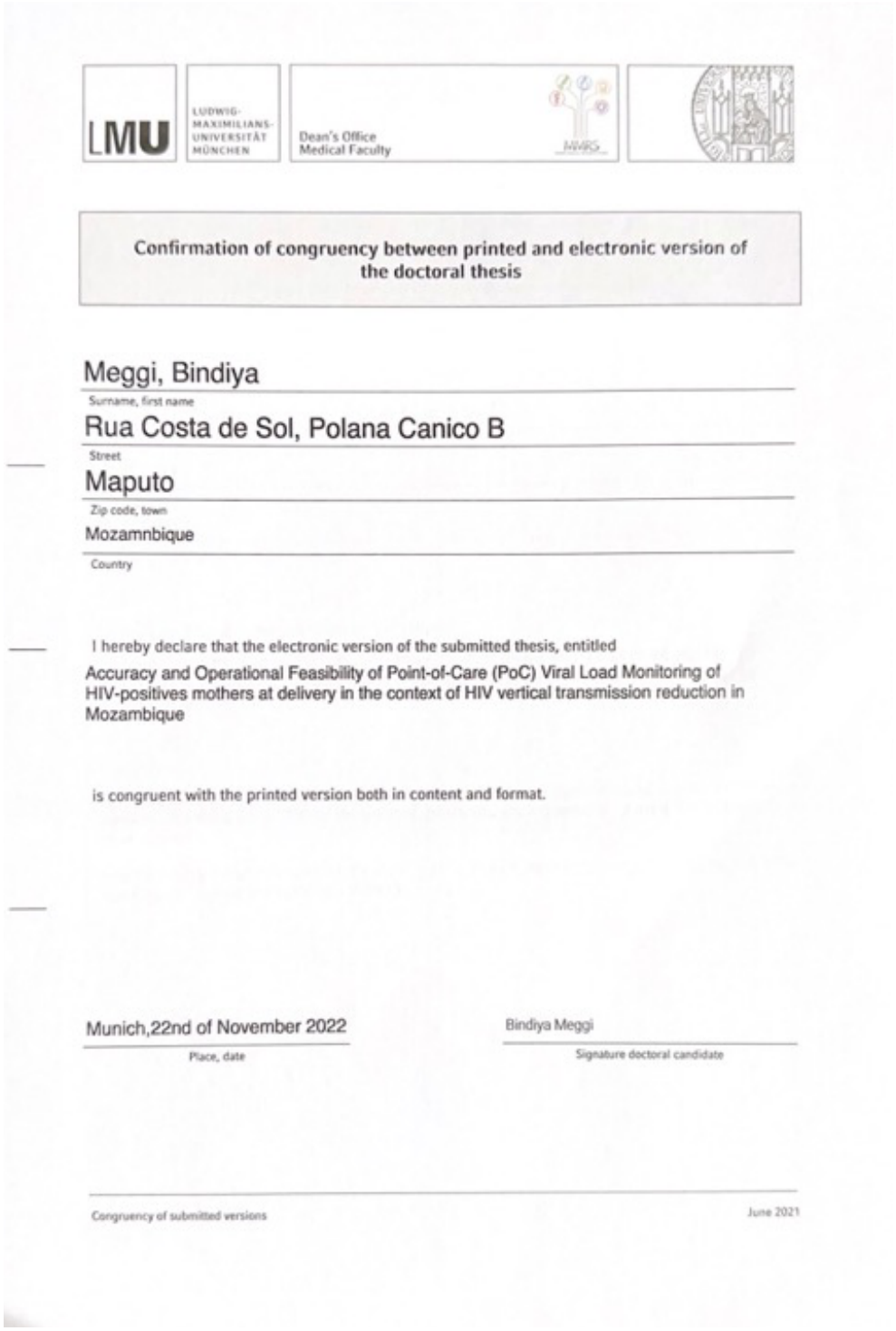

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Key Words

HIV transmission

Point-of-Care

Viral load

Diagnostic accuracy

Abstract

Background: Many resource-limited countries face challenges in implementing HIV viral load testing within their public health programs due to suboptimal laboratory infrastructure and limited human resources. HIV point-of-care viral load (PoC VL) testing in pregnant and breastfeeding women could provide an opportunity for faster identification and management of virologic failure in mothers, which in turn may contribute to higher effectiveness in preventing mother-to-child transmission. The objective of this research project was to describe the diagnostic accuracy, feasibility and usability of PoC VL for pregnant and breastfeeding women in primary health care clinics in southern and central region of Mozambique.

Methods: HIV-infected pregnant and postpartum women were included in the first cross-sectional study. Each participant was tested using both on-site m-PIMA PoC VL and referral laboratory-based VL assays. In the second study, mother/child pairs were recruited in maternity wards in 14 primary health facilities. Half of those mothers were tested with PoC VL at delivery (Intervention Arm). The other half (Control Arm) saw samples collected and sent to the central laboratory for referral viral load testing. Three months post-delivery, all mothers had a viral load performed (laboratory based or PoC). Linear regression analysis and Bland-Altman plots were used to describe diagnostic accuracy in the first study and generalized linear mixed-effects models were used for to account for clustered data in the second study.

Results: The sensitivity and specificity of the m-PIMA PoC VL assay were 95.0% (95% CI: 91.6-97.3%) and 96.5% (95% CI: 94.2-98.0%), respectively at a threshold of 1,000 copies/mL. In the intervention arm, 1906 (92.7%) of women had a viral load processed via PoC VL on site by nurses and of which 1891 (99.2%) results were communicated to the patients on the same day. There was no effect of PoC VL (intervention arm) in terms of viral suppression at week 12 [OR 1.25 (95% CI: 0.86-1.82); p=0.235] nor in transmission rate at by week 12 in the intervention arm compared to the control arm [1.69 (95% CI: 1.11-2.26) versus 1.49 (95% CI: 0.92 -2.05)].

Conclusions: M-PIMA PoC VL is accurate and operationally feasible in maternity wards of primary healthcare settings in Mozambique. Nevertheless, having the viral load result available might not be sufficient to have an impact on maternal viral suppression rate or in transmission rates at 12 weeks post-delivery. Other operational aspects should be considered such as quality of adherence

counselling and social support for improved adherence and early second line regimen switches to see a greater impact of PoC viral load monitoring at delivery.

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AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral Treatment
CISPOC	Centro de Investigação em Saúde de Polana Caniço
EID	Early Infant Diagnosis
EMTCT	Elimination of Mother to Child Transmission
DBS	Dried Blood Spot
DTG	Dolutegravir
HF	Health Facility
HIV	Human Immunodeficiency Virus
INS	Instituto Nacional de Saúde
LMIC	Low-Middle Income Countries
LOD	Limit of Detection
MCH	Mother and Child Health
MTCT	Mother-to-child transmission
NNRTI	Non-Nucleoside reverse transcriptase inhibitors
NRTI	Nucleoside reverse transcriptase inhibitors
PoC	Point-of-Care
PoC VL	Point-of-Care Viral Load
RNA	Ribonucleic acid
UN	United Nations
UNAIDS	Joint United Nations Programme on HIV/AIDS
VL	Viral Load
WHO	World Health Organization

1. Introduction

Despite many years having passed since its beginning, the HIV pandemic is still a public health issue around the world and specially in sub-Saharan Africa. In 2020, 1.5 million people were newly infected with HIV and of those 60% were in sub-Saharan Africa. In this region, 25% of HIV infections where in adolescent girls and young women (aged 15-24 years). Children below 15 years accounted for 150,000 new infections. Those children were mainly infected through mother-to-child transmission (MTCT) (1). The global 90-90-90 targets[1] set for 2020 have helped the world to make progress in testing and treatment, but those targets were not equally met worldwide. Due to the global roll out of treatment, the AIDS-related deaths reduced have by 47% since 2010 (1).

Since 2016, WHO (World Health Organization) has recommended lifelong antiretroviral treatment (ART) regardless of CD4 count for all HIV infected persons including HIV-infected pregnant and breastfeeding women. This not only increases quality of life for women but also has an important impact in preventing of mother-to-child transmission which can occur either during pregnancy, labour and delivery or during breast feeding (2).

Initiating a lifelong treatment requires a good monitoring. This is crucial to identify adherence issues and treatment regimen switches needed in the event of treatment failure. Good monitoring also helps patients to have control over their care and provides motivation to continue to adhere to the treatment (3).

The use of viral load (VL) testing as the preferred approach to monitoring ART and identifying treatment failure has been recommended since 2016 (2,3). Since then, many low and middle-income country programmes have worked towards adopting routine viral load testing for treatment monitoring. Because of this, for the first time testing volumes in lower and middle income countries (LMIC) passed a 20 million tests milestone for viral load in 2019, with a global coverage of 70% (4). Many strategies have been promoted to improve access for HIV viral load monitoring, including scaling up laboratory capacity and strengthening specimen referral networks. However, many challenges remain: inadequate access, limited infrastructure, lack of skilled human resources, long turnaround times for results and imperfect clinical utilization of them (3).

[1] The 90–90–90 targets are: 90% of people living with HIV know their HIV status, 90% of people who know their HIV-positive status are accessing treatment and 90% of people on treatment have suppressed viral loads.

The UNAIDS has set a new target 95-95-95 for 2025. This means 95% of people living with HIV know their HIV status, 95% of people who know their HIV-positive status are accessing treatment and 95% of people on treatment have suppressed viral loads. In order to reach the third 95%, the viral load monitoring is crucial. The benefit of VL monitoring is influenced by the effective turn-around time from specimen collection to result return, as well as the intervention strategies prescribed for patients with an elevated viral load. Point-of-care VL (PoC VL) testing could effectively eliminate turnaround times (5). WHO conditionally recommends the use of PoC VL testing to monitor ART treatment success. The guidelines also identify six priority populations most likely to receive the greatest benefit from rapid VL results return, including pregnant and breastfeeding women (3). They also recommend that viral load testing should be performed in all pregnant women at 34-36 weeks of gestation or at least at delivery. For all breastfeeding women, a viral load test is recommended three months after delivery and every six months. Women considered high risk for HIV vertical transmission are those with a viral load above 1000 copies/mL. If that is the case, the infant should be tested immediately after birth (already in the maternity ward, if possible) and given enhanced postnatal prophylaxis (3).

Currently there are two WHO prequalified commercial PoC products for HIV viral load monitoring available: Cepheid GeneXpert (Cepheid, Sunnyvale, CA) and Abbott m-PIMA (Abbott, Chicago, IL) (6,7).

To date, there is limited data on how on-site same-day PoC VL testing could be implemented for pregnant and breastfeeding mothers and what the impact would be for this high priority group. In this research we aim to describe the accuracy, operational feasibility and usability of PoC VL at maternity wards performed by nurses in primary healthcare facilities in south and central region of Mozambique.

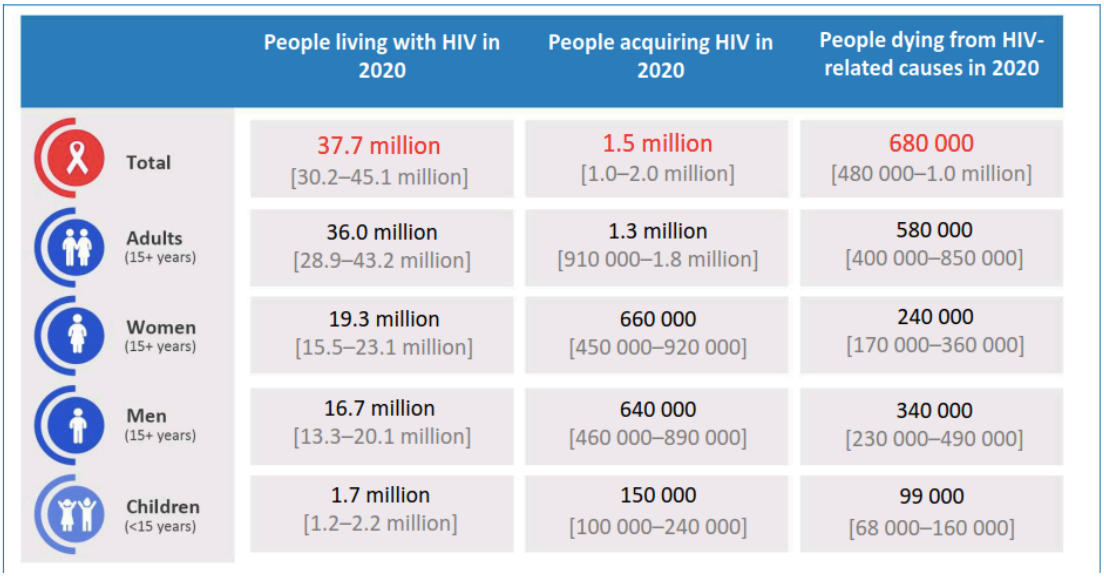
[2].Dual regimen of AZT and NVP up to 12 weeks

1.1 Literature Review

1.1.1 HIV global burden

Even four decades after the virus was first isolated, HIV continues to be the deadliest pandemic of the modern era. Around 36 million people have died from AIDS-related illness until now (1).

In 2020, 1.5 million [1.0 million–2.0 million] new HIV infections and 680 000 [480 000–1 000 000] deaths from AIDS-related causes have occurred. There were 37.7 million [30.2 million–45.1 million] people living with HIV by end of 2020. From those 19.3 million were woman (15+age) (1). (Figure 1)



	People living with HIV in 2020	People acquiring HIV in 2020	People dying from HIV-related causes in 2020
Total	37.7 million [30.2–45.1 million]	1.5 million [1.0–2.0 million]	680 000 [480 000–1.0 million]
Adults (15+ years)	36.0 million [28.9–43.2 million]	1.3 million [910 000–1.8 million]	580 000 [400 000–850 000]
Women (15+ years)	19.3 million [15.5–23.1 million]	660 000 [450 000–920 000]	240 000 [170 000–360 000]
Men (15+ years)	16.7 million [13.3–20.1 million]	640 000 [460 000–890 000]	340 000 [230 000–490 000]
Children (<15 years)	1.7 million [1.2–2.2 million]	150 000 [100 000–240 000]	99 000 [68 000–160 000]

Figure 1- Summary of the global HIV epidemic, 2020
Source: UNAIDS/WHO estimates 2021 (8)

From the total daily new infections in 2020, 60% were in sub-Saharan Africa and 90% were among adults aged 15 years and above. From these 51% are among woman of all age groups and 20% among young women (15-24 years). Women are biologically more susceptible to HIV but gender inequalities, restrictive access to care and sexual violence contribute to aggravate the scenario (1,9).

Many efforts have been made to reduce mortality and transmission of HIV and some progress has been seen. In 2020, there was a 47% decline in AIDS-related death compared to 2010. But there was only 31% of decline in global new infections compared to 2010, far behind what was the target for both indicators for the year 2020 (75%). Nevertheless, a notorious reduction in new infections has been seen in sub-Saharan Africa and the Caribbean (1).

In 2016, the United Nations (UN) general assembly adopted the 90-90-90 global targets for 2020. Although globally these targets were not achieved (Figure 2), 8 countries reached all the targets and 11 reached the target of 73% of viral suppression in people living with HIV. This showed that targets were achievable and possible in a diversity of settings (1).

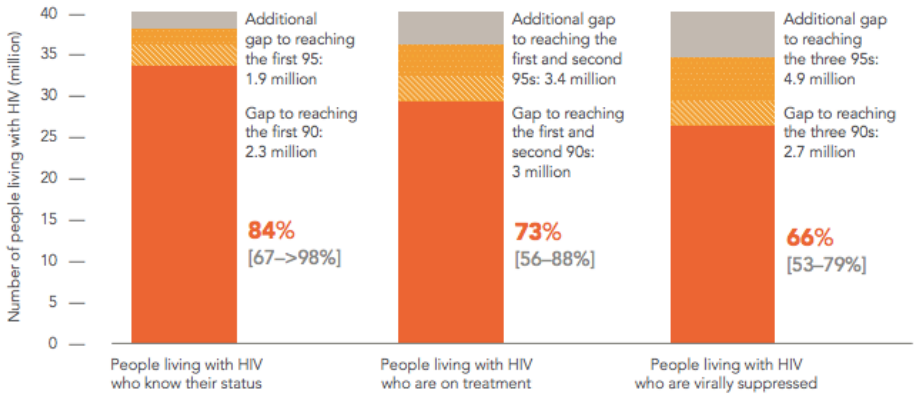


Figure 2- HIV Testing and Treatment cascade, global, 2020
Source: UNAIDS data 2021 (1)

Since 2016, the majority of the countries has adopted the "treat-all" policy suggested by WHO and that has strongly increased the number of HIV positive people that are on treatment (2). In 2020, 73% [56–88%] of all people living with HIV were accessing treatment, including 85% [63– >98%] of pregnant women living with HIV (10). The recommended preferred first-line regimen for adults (including for pregnant and breastfeeding woman) and adolescents is Dolutegravir (DTG) in combination with an NRTI backbone (3). For those DTG-based regimen is failing, the preferred second-line regimen is boosted protease inhibitors in combination with an optimized nucleoside reverse-transcriptase inhibitor backbone (Table 1).

Table 1:Summary of treatment options for first-line, second line and third-line ART regimens for adults, adolescents and children

Populations	First-line regimen	Second-line regimen	Third-line regimen
Adults and adolescents	Two NRTIs + DTG	Two NRTIs + ATV/r (or LPV/r)	DRV/r[a] + 1–2 NRTIs ± DTG[b] Optimize the regimen using a genotype profile (if LPV is used in second-line ART)
		Two NRTIs + DRV/r	Optimize the regimen using a genotype profile
	Two NRTIs + EFV	Two NRTIs + DTG	Two NRTIs + (ATV/r, DRV/r or LPV/r) ± DTG[b]
Children	Two NRTIs + DTG	Two NRTIs + LPV/r (or ATV/r[c])	DRV/r[a,d] + 1–2 NRTIs ± DTG[b,e] Optimize the regimen using a genotype profile for children younger than three years
	Two NRTIs + LPV/r	Two NRTIs + DTG	DRV/r[a,d] + 1–2 NRTIs ± DTG[b,e] Optimize the regimen using a genotype profile for children younger than three years
	Two NRTIs + NNRTI	Two NRTIs + DTG	Two NRTIs + (ATV/r, LPV/r or DRV/r[d]) ± DTG[e]

[a] 600/100 mg twice daily.
[b] 50 mg twice daily.
[c] Boosted PI.
[d] DRV cannot be used for children younger than three years.
[e] For age and weight groups with approved DTG dosing (<20 kg).

Source: Consolidated Guidelines On HIV Prevention, Testing, Treatment, Service Delivery And Monitoring: Recommendations For a Public Health Approach (3)

The new targets set for 2030 by United Nations, 95-95-95 are challenging specially for the third 95. With these targets, we expect that 95% of people living with HIV know their status, 95% of people who know their status are receiving treatment, and 95% of people on HIV treatment have a suppressed viral load. Countries should put in place strategies to be able to reach these targets even though many did not even reach the 90-90-90 target for 2020 (11).

In order to support countries achieving the 95-95-95 targets, the UNAIDS Global AIDS Strategy 2021-2026, included AIDS targets for 2025. This targets included targets for services, integration and societal enablers (Figure 3) .

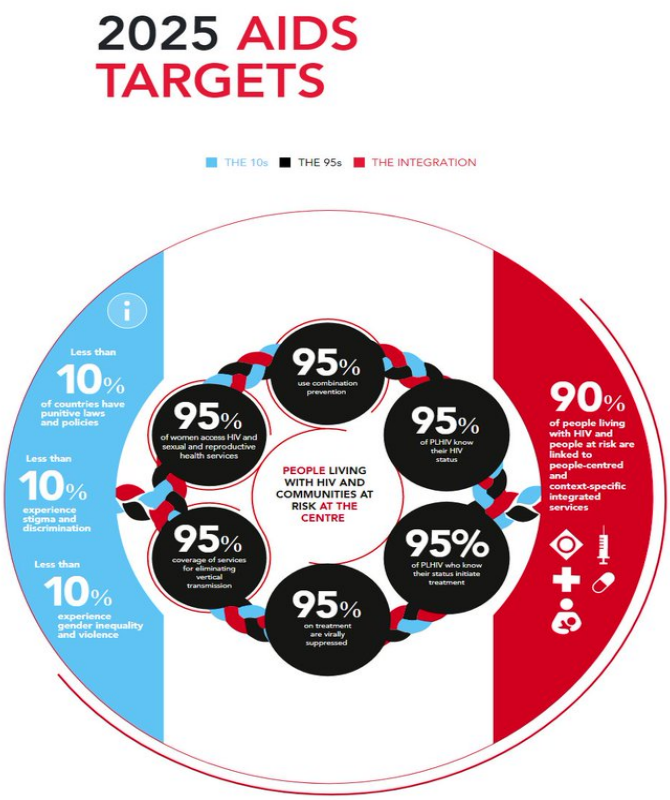


Figure 3-2025 AIDS targets: the next generation of goals for the global AIDS response

Source: aidstargets2025.unaids.org

1.1.2 HIV Mother-to Child transmission

The target of 20 000 new HIV infections in children resulting from mother-to-child transmission (MTCT) for 2020 was globally missed. Although there was a drop in these infections from 190 000 in 2015 to 150 000 in 2020, it was far behind from the ambitious target (1). This decline was mainly due to increased coverage of treatment for pregnant and breastfeeding woman. Without treatment approximately 15–30% of infants will become infected with HIV during gestation and delivery and 5–15% breastfeeding (12).

In 2020, 89 % of new HIV paediatric infections and 88 % of children and adolescents living with HIV worldwide were from sub-Saharan Africa (1).

It is estimated that 1.3 million women and girls living with HIV become pregnant each year (13). In 2020, 85% of these women and girls had access to ART to prevent MTCT(1). But this high ART coverage levels did not reflect that they maintained in treatment throw out the transmission period. This transmission period includes pregnancy, delivery and breastfeeding.

The elimination of mother-to-child transmission requires a full integration of the prevention interventions into maternal, newborn child and adolescent health services. The high coverage of treatment is not the only player in this setting. The actions should be focusing in preventing HIV infections among woman, testing and treating for HIV and maintaining them suppressed.

The final diagnosis of an HIV-exposed child is done at 18 months of age or 2 weeks after cessation of breastfeeding. This means the mother should be retained in care and maintained suppressed by then to eliminate the transmission.

To recognize the effort made by High-Burden Countries toward elimination of mother to child transmission (EMTCT) of HIV and Syphilis, WHO provided guidance on developed criteria to measure the progress on the path of elimination. The updated guidance describes the impact and process indicators for achieving tiers on the path of elimination (14) (Figure 4).

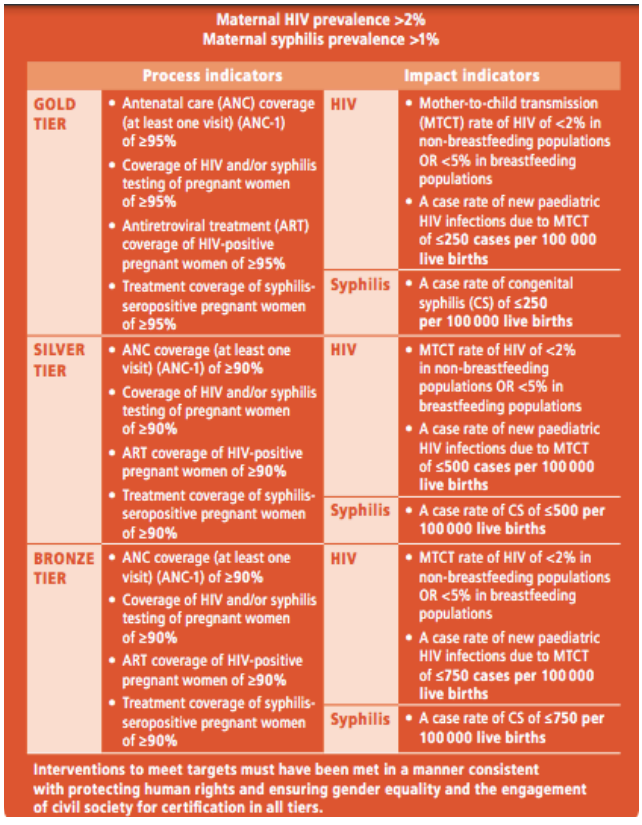


Figure 4-Indicators for certification on the Path to Elimination of MTCT of HIV and/or syphilis (high-prevalence countries)

Source: Criteria and processes for validation: EMTCT of HIV and Syphilis 2017 (14)

Worldwide, 15 countries have been certified for eliminating mother-to-child HIV transmission. In 2021, Botswana became the first high-burden country to be certified for achieving the “silver tier”

status by the World Health Organization (WHO). This is very encouraging for other Sub-Saharian countries to achieve EMTCT of HIV.

1.1.3 HIV Treatment Monitoring

The main objective of highly active antiretroviral therapy (HAART) is to reduce the plasma viral load below a certain threshold and reinstitute and safeguard the immunological function. Achieving this means that the quality of life of the patient is improved and the risk of transmission reduced (2,15). HIV treatment monitoring is very crucial to know whether treatment is working or not. In case of failure, it will also help to identify adherence issues or the need to change the ART regimen (3). Treatment failure can be identified based on clinical, immunological, or virological criteria.

Since 2016, WHO has strongly recommended the use of viral load testing as the preferred approach to monitor HIV treatment over the clinical and immunological approach. Viral load testing enables an early and accurate identification of treatment failure and reduces the likelihood of increased drug resistance mutations (2).

An updated treatment monitoring algorithm was developed and recommended by WHO in 2021 (Figure 5). This algorithm should help early detection of treatment failure and identification of those needing to switch to second line ART. The main changes in this new algorithm are:

- Timing of the first viral load: the first viral load result should be available by 6 months after ART initiation.
- Timing of repeated viral load after elevated viral load: the second viral load test should be done three months following a first elevated viral load, early enough to prevent possible selection of drug resistance and transmission of drug-resistant virus.
- Immediate (based on a single viral load result) switching for those receiving NNRTI-based regimens, considering that these regimens carry a high resistance profile in LMIC.
- Treatment failure threshold: anyone with a viral load above 1000 copies/mL three months after a first viral load result equally above 1000 copies/mL is considered a treatment failure. Those with low-level viremia (50–1000 copies/mL) are considered at risk of failing and need to be closely supervised.

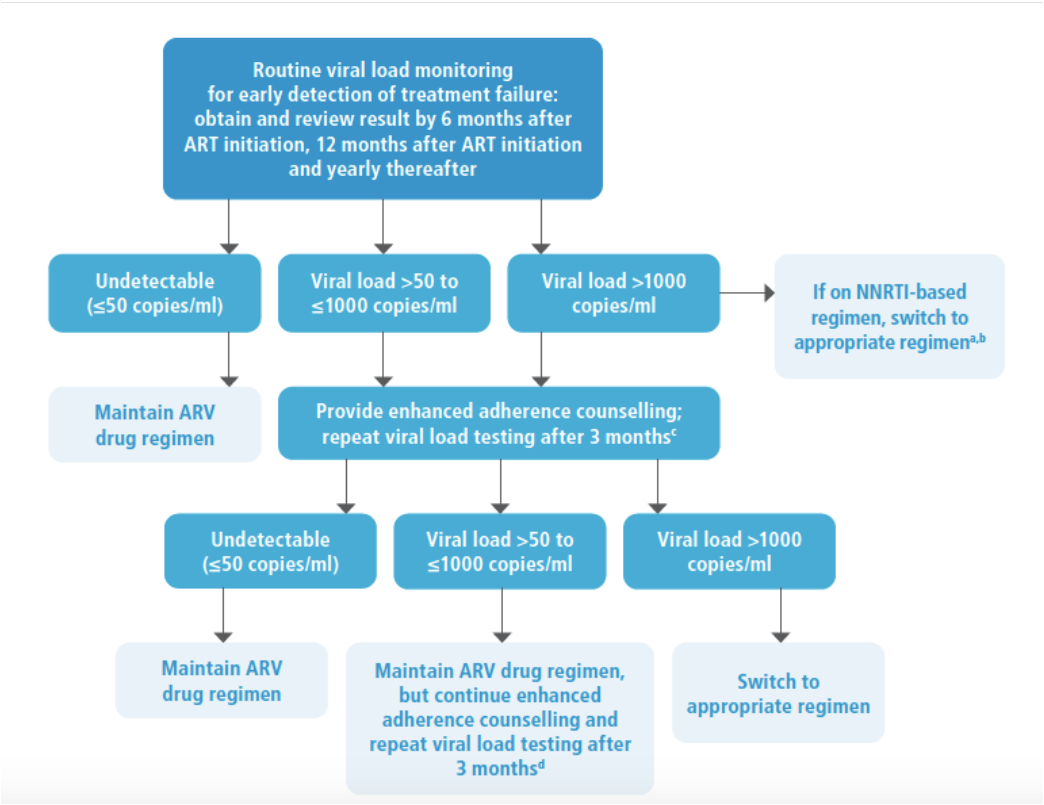


Figure 5- HIV Treatment Monitoring Algorithm

Source: Consolidated Guidelines On HIV Prevention, Testing, Treatment, Service Delivery And Monitoring: Recommendations For a Public Health Approach (3)

For pregnant and breastfeeding women, WHO recommends that, regardless of ART initiation timing, viral load testing should be performed at 34-36 weeks of gestation or at latest at delivery.
If at this time, the viral load is above 1000 copies/mL, the women are considered at high risk and enhanced prophylaxis should be given to the infant. If possible, a nucleic acid testing at birth should be done.
For those pregnant women who were on ART before conception, viral load testing should be done at first ANC visit. For those who started ART during pregnancy, the viral load should be measured three months after treatment initiation. In both cases where viral load is above 1000 copies/mL, nucleic birth testing of the infant should be considered.
For all breastfeeding women, viral load should be measured three months after delivery and every six months thereafter.

In 2019 for the first time, the number of viral load tests conducted by LMIC passed 20 million and represented a global coverage of 70% (4) (Figure 6).

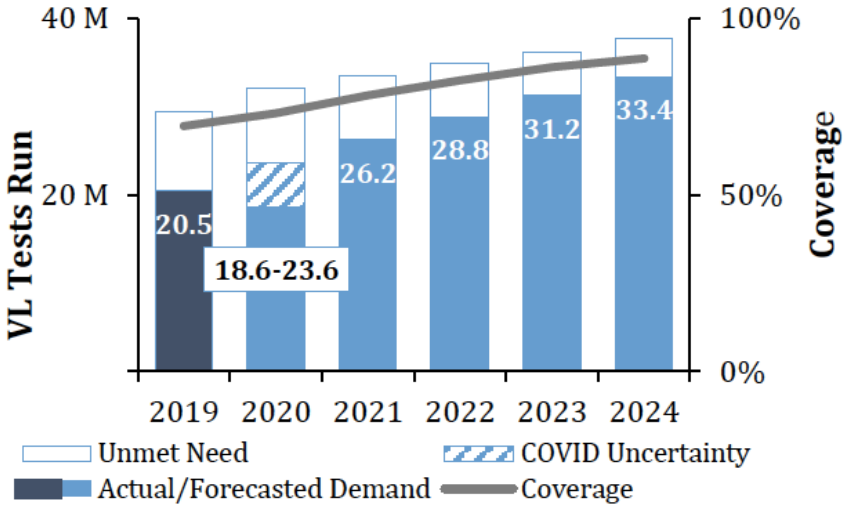


Figure 6- LMIC Viral Load Demand Forecast

Source: CHAI Market Report 2020 (4)

Many LMIC have made tremendous effort in implementing viral load testing for HIV monitoring. To expand the access, they rely on dried blood spot or point-of-care technologies.

The diagnostic accuracy of these alternatives viral load methods varies and not all has good performance at lower treatment failure thresholds (Table 2).

Table 2: Diagnostic accuracy of alternative sample types or point of care technologies at lower thresholds

Sensitivity (copies/mL)	Abbott 1-spot[a]	Abbott 2-spot[a]	Biocentric	bioMerieux[b]	Hologic[c]	Roche FVE[d]	Roche SPEX[d]	Siemens	Cepheid
1000	88 (50–98)	93 (84–97)	95 (71–99)	83 (78–87)	85 (44–98)	95 (85–98)	98 (96–99)	91 (69–98)	96 (95–97)
800	92 (5–100)	93 (83–97)	99 (44–100)	85 (80–89)	93 (31–100)	95 (87–98)	99 (96–100)	91 (75–97)	97 (96–98)
600	93 (0–100)	93 (84–97)	99 (60–100)	89 (84–92)	95 (28–100)	94 (84–98)	99 (96–100)	93 (84–97)	97 (96–98)
500	93 (0–100)	93 (84–97)	98 (67–100)	89 (85–92)	95 (29–100)	93 (82–98)	99 (96–100)	97 (66–100)	97 (96–98)
400	94 (0–100)	92 (84–97)	98 (60–100)	90 (86–93)	95 (28–100)	92 (81–97)	99 (95–100)	97 (63–100)	96 (95–97)
200	97 (0–100)	91 (83–95)	98 (65–100)	89 (84–93)	95 (22–100)	89 (76–96)	99 (95–100)	98 (72–100)	95 (93–97)
Detectable	93 (63–99)	93 (76–98)	98 (60–100)	88 (75–95)	75 (52–90)	97 (58–100)	99 (95–100)	90 (84–94)	93 (88–96)

Specificity (copies/mL)	Abbott 1-spot[a]	Abbott 2-spot[a]	Biocentric	bioMerieux[b]	Hologic[c]	Roche FVE[d]	Roche SPEX[d]	Siemens	Cepheid
1000	99 (68–100)	91 (82–96)	55 (35–74)	95 (89–98)	73 (31–94)	94 (72–99)	48 (23–75)	88 (75–94)	97 (93–98)
800	99 (24–100)	92 (83–96)	38 (11–76)	96 (91–98)	72 (42–90)	93 (65–99)	38 (13–70)	87 (68–95)	97 (93–99)
600	99 (12–100)	93 (81–97)	28 (6–71)	95 (91–97)	89 (50–99)	93 (68–99)	33 (12–65)	79 (61–90)	96 (92–98)
500	99 (9–100)	93 (82–98)	24 (4–68)	95 (91–98)	89 (50–98)	92 (68–98)	30 (10–62)	66 (31–89)	95 (90–98)
400	99 (8–100)	93 (80–98)	11 (1–73)	96 (91–98)	88 (48–98)	92 (68–98)	28 (9–60)	65 (25–91)	96 (93–98)
200	99 (5–100)	97 (92–99)	15 (1–70)	93 (89–95)	81 (72–89)	92 (71–98)	25 (8–58)	65 (26–90)	98 (95–99)
Detectable	93 (66–99)	79 (8–99)	19 (5–51)	93 (90–96)	87 (67–96)	58 (6–97)	4 (0–54)	69 (41–88)	81 (65–90)

[a] Abbott RealTime HIV-1.
[b] bioMerieux NucliSENS® EasyQ® HIV-1 v2.0
[c] Hologic Aptima HIV-1 Quant Dx Assay.
[d] Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, version 2.0

Source: Consolidated Guidelines On HIV Prevention, Testing, Treatment, Service Delivery And Monitoring: Recommendations For a Public Health Approach (3)

1.1.4 Point-of-Care Viral Load

Molecular diagnostic laboratories require high levels of infrastructure, equipment and technical expertise. Such laboratories are not easy to establish and run. There are now new PoC viral load technologies becoming available that will enable measurement of viral load in HIV patients within minutes or a couple of hours on site in the clinic, operated by non-technical staff and without laboratory infrastructure. These PoC viral load technologies may be useful to implement in settings with basic infrastructure or for use in specific populations that need rapid results more urgently.

WHO conditionally recommends the use of PoC VL testing to monitor ART treatment success. The guidelines also identify six priority populations most likely to receive the greatest benefit from rapid VL result return; pregnant and breastfeeding women are considered one of these priority populations (3).

Point-of-Care VL testing at birth would provide an opportunity to test women shortly after delivery and the results would inform actions toward reducing the risk of vertical transmission.(16) These actions could be to reinforce enhanced adherence or switch to a different treatment regimen for the mother and enhanced prophylaxis for the exposed infant.

Currently there are two WHO prequalified PoC products for HIV viral load monitoring: Cepheid GeneXpert (Cepheid, Sunnyvale, CA) and Abbott m-PIMA (Abbott, Chicago, IL) (6,7). Both of these technologies require plasma separation from whole blood and both platforms are currently being used for other diagnostic purposes such as TB (GeneXpert) and early infant HIV diagnosis (m-Pima and GeneXpert). There are differences in terms of operational characteristics of both technologies regarding power supply, sample volume and limit of detection threshold (Table 3).
Cepheid GeneXpert was one of the first PoC VL products to be prequalified and many studies have shown its diagnostic accuracy. A recent systematic review on Cepheid GeneXpert implementation studies showed a pooled sensitivity of 96.5% (95% CI: 95.1–97.5) and pooled specificity of 96.6% (95% CI: 92.9–98.4) for a threshold of 1000 copies/mL. The mean bias was 0.04 log copies/mL (17).
The diagnostic accuracy of Abbott m-PIMA has only been evaluated more recently and only two studies are available. Both of them showed good concordance of results and accuracy at the 1000cp/mL threshold (18,19). However, none of these studies included pregnant or breastfeeding women nor were the operators nurses.

Only one randomized trial has examined the clinical impact of PoC VL (GeneXpert) in HIV positive adults (20). There is presently no study available on clinical impact in pregnant or breastfeeding women.

Table 3: Operational characteristics comparison of Xpert and m-PIMA HIV PoC VL

	Xpert HIV-1 PoC VL (Cepheid)	**m-PIMA HIV-1/2 VL (Abbott)**
WHO prequalification year	2017 (7)	2019(6)
Test System	Nucleic-acid Amplification of HIV-1 Group M/N and O	Nucleic-acid Amplification of HIV-1 Group M/N and O, and HIV-2
Sample volume	1 mL of plasma	50 µL of plasma
Analysis time	~90 minutes	~70 minutes
Power	Plug Operated	Plug/battery Operated
Lower detection threshold	40 copies/mL	800 copies/mL
Upper detection threshold	10.000.000 copies/mL	1.000.000 copies/mL

1.1.5 HIV burden in Mozambique

Mozambique is a country of sub-Saharan Africa with a population of 28 861 863 in 2017 that is severely affected by HIV (10,21). Mozambique had an HIV prevalence of 11.5% (eighth-highest in the world), with around 2.1 millions of people living with HIV (second highest position in the world) in 2020 (1). During that period, it was estimated that 98 000 new HIV infections occurred in Mozambique with 56% among woman.

According to a national population-based survey - *Inquérito de Indicadores de Imunização, Malária e HIV/SIDA* (IMASIDA) conducted in 2015, the prevalence of HIV in the population from 15 to 49 years of age was 13,2%. It was higher in women (15,4%) compared to men (10,1%). The provinces of Tete (5.2%), Nampula (5.7%) and Niassa (7.8%) had the lowest prevalence. The

provinces of Gaza (24.4%) and Maputo (22.9%), plus Maputo City (16.9%) had the highest prevalences (22) (Figure 7).

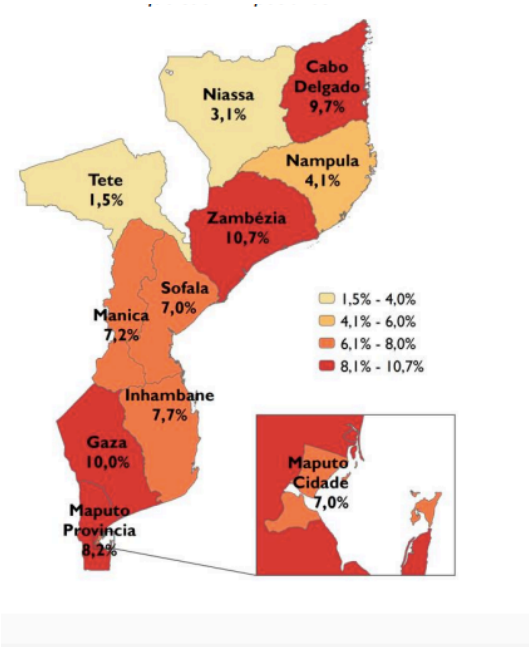


Figure 7- HIV prevalence per province of Mozambique in 2015,
Source: IMASIDA 2015 (22)

Regarding the 90-90-90 goals, 81% of people living with HIV knew their status, 68% were on treatment and 55% were suppressed in 2020 (1).

Mozambique is one of the countries where important efforts were made towards elimination of mother-to-child transmission. It was estimated that 100% [82%-100%] of pregnant woman living with HIV had access to antiretroviral medicines. The final vertical transmission rate including breastfeeding went from 33,5% [27,5%-51%] in 2010 to 13,5% [11,1%-17,6%] in 2020, yet these results are still far from the aspirational target of less than 5% set to eliminate vertical transmission (1,23) (Figure 8).

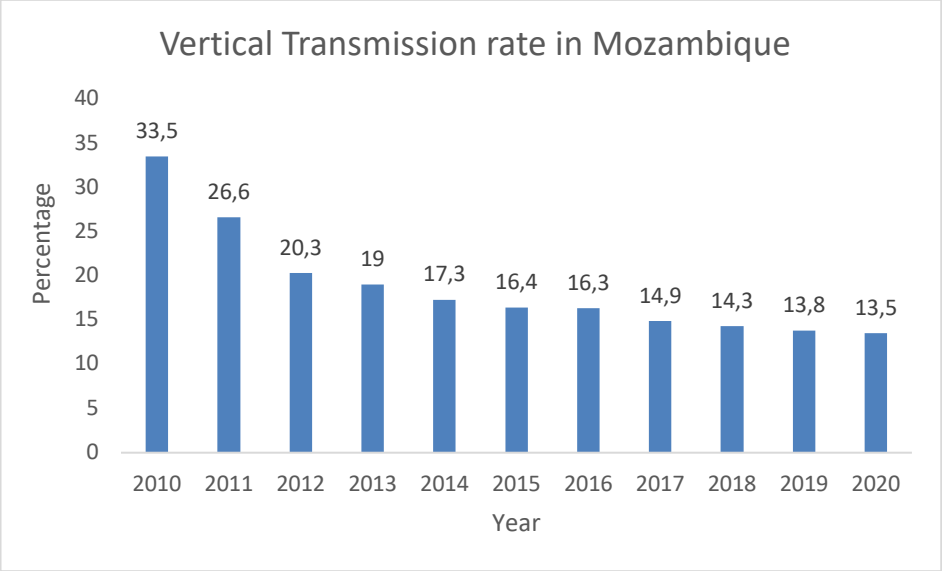


Figure 8-Vertical Transmission Rate by Year in Mozambique

Source: NHP Report 2020 (23)

In Mozambique, the measurement of HIV viral load for monitoring patients on anti-retroviral therapy (ART) is not widely accessible. Since increasing numbers of patients are initiated into ART, the demand for this test to identify virologic treatment failures or non-adherence is increasing. Viral load testing is conducted using molecular diagnostics and is only available in a limited number of central laboratories, with many parts of the country lacking access to viral load testing on-site. Test samples are transported over large distances which introduces delays especially for patients in rural and remote areas. To overcome the sample transportation issue, Mozambique opted for Dried Blood Spots (DBS) samples for viral load measurements. Some progress has been made to increase de coverage of viral load testing.

The data from the Laboratory Information System (LIS) for viral load in Mozambique showed that from 2015 to 2019, there was an increase in health facilities (HF) that performed and provided VL results. The data indicate that in 2015 a total of 94 HF reported performing VL tests, and in 2019 this number increased to 1,426 HF. The proportion of VL tests by active on ART increased from 51% in 2018 to 63% in 2019.

According to the routine program data, almost 800 000 viral load tests were conducted in 2020. From those, only 70% of the results were received by the caregiver and 52% of patients tested actually underwent suppression (23) (Figure 9). The suppression rate was not equal in all provinces of Mozambique. They ranged from 45% in Niassa to 62% in Tete (Figure 10).

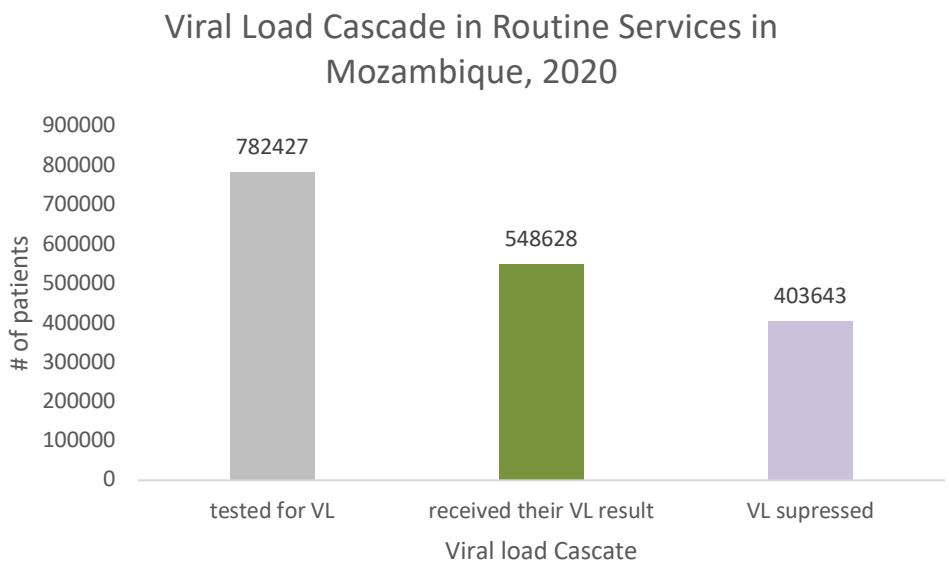


Figure 9- Viral load Cascade in Routine Services in Mozambique, 2020

Source: NHP Report 2020 (23)

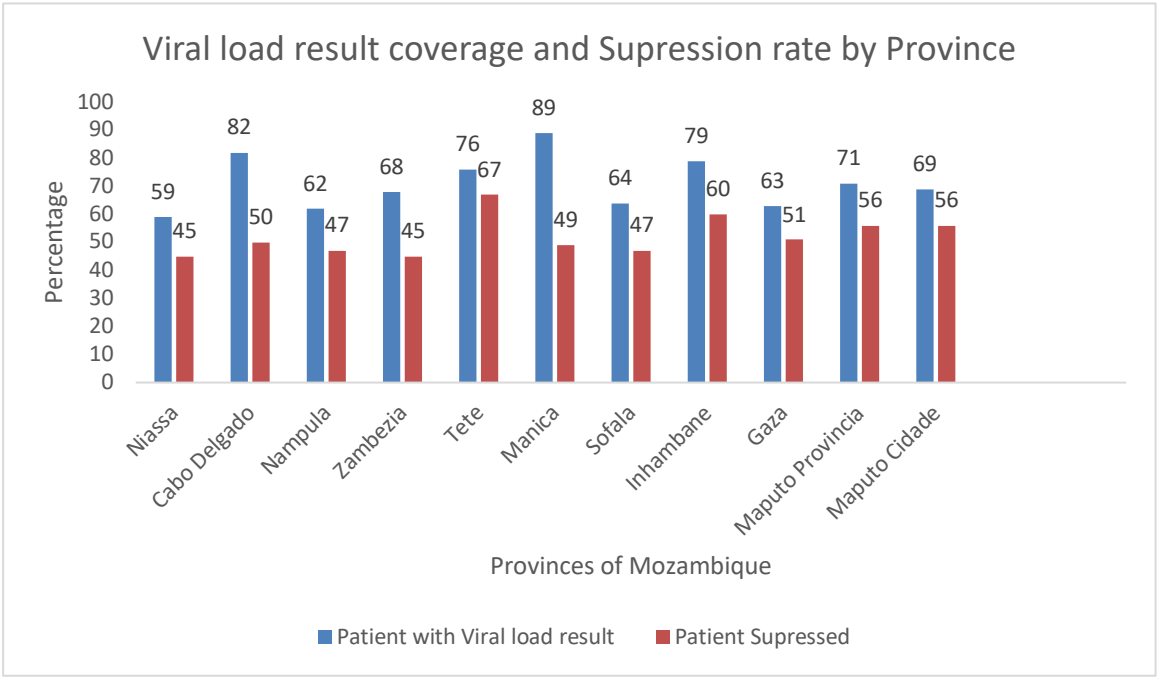


Figure 10-Percentage of Patients that received their result and Suppression rate by Province in Mozambique

Source: NHP Report 2020 (23)

In Mozambique, approval of the switch to second-line regimen is a decentralized competence in all provinces approved by ART committees. Since mid-2019, with the introduction of new optimized regimens containing Dolutegravir (TLD), a gradual transition to a new first line has begun in almost all adult and child patients (weight >20Kg) on ART.

In this sense, the number of requests submitted for second line approval went from 22,373 in 2019 to 7,481 in 2020, which corresponds to a reduction of 14,892 (67%) requests. In the same order, the proportion of approved orders for line change also experienced a reduction of 90% in 2019 against 78% in 2020 (Table 4).

Table 4: Cases of First-Line Treatment Failure submitted and approved for second-line, 2019-2020

	2019		2020	
	Submitted Requests	Approved Requests	Submitted Requests	Approved Requests
Niassa	359	312	66	41
Cabo Delgado	780	696	381	256
Nampula	2,575	2,411	748	641
Zambezia	6,061	5,474	1,236	886
Tete	685	605	259	203
Manica	483	418	105	68
Sofala	1,318	1,203	1,399	1284
Inhambane	974	781	145	114
Gaza	2,409	2,161	236	181
Maputo Provincia	4,054	3,734	1,151	821
Maputo Cidade	2,675	2,429	1,755	1365
Total	22,373	20,224	7,481	5,840

Source: NHP Report 2020 (23)

1.2 Rationale and Objectives

Viral load monitoring coverage is increasing in LMIC with efforts made in new laboratory establishments and alternative sample usage (DBS). Nevertheless, the turnaround times are still long and could be the reason of missed opportunities to act on adherence issues or treatment failures for priority populations like pregnant and breastfeeding women. PoC VL could help in these special situations. WHO has conditionally recommended the usage of PoC VL. Currently, there are two technologies prequalified by WHO for PoC VL. Although accuracy data for GeneXpert is

widely available, this is not the case for m-PIMA. Data related to feasibility and clinical impact of PoC VL use in pregnant and breastfeeding women is also not available.

The aim of this research was to first evaluate the accuracy of the m-Pima PoC viral load test (Abbot) as compared to standard VL testing (Roche TaqMan System) for PoC VL monitoring in HIV-infected pregnant women around the time of delivery (Study 1). Following this methodological evaluation, we aimed to demonstrate the operational feasibility of nurse based PoC VL monitoring at primary health facilities, and to assess the resulting impact on post-partum maternal viral load evolution in conjunction with an expected effect on MTCT prevention (Study 2). In order to dissect this translational clinical impact, we adopted a cluster randomized design, in which half of the health facilities (=cluster) implemented maternal PoC VL monitoring at birth, whereas the other half of the health facilities received standard of care (SoC) as provided by the health system in Mozambique.

1.2.1 Primary Objective

- To determine the diagnostic accuracy of m-PIMA HIV-1/2 Viral Load Test (Abbott, Chicago, IL, USA) operated by maternal-child health (MCH) nurses- **Study 1**
- To determine the operational feasibility and usability of m-Pima Viral Load test in the maternity wards for pregnant women close to the delivery point. **Study 2**

1.2.2 Secondary Objective

- To compare the diagnostic accuracy between capillary blood and venous blood of m-PIMA HIV-1/2 Viral Load Test (Abbott, Chicago, IL, USA)- **Study 1**
- To describe the impact of the VL monitoring at delivery for the mother's clinical management and association with transmission rates to their infants- **Study 2**.

2. Material and Methods

This research project included two studies: one on diagnostic accuracy and the other forming part of a larger study which is a cluster randomized trial on early treatment of HIV in neonates and its clinical impact in terms of mortality and morbidity.

2.1 Study 1

2.1.1 Study setting and design

This study was conducted in in a peri-urban area of Maputo city, the capital of Mozambique. It was coordinated by Polana Caniço Health Research and Training Center (CISPOC). CISPOC is a clinical research centre under the Instituto Nacional de Saúde of Mozambique with a mission of generating and promoting the incorporation of scientific and technological solutions to the main health problems and conditions in Mozambique. Maputo city had a HIV prevalence of 16,9% in 2015 and is thus one of the provinces in Mozambique with high HIV prevalence.
In this cross-sectional study, participants were recruited from two primary healthcare facilities in Maputo City, Mozambique: *Polana Caniço* and *1º de Maio Health Centres*. These two health facilities were selected as recruitment sites due to proximity to CISPOC where data was processed and samples for laboratory referral were prepared. Pregnant and Postpartum women were invited to participate in the study during their Antenatal Care or Postnatal Care consultation.

2.1.2 Study Participants

Participants with the following eligibility criteria were included:

2.1.2.1 *Inclusion Criteria*:

- Being pregnant and post-partum women coming for a routine consultation in the health facility
- 18 years of age or above
- Having documented HIV infection irrespective of being on ART.

2.1.2.2 *Exclusion Criteria*:

- having a serious medical conditions which would make testing dangerous for the patient such as severe anaemia or Pre-Eclampsia (or uncontrolled hypertensive disorder);

- having any other medical condition that would render inclusion into the study unethical as judged by the medical team.

In order to recruit patients in a spectrum of low to high viral load, four groups were considered in the following ranges: undetectable, detectable below 1,000 copies/mL, between 1,000-10,000 copies/mL and above 10,000 copies/mL. In order to have at least 100 participants in each group, participants were intentionally targeted to represent one of the above groups based on:

- time on antiretroviral treatment (ART): ≥ 12 months, patients on ART at 1 to 12 months and patients without ART or just starting ART
- suspected treatment failure.

Although baseline viral loads were not routinely performed in Mozambique, in this study baseline testing was done to allocate participants in the high viral load range.

2.1.3 Sample Size and Study Sampling

Sample size calculations were based on the need of adequate sensitivity of PoC viral load technology and on the fact that viral load results will be categorized using 3 thresholds namely (1) undetectable, (2) 1 000 copies/mL and (3) 10 000 copies/mL.

Each participant would be allocated in one of the following groups; based on the time on ART: ≥ 12 months, 1 to 12 months and patients without ART. The study had to recruit 699 participant in total (233 in each group) if 95% is the lowest acceptable sensitivity for each viral load threshold and assuming 8% sample loss due to errors or poor handling. The study was initiated including consecutive participants but also had to target specific participants in order to increase the number of participants in certain groups.

2.1.4 Study Procedures

2.1.4.1 Recruitment and Sample/Data Collection.

Participants were identified in the pre or post-natal clinical consultation area of the site by study personnel. After the routine consultation, potential volunteers were invited to a confidential room, where a detailed explanation of the study objectives was provided and informed consent was obtained. If the participant was willing to participate in the study, she was assessed for eligibility. If the patient was not willing to participate in the study, normal clinical care was proceeded with as usual. A study unique identification code was assigned to each participant. One m-PIMA PoC VL device was allocated in the consultation room of each site.

Routine mother-child health care (MCH) nurses were trained in research ethics, human subject protection and study procedures including blood collection and testing operations on the m-PIMA PoC VL device.

For each participant, venous blood was collected using a 6mL K_2 EDTA tubes (Becton Dickinson, Franklin Lakes, NJ, USA) by the clinical routine task nurse (Table 5). The following data were collected from each participant: date of birth, date of ART initiation and ART treatment regimen. For participants included in the capillary blood sub-study, additionally 5-6 drops of finger capillary blood were collected directly into the 0.5mL EDTA microtainer tubes.

Table 5- Summary of type of sample, operator, platform and limit of detection (LOD) for viral load testing used in the study

Quantity and Sample Type	Operator / placement	Platform	Procedure	LOD
50µL of venous plasma	Nurse / consultation room	m-PIMA	500µL of whole blood was transferred to a microtube. The microtube was centrifuged for 5 minutes for plasma separation. using a mini centrifuge with fixed rotational speed of 6000rpm	800 copies/mL
50µL 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 mini centrifuge with fixed rotational speed of 6000rpm	800 copies/mL
One dried blood (80µl prepared from whole venous blood)	Lab technician / centralized molecular virology lab at the Instituto Nacional de Saúde	Roche Cobas Ampliprep/Cobas TaqMan 96 HIV-1 v2 (CAP/CTM) automated instrument or Abbott RealTime HIV-1 system	DBS card was prepared by nurse using venous whole blood and sent to centralized lab for processing.	400 copies/mL-Roche CAP/CTM 837 copies/mL
1.1 mL of frozen venous plasma	Lab technician / centralized molecular virology lab 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 lab where it was centrifuged and the plasma was stored for batch testing.	20 copies/mL

Source: Meggi, et al 2021(24)

2.1.4.2 Venous Blood m-PIMA PoC VL testing

After blood collection, MCH nurses, transferred 0.5mL of whole blood of each participant to 0.5mL EDTA microtainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA). The labelled microtainer tube was allocated individually into a microcentrifuge (myFuge® Mini Centrifuge, Benchmark Scientific, Sayreville, NJ, USA) for plasma separation at 5,000 rpm for five minutes. The microcentrifuges were not part of m-PIMA PoC VL manufacturer's product kit and were purchased by the study (Figure11).

From the centrifuged plasma, 50 microliters were transferred into the PIMA HIV-1/2 Viral Load cartridge (Abbott, Chicago, IL, USA) using a Pasteur pipette. Immediately, the cartridge was introduced into the m-PIMA analyser for processing. The analyser is automatic and has a touch screen to introduce sample ID, operator name and some other settings. The results are automatically available after approximately 70 minutes. The m-PIMA is capable of quantifying HIV-1 groups M/N and O or HIV-2 RNA. A portable printer and external battery are provided by the manufacturer. The device also allows for exporting data using an external data sharing device.

Figure 11-Study Equipment including mini-centrifuge provided by the study
Source: Bindiya Meggi (2018)

2.1.4.3 Capillary Blood m-PIMA PoC VL testing

The capillary blood (5-6 drops) that were collected directly into 0.5mL EDTA microtainer tubes, was centrifuged at 5,000 rpm for five minutes for plasma separation using the above-described study microcentrifuge. All the following procedures were the same as described for venous blood.

2.1.4.4 Laboratory-Based VL testing

After using the amount of blood for PoC VL testing, the remaining venous whole blood was used to prepare Dried Blood Spots (DBS) and the rest was transported within four hours to Instituto

Nacional de Saúde-INS (the HIV VL Reference laboratory). DBS was prepared because this is the routine for viral load measurements in the national system and to assess the diagnostic accuracy of DBS type of sample. At INS, the blood was centrifuged for plasma separation within six hours of collection. The plasma was stored at –80^{o}C and processed in batches using the Roche CAP/CTM 96 HIV-1 Quantitative Test v2 (Roche Molecular Diagnostics, Branchburg NJ, USA), according to the manufacturer's instructions. The testing was done by a qualified trained laboratory technician using around 1.1mL of frozen sample.

The DBS was processed using either on Abbott m2000 real time or Roche CAP/CTM as part of routine system.

2.1.5 Data Management and Analysis

All filled study forms including laboratory results were assessed for completeness by a data manager. After that they were double entered into a customized data base created using PostgreSQL (25).

To determine the diagnostic accuracy the following parameters were calculated: sensitivity, specificity, positive and negative predictive values, bias, limits of agreement, linearity, correlation, and coefficient of determination (R^2).

Using a two-way contingency table, data was summarized to allow determination of sensitivity, specificity, positive and negative predictive values at different thresholds (Not detected, 800, 1000 and 10000 copies/mL.)

For correlation analysis, all PoC VL and laboratory-based test results were log10-transformed to reduce skewness. To allow log transformation of special cases like undetectable viral load and below limit of detection of each technology, some conventions were adopted: samples with undetectable viral load were assigned a value of 1 copies/mL and those below the limit of detection (LOD) were assigned the value of LOD. Linear regression analysis (Correlation Coefficient and R-squared) and Bland-Altman plots (mean bias ± 1.96 standard deviation) (26,27) were used to determine the level of agreement between PoC VL and plasma laboratory-based results.

For the above analyses, RStudio 2016 (Boson, MA, USA) (28) and Microsoft Excel 2011 v14.1.0 (Microsoft Co., Redmond, WA, USA) were used.

2.1.6 Quality Assurance

MHC nurses were trained by the study team to operate the m-Pima PoC VL. The study team received a training of trainers (TOT) by the manufacturer. The training for nurses had a duration of

5 days that included theory and practical sessions. At the end of the training, competency was assessed regarding sample processing, device handling and results interpretation.
The laboratory technician at INS was formally trained to use the technologies used to process viral load in the reference centralized laboratory. The laboratory participates in the external quality assurance program provided by the US Center of Disease Control and Prevention (CDC).
Both MHC nurses and laboratory technicians were blinded in regards to the result of other parallel test.

2.1.7 Ethical Consideration

This study was approved by the Mozambique's National Health Bioethics Committee (*Comité Nacional de Bioética para Saúde de Moçambique*: 281/CNBS/2018). All participants were asked to provide written permission to participate using an Informed Consent Form in Portuguese. In addition, an impartial literate witness was present during the consent process if the patient was illiterate. No participant was denied care or faced any negative consequences as a result of refusing to participate in the study. All participants were free to withdraw from the study at any time and for any reason. All subjects' information was treated in a strictly confidential manner and anonymized by unique study ID number. All files were stored at a secure and locked place to which only authorized study staff had access. The risks related to study participation was considered low. No compensation was provided to the participants for their study participation.

2.2 Study 2

2.2.1 Study setting and design

This sub study is part of another larger study identified under the acronym "LIFE "that is being conducted in Mozambique and Tanzania. In Mozambique, this study is being conducted in two provinces: Sofala and Manica. It is coordinated by Beira Operational Investigational Center (CIOB). CIOB is an operational research centre under the Instituto Nacional de Saúde of Mozambique. Sofala and Manica had an overall HIV prevalence of 7% and 7,2 % in 2015, respectively.

The LIFE study is a clustered randomized clinical trial conducted in 28 health facilities, 14 in Mozambique and 14 Tanzania . The primary objective of the LIFE study is to establish the clinical impact of a PoC-EID for infants and neonates at birth and at 4-8 weeks, linked with nurse-supported immediate ART initiation in HIV-infected neonates, versus standard-of-care (SoC) on the primary and secondary endpoints. Combined clinical outcomes include mortality, morbidity

(WHO Stage 2 or above, severe infant medical conditions), hospitalization, toxicity (Grade 3 or above laboratory abnormality), poor antiretroviral treatment response (confirmed virological failure, treatment termination or prolonged interruption), or loss to follow up of HIV-infected infants.

In Mozambique the following health facilities (Table 6) were selected based on their numbers of HIV infected pregnant women, MCTC rate and logistically operational. They were randomized to either be in the control or in the intervention group. Stratification during the randomization process was done for country and for delivery volume.

Table 6: Health Facilities participating in the LIFE study in Mozambique

Province	District	Health Facility	Study Arm*
Sofala	Cidade da Beira	CS MANGA-LOFORTE	Control
Sofala	Cidade da Beira	CS M.MASCARENHAS	Intervention
Sofala	Cidade da Beira	CS MACURRUNGO	Control
Sofala	Cidade da Beira	CS NHACONJO	Control
Sofala	Cidade da Beira	CS CHINGUSSURA	Control
Sofala	Cidade da Beira	CS PONTA GEA	Intervention
Sofala	Cidade da Beira	CS MUNHAVA	Intervention
Sofala	Dondo	CS MAFAMBISSE	Intervention
Sofala	Dondo	CS DONDO	Control
Manica	Cidade de Chimoio	CS 1 DE MAIO	Intervention
Manica	Cidade de Chimoio	CS 7 DE ABRIL	Intervention
Manica	Cidade de Chimoio	CS NHAMAONHA	Control
Manica	Cidade de Chimoio	CS VILA NOVA	Control
Manica	Gondola	HD GONDOLA	Control

*Health facilities were randomized to either be control or intervention

Mother/child pairs were recruited at maternity wards of these primary healthcare facilities (clusters). Half of the health facilities (Intervention Arm, or Arm A) tested mothers with PoC VL at birth and their babies with PoC EID by nurses, with indication for immediate treatment for infants with HIV-positive results. The other half (Control Arm, or Arm B) collected samples from the mothers for processing at a central reference laboratory for viral load. The babies from arm B only did early infant diagnosis testing at week 4-6 but collected DBS at birth for retrospectively testing if found positive at week 4-6. The viral load results for mothers in Arm B were given to the mother

at her next visit, if available. The mother-child pairs were followed up over two visits: visit 2 (week 4-8) and visit 3 (week 11-16) of life. In both visits, EID testing was performed for all previously negative babies. At visit 3, all mothers had a viral load performed (central laboratory or PoC). Mothers in Arm A with viral load results above 1,000 copies/mL at delivery were referred for adherence counselling according to national guidelines. For Arm B, all positives and lost to follow up after visit 1, had their DBS retrospectively processed to know the status at birth (Figure 12).

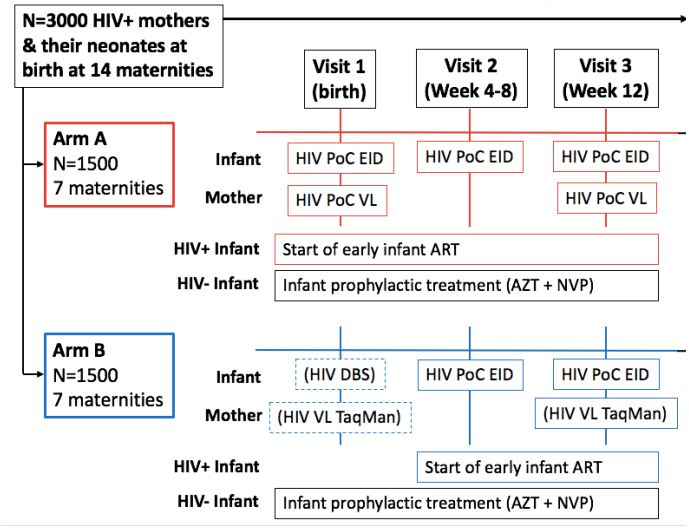


Figure 12-LIFE study Scheme in Mozambique (Phase 1)

2.2.2 Study Participants

The participants were included in the LIFE study using the following eligibility criteria:

2.2.2.1 Inclusion Criteria

- Voluntary and informed consent of the mother for her own study participation (if applicable).
- Voluntary and informed consent of the legal guardian of the child for participation of the child in the study.
- Mothers/legal guardians ≥18 years of age.
- Documented maternal HIV infection.
- Willingness to consent to HIV testing for the child and herself.
- Willingness to consent to active tracing including home tracing.

2.2.2.2 Exclusion Criteria

- Deficiency in the mother, rendering it difficult, if not impossible, for her or her infant to take part in the study or understand the information provided to her. This includes alcoholism, drug dependency as well as psychiatric illnesses, suicidal tendencies or any other serious inability.
- Having delivered more than 72h (3 days) ago
- Prison inmates
- Women presenting with an emergency requiring immediate medical assistance not resolved at study inclusion
- Stillbirths
- Infants requiring emergency care (e.g. immediate or rapid occurring life threatening conditions, resuscitation, prolonged obstetric related intensive care, severe jaundice) or born with severe malformation.
- If within the discretion of the investigator based on recommendation of the gynaecologist or pediatrician in charge study participation would possibly add not acceptable risk or burden to the mother or infant (e.g. significant congenital malformation, health deficiencies, very low birth weight less than 1500g)
- Unlikely to comply with protocol as judged by the principal investigator or his designate

2.2.3 Sample Size and Study Sampling

The sample size was calculated for the primary endpoints of the LIFE study. It was calculated assuming a combined endpoint (death, WHO Stage B/C disease, severe infant medical conditions, any grade III/IV lab abnormality, confirmed virological failure, ART toxicity) of 30% versus 14% in the SoC Arm B and intervention Arm A respectively. In order to achieve 80% power to show a significant difference between arms at the 95% level of confidence, it would be necessary to randomize 112 HIV-infected infants into each arm (244 overall). Assuming a 4% HIV transmission rate by 12 weeks of age, and 5% loss to follow-up, this means that we would need to randomize overall 6000 HIV-exposed babies (3000 per arm). In Mozambique, it was calculated to recruit 1500 women in Arm A and 1500 women in Arm B to be consecutively included in the study. The aspect of determining the impact of PoC VL in the MTCT rate was a secondary objective for which the study was insufficiently powered. Therefore, we assumed a descriptive analysis outcome. Due

to a lower-than-expected transmission rate in Tanzania, the sample size was increased to allow for an additional 1000 participants in Mozambique.

2.2.4 Study Procedures

2.2.4.1 Recruitment and Sample/Data Collection.

HIV positive pregnant women presenting for delivery at their obstetric health facility were invited to participate in the study. Once a participant had signed a consent form and eligibility criteria were confirmed, their details were registered in a Study Master Form. Each mother and infant pair received a unique identification number with separate ID's for the mother and each infant that could be linked. A study participation card including the study ID, details to contact study personnel and study visit dates were provided to mothers, who were asked to present the card at each visit for identification.

The following clinical assessments were documented:

- Demographic data (data of birth or age, education)
- Information if the HIV status was disclosed to family members
- HIV related information (e.g. date of HIV diagnosis, current ART and ART history, start or stop date of ART, last CD4-count, last viral load if available)
- Self-reported ART adherence and knowledge about infant HIV transmission
- Pregnancy history (e.g. ANC attendance during current pregnancy, gravida, parity)
- Current pregnancy and obstetric information including: First day of last menstrual period, method and location of delivery (e.g. vaginal or vaginal assisted, at home or at the clinic, date and time of delivery; gestational age at delivery; single, twin, triplet or above birth; premature rupture of the membrane; complications during delivery).

For viral load testing, venous blood was collected into 3mL K_2 EDTA tubes (Becton Dickinson, Franklin Lakes, NJ, USA) from the mothers

2.2.4.2 Abbott m-PIMA Viral Load Testing

Abbott m-PIMA viral load testing was performed by nurses, who collected and transferred 0.5mL of whole blood to labelled 0.5mL EDTA microtainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA). The testing procedure was the same as described in section 2.1.4.2. For this study, seven m-PIMA devices for Arm A were deployed to maternity wards. The Arm B health facilities used m-PIMA devices available from the routine early infant diagnosis program at Child at Risk consultation rooms.

2.2.4.3 Training of PoC viral load testing and Sample Prioritization

Nurses received a five-day training on how to operate PoC devices for viral load testing, as well as sample collection, centrifugation and how to pipette plasma into the test cartridge. Post-training support was offered through routinely planned supervision visits, with the first supervision being conducted one month after initial trainings and bimonthly thereafter.

Since PoC VL was processed on the same devices used for early infant diagnosis (EID), priority was given to EID testing. This did not affect the testing of PoC VL, as patient flow was managed in a way that ensured that infants who needed diagnosis were prioritized while ensuring that baseline viral loads could also be tested on the same day.

2.2.5 Data Management and Analysis

All data were recorded on paper, double entered into an OpenClinica® database (25), and corrected for data entry errors. Data management was performed at each country in collaboration with the central data management unit (DMU) in Munich, Germany. Access to the database for data entry was individual-specific username and passwords. The central data management unit (DMU) was located at LMU who will also host the central OpenClinica server and secure on data back-up provisions. The LMU DMU served as the centralized location assuring data quality using automatic range checks and validations and query generation tools within the database. Query reports was sent back to sites to flag data errors and inconsistencies where site staff had responsibility for responding to the queries and correcting the data on the database as needed.

Descriptive summaries of baseline demographic and HIV characteristics are reported as percentages and stratified by intervention group. Generalized linear mixed-effects models were used to account for clustering of data by health facility. Comparisons of proportions between intervention and control Arms were based on Pearson's Chi-square tests with Yates' continuity correction. To assess the impact of the intervention on maternal viral suppression at week 12 post-delivery, only participants with both delivery and week 12 viral load results were included. Viral suppression, defined as <1000 copies/mL, at week 12 was used as the outcome in a mixed-effects model in which intervention group and baseline viral suppression status were included as a fixed effects and health facility was included as a random effect. Odds ratios by intervention group are reported.

We used the Kaplan-Meier method to estimate the cumulative probability of HIV acquisition in infants between intervention groups up to 12 weeks of age. Intervention group and clustering by health facility were included in a proportional hazards regression model. Though the study was designed to have the final follow-up at 12 weeks, we allowed for study visits up to 16 weeks. Transmission rates at week 12 as proportions and the hazard ratio for intervention effect are reported. Data analysis was performed in R (29).

2.2.6 Quality Assurance

Supervisions served to ensure quality in operating the PoC device, sample processing and results interpretation. Where retraining needs were identified, staff were offered corrective training. Data quality was also monitored remotely in real time through online results databases made possible through connectivity solutions (m-PIMA: Sympheos Datapoint). Monitoring visits were also conducted to ensure source document verification for results. Study monitoring included monitoring by internal monitors assigned at each country's research institution. Internal monitoring included quality control and assurance of e.g. proper informed procedures, completeness of DCF documentation, completeness of essential document maintenance, adequate timing of HIV diagnostic result dissemination, and adequate storage of biorepositories. External monitoring was performed at least annually by the sponsor's monitoring team.

2.2.7 Ethical Consideration.

This study was approved by the Mozambique's National Health Bioethics Committee (Reference number 281/CNBS/2018), the Tanzanian National Health Research and Ethics Review committee (Reference number NIMR/HQ/R.8a/Vol. IX/3071) and the Institutional Review Board of the University of Munich, Germany (Reference number 19-441). Written informed consent was obtained from each participant prior to conducting any study procedure. Study participation did not influence the standard procedure for HIV diagnosis, care and prophylactic treatments. As a "more than minimal risk but with the potential of direct benefit" study participant insurance was not in place. Permission for direct access to subject's data was sought in writing by the Investigator as part of the informed consent procedure. No directly identifiable participant data was held in the study database; participants were identified by an anonymous study ID. However, data that could be considered as indirectly identifiable (date of birth) or sensitive (date of medical events) was part of the data set and was handled within the project in a secure manner. All staff who handled personal data during their activities were appropriate trained.

3. Results

3.1 Study 1

3.1.1 Patient demographics and clinical characteristics

From September 2018 to April 2019, a total of 699 pregnant and postpartum women were included in the study. From those, 692 actually met the eligibility criteria (Figure 12). The majority (74%) of participants were less than 35 years of age (median age [IQR]: 29 [25 - 34] years) and 55% were on treatment for HIV for more than one year (median time on ART [IQR]: 1.64 [0.24 - 4.65] years). According to laboratory-based plasma testing, the viral load results ranged from undetectable to 2,454,892 copies/mL. A total of 427 participants (62.7%) had viral loads below 1,000 copies/mL. From those, 251 (58.8%) were not detected, 85 (19.9%) were detected below 20 copies/mL (LOD) and 91 (21.3%) were detected between 20 and 1000 copies/mL. Only 60 (8.7%) participants had a viral load between 1000 and 10,000 copies/mL and 202 (29,2%) participants had above 10,000 copies/mL (Table 7). The initial error rate of m-PIMA was 4.3 but as more plasma was available on site it was possible to repeat the testing without new collection. At the end only 3 (0,4%) of participants did not get any valid result (Figure 13).

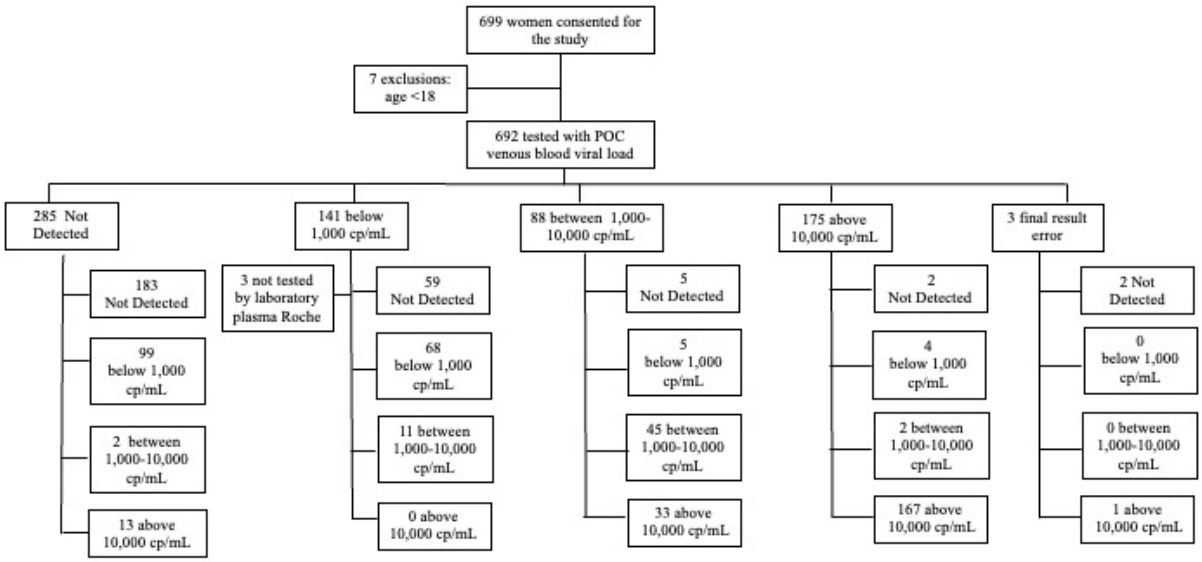


Figure 13- Flow diagram of participants of the study

POC = Point-of-Care; cp/mL = copies per millilitre

Source: Meggi, et al 2021(24)

Table 7: 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%)
1,000 – 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%)

Notes: *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, Abbott DBS=837 cp/mL ‡Centralized laboratory-based testing. ‡‡Errors for PoC are those whose final result were designated as error due to repeated errors or not enough sample to repeat.

Source: Meggi, et al 2021(24)

3.1.2 Performance of venous blood plasma m-PIMA compared with plasma laboratory-based technology

From 692 participants, 686 (99.1%) of participants had valid results for both m-PIMA PoC VL and plasma laboratory-based testing.

The sensitivity of detecting nucleic acids of m-PIMA (venous plasma) was 76.7% (95% CI: 72.4 – 80.5%) and specificity was 73.5% (95% CI: 67.6 – 78.9%) (Table 8). There were 102 samples with undetectable virus by m-Pima that were actually detectable by laboratory plasma-based testing. But from those 58 (56,9%) had detectable below 20 copies/mL in the laboratory plasma-based testing. In the other way. 66 samples had detectable viral load by m-Pima and had undetectable by laboratory plasma-based testing. From those 53 (80.3%) were below 800 copies/mL in m-Pima.

The sensitivity of venous blood plasma m-Pima at a threshold of 1000 copies/mL was 95.0% (95% CI: 91.6 – 97.3%) and specificity was 96.5% (95% CI: 94.2 – 98.0%) when compared with plasma laboratory testing (Table 8). The positive predictive value and negative predictive value at the

same threshold were 94.3% (95% CI: 90.8 – 96.8%) and a 96.9% (95% CI: 94.8 – 98.4%), respectively.

The correlation coefficient between the two viral load results was 0.922 (95% CI: 0.902 – 0.939) with an r^2 of 0.850 and a mean bias of 0.202 log copies/mL (95% LOA: -0.366 – 0.772 log copies/mL) (Figure 14A and 14B).

The sensitivity of venous blood plasma m-Pima at a threshold of 10 000 copies/mL was 83.6% (95% CI: 77.7 – 88.4%) and specificity was 98.6% (95% CI: 97.0 – 99.4%) when compared with plasma laboratory testing (Table 8). The positive predictive value and negative predictive value at the same threshold were 96.0% (95% CI: 91.9 – 98.4%) and a 93.5% (95% CI: 91.0 – 95.5%), respectively

Table 8: 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	255	8	398	25	97.0%	94.1%	91.1%	98.0%
1,000	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%

Notes: TP = true positive; FN = false negative; TN = true negative; FP = false positive; PPV = positive predictive value; NPV = negative predictive value

Source: Meggi, et al 2021(24)

3.1.3 Agreement between viral loads obtained using venous and capillary blood plasma on the m-PIMA assay

A subset of 93 participants, tested two types of blood on M-Pima: capillary and venous blood. The correlation coefficient between these two sample types was 0.983 (95% CI: 0.963 – 0.992) with an r^2 of 0.966. The mean bias was 0.021 log copies/mL (95% LOA: -0.233 – 0.276 log copies/mL) (Figure 14C and 14D).

3.1.4 Performance of DBS laboratory-based testing compared with plasma laboratory-based testing

For this secondary analysis, 687 participants had both valid DBS and plasma laboratory-based testing results. The sensitivity of DBS laboratory-based testing at a threshold of 1000 copies/mL

was 78.2% (95% CI: 72.8 – 83.1%) and a specificity of 98.6% (95% CI: 97.0 – 99.5%) when compared with plasma laboratory testing. The positive and negative predictive value at same threshold were 97.2% (95% CI: 93.9 – 98.9%) and 88.0% (95% CI: 84.8 – 90.8%), respectively. The correlation coefficient between the two viral load results 0.741 (95% CI: 0.676 – 0.795) and r^2 of 0.549 with a mean bias of 0.627 log copies/mL (95% LOA: -0.325 – 1.479 log copies/mL) (Figure 14E and 14F).

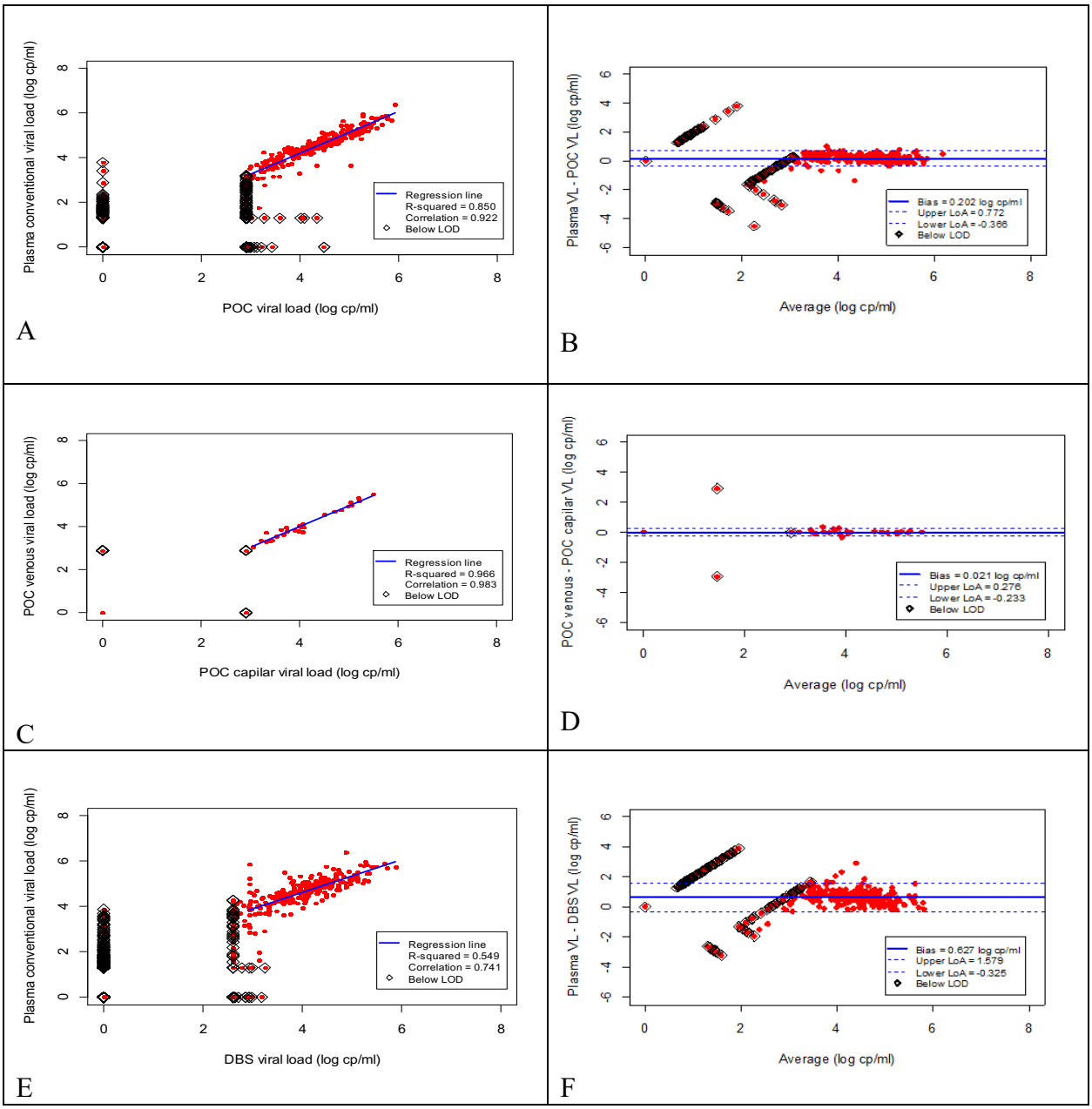


Figure 14- Agreement analysis viral load technologies: Linear regressions and Bland-Altman diagrams

Paired Venous blood plasma m-PIMA and conventional plasma samples (*A* and *B*)

Paired Venous blood and capillary plasma m-PIMA (*C* and *D*)

Paired DBS and conventional plasma samples (*E* and *F*)

Source: Meggi, et al 2021(24)

3.2 Study 2

3.2.1 Patient demographics and clinical characteristics

From October 2019 to July 2021, a total of 3952 mothers, 2,057 (52.0%) intervention Arm A and 1895 (48.0%) control Arm B from Mozambique were included in this analysis. These mothers were included in the LIFE study from October 2019 to July 2021. A total of 2,267 (57.4%) of mothers were below 30 years of age, 1,871 (47.3 %) had primary education and 2,527 (63.9%) had two antenatal consultations. A total of 2,455 (62.1%) were diagnosed with HIV for more than a year before their enrolment in the study, 3,667 (92.8%) reported having disclosed their status, and 3,232 (81.8%) were previously on or started a Dolutegravir-based ARV regimen at delivery. A total of 1,216 (30.8%) women had a viral load above 1,000 cp/mL at delivery. When comparing viral loads below 1,000 cp/mL (supressed), the intervention Arm had a significantly higher proportion of supressed compared to the control Arm (72.4% versus 62.5%; p <0.0001) (Table 9).

3.2.2 Operational Feasibility of PoC VL

In the intervention Arm, 1,908 (92.7%) patients had a viral load processed using the PoC system with samples collected on the same day of whom 1906 (99.9%) had valid viral load results. From those, 1,891 (99.2 %) were communicated to the mothers on same day, with an overall 91.9% of same day results received by all patients (Figure 15).

The initial error rate for m-PIMA was 6.1% but, due to extra plasma availability, the majority of the VL tests were repeated using the same sample to obtain a valid result. As the study progressed and nurses became more experienced running PoC VL, the error rate dropped to 4%, resulting in an overall error rate of 5%. Most of the errors (79%) were either cartridge or software related and not due to the operator.

Table 9: Demographic characteristics of the included women at delivery

	Intervention (N=2057)	Control (N=1895)	Overall (N=3952)
Mother's age			
18-25y	459 (22.3%)	457 (24.1%)	916 (23.2%)
25-30y	690 (33.5%)	661 (34.9%)	1351 (34.2%)
30-35y	538 (26.2%)	460 (24.3%)	998 (25.3%)
35y+	370 (18.0%)	312 (16.5%)	682 (17.3%)
Not available	0 (0%)	5 (0.3%)	5 (0.1%)
Education level			
None	283 (13.8%)	250 (13.2%)	533 (13.5%)
Primary school	780 (37.9%)	767 (40.5%)	1547 (39.1%)
Secondary school or higher	993 (48.3%)	878 (46.3%)	1871 (47.3%)
Not available	1 (0.0%)	0 (0%)	1 (0.0%)
Antenatal care			
1 visit	230 (11.2%)	338 (17.8%)	568 (14.4%)
2 visits	1464 (71.2%)	1063 (56.1%)	2527 (63.9%)
3 visits	360 (17.5%)	396 (20.9%)	756 (19.1%)
None	3 (0.1%)	98 (5.2%)	101 (2.6%)
HIV disclosure			
No	108 (5.3%)	177 (9.3%)	285 (7.2%)
Yes	1949 (94.7%)	1718 (90.7%)	3667 (92.8%)
Time since HIV diagnosis			
1y or more	1285 (62.5%)	1170 (61.7%)	2455 (62.1%)
Less than 1y	768 (37.3%)	718 (37.9%)	1486 (37.6%)
Not available	4 (0.2%)	7 (0.4%)	11 (0.3%)
ART regimen			
TDF + 3TC/FTC + DTG	1722 (83.7%)	1510 (79.7%)	3232 (81.8%)
TDF + 3TC/FTC + EFV	302 (14.7%)	360 (19.0%)	662 (16.8%)
TDF + 3TC + LPV/r	1 (0.0%)	1 (0.1%)	2 (0.1%)
Other	1 (0.0%)	2 (0.2%)	3 (0.1%)
None	31 (1.5%)	22 (1.2%)	53 (1.3%)
Viral load at delivery			
Suppressed <1000c/ml	1490 (72.4%)	1185 (62.5%)	2675 (67.7%)

	Intervention (N=2057)	Control (N=1895)	Overall (N=3952)
Not suppressed >1000c/ml	566 (27.5%)	652 (34.4%)	1218 (30.8%)
Not available	1 (0.0%)	58 (3.1%)	59 (1.5%)

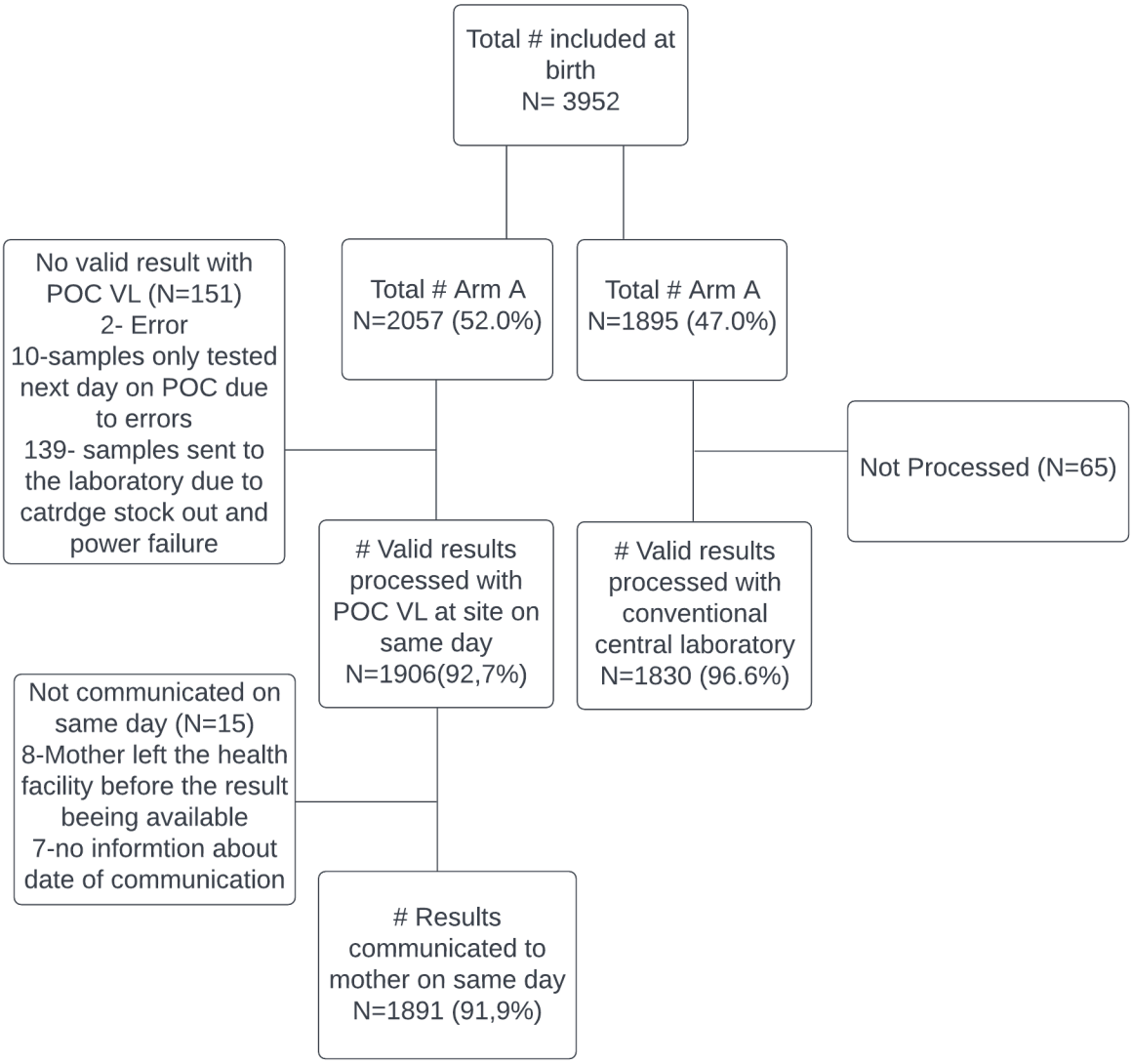


Figure 15- Flow Chart of PoC VL results

3.2.3 Impact of PoC VL at birth on suppression rate at week 12

A total of 2,722 mothers had both delivery and week 12 viral load data (1442 in the interventional Arm and 1,280 in the control Arm). Within this subgroup, the intervention arm had a suppression rate of 74.5 % at birth and 81.3% at week 12. The control Arm had a suppression rate of 65.4% at birth and 74.4% at week 12 (Table 10).

Among mothers with suppressed VL at delivery, the proportion remaining suppressed at Week 12 was higher in Arm A vs. B (91.6% vs. 84.3%; p <0.0001). Among mothers with high VL at delivery, there was no significant difference in the proportions with suppressed VL at Week 12 between Arms (51.4% vs. 55.5%; p=0.236) (Table 11).

Table 10: Maternal viral load comparison of delivery and week 12

	A (N=1442)	B (N=1280)	Overall (N=2722)	p-value‡
Delivery				
Not suppressed†	368 (25.5%)	443 (34.6%)	811 (29.8%)	ref
Suppressed†	1074 (74.5%)	837 (65.4%)	1911 (70.2%)	0.017**
Week 12				
Not suppressed	269 (18.7%)	328 (25.6%)	597 (21.9%)	ref
Suppressed	1173 (81.3%)	952 (74.4%)	2125 (78.1%)	0.076

†Not suppressed: >1000c/ml; Suppressed: <1000c/ml; ref: reference category
‡Chi-square test with Yates continuity correction adjusted for clustering by health facility

Overall, the Arm A VL PoC intervention did not have any effect on the suppression rate after adjusting for viral load at delivery and clustering by health facility on suppression rates at week 12 (OR 1.25 [95% CI: 0.863, 1.82]; p=0.235). However, viral suppression at delivery was significantly associated with maintaining viral load suppression at week 12 (OR 6.51 [95% CI: 5.330, 7.950]; p<0.0001).

Table 11: Maternal viral load comparison from delivery to week 12 by viral suppression status at delivery

Arm A				Arm B				
		Week 12				Week 12		p-value‡
		Low (N=1173)	High (N=269)			Low (N=952)	High (N=328)	
Delivery	Low (N=1074)	984 (91.6%)	90 (8.4%)	Delivery	Low (N=837)	706 (84.3%)	131 (15.7%)	<0.0001***
	High (N=368)	189 (51.4%)	179 (48.6%)		High (N=443)	246 (55.5%)	197 (44.5%)	0.236

‡Chi-square test with Yates continuity correction adjusted for clustering by health facility of proportion Low and High at delivery remaining Low and High vs. switching VL categories between Arms

3.2.4 Impact of PoC VL at birth on mother clinical management and transmission rates by week 12.

During the study period, there was a change in local guidelines and all adults changed their first line treatment to a Dolutegravir based regimen. This included post-partum women. But no women changed their regimen based on birth viral load before week 12. Only adherence counselling was performed.

Table 12A shows the number of transmissions and transmission rate by intervention group from birth to week 16. Table 12B shows only those transmissions that occurred after birth (i.e. excluding infants testing positive by PoC EID in the intervention Arm and by retrospective PCR performed at the central laboratory from DBS in the control group). In Arm A, a total of 32 infants were positive at birth (PoC EID), and an additional 31 infants were diagnosed positive from 4-16 weeks of age. In Arm B, a total of 20 infants were positive at birth (retrospective PCR performed at the central laboratory from DBS) and another 26 were diagnosed positive from 4-16 weeks of age (Table 12 and Figure 16). The MTCT from birth to week 16 was 3.02 (95% CI: 2.28, 3.75) in the intervention arm, 2.39 (95% CI: 1.71, 3.07) in the control Arm, and 2.72 (95% CI: 2.21, 3.22) overall. The post-partum MTCT rate was 1.69 (95% CI :1.11, 2.26) in the intervention Arm versus 1.49 (95% CI: 0.92, 2.05) in the control Arm. There was no statistically significant difference in transmission rate between arms (HR 0.82 [95% CI: 0.56, 1.19]; p=0.297) (Figure 16).

Table 12: Number of events (MTCT) and transmission rate (95% CI§)

Table 12A: Number of events (MTCT) and transmission rate (95% CI§) at 12 weeks

	Arm A	**Arm B**	**Overall**
N (Total)	N= 2087	N= 1926	N= 4013
n (Number of events)	n= 63	n= 46	n= 109
Proportion infected at W12†	3.02 (2.28, 3.75)	2.39 (1.71, 3.07)	2.72 (2.21, 3.22)

§Wald 95% Confidence Intervals calculated using binomial distribution
†Pearson's Chi-squared tests with Yates' continuity correction for proportion infected at W12 Arm A vs. B p=0.29

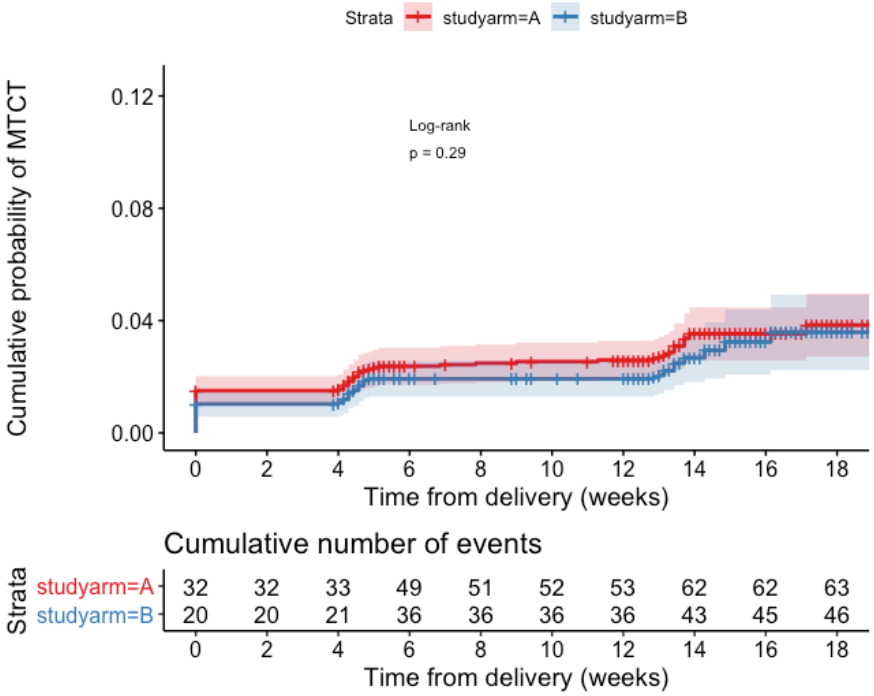
Table 12B: Post-partum number of events (MTCT) and transmission rate (95% CI§) at 12 weeks (i.e. excluding infants infected at birth**)**

	Intervention	**Control**	**Overall**
N (Total)	N= 1899	N= 1749	N= 3648

n (Number of events)	n= 32	n= 26	n= 58
Proportion infected at W12†	1.69 (1.11, 2.26)	1.49 (0.92, 2.05)	1.59 (1.18, 1.20)

§Wald Confidence Intervals calculated using binomial distribution

†Pearson's Chi-squared tests with Yates' continuity correction for proportion infected at W12 Arm A vs. B p=0.68



**p*-value was calculated by log-Rank test

Figure 16: Cumulative probability of MTCT by intervention group.

4. Discussion

Results of study 1 show that m-PIMA PoC VL testing is accurate and feasible at primary healthcare clinics in a resource-limited setting. It also shows that a routine nurse operator can perform the testing within their daily activities and can provide reliable same-day results for pregnant and breastfeeding women. Providing accurate and timely result to this high-risk group is crucial to reduce vertical transmission of HIV.

Using 1000 copies/mL as the threshold for virological failure, as defined by WHO, the diagnostic accuracy of m-PIMA PoC VL as compared to the standard TaqMan VL assay is good (sensitivity of 95.0%, specificity of 96.5% and mean bias of 0.20 log copies/mL). A study in Kenya also had similar results for m-PIMA (sensitivity 95.4% and a specificity of 96.0%) (18). Regarding to the mean bias, another study from Brazil showed a mean difference of -0.20 Log copies/mL between the mPima and the reference test (19). Although in both of these studies, the reference test used was Abbott™ RealTime HIV-1 quantitative test and not Roche CAP/CTM 96 HIV-1 Quantitative Test, both had comparable results. The performance is also similar to the GeneXpert viral load assay that has a sensitivity ranging from 95%-97% and specificity from 93%-98% at 1000 copies/mL (17,30,31).

The sensitivity and specificity of m-PIMA to detect treatment failure at detectable threshold was low (76.7% and 73.5%, respectively). The study in Kenya had similar results with m-PIMA, with a sensitivity of 56,2% and specificity of 90.26% (18). The GeneXpert has also low performance at that threshold albeit better than m-PIMA. A systematic review showed that the pooled sensitivity and specificity of GeneXpert Viral load at detectable threshold was 93.3% and 80.6%, respectively (17). The main reason for this low performance may be the volume used in m-PIMA. In M-PIMA only 50µL of plasma is used compared to 1,100µL in both the Roche and GeneXpert Viral Load assays. And the LOD of both technologies is also very different. The LOD of m-PIMA is much higher (800 copies/mL) compared to GeneXpert (40 copies/mL) which is much closer to the Roche (20 copies/mL).

While comparing the performance of the venous blood plasma PoC VL and the DBS laboratory-based test compared to the reference test, we can see that PoC VL is superior to DBS in terms of the correlation coefficient (0.922 vs 0.741), meaning that PoC VL has a better agreement with gold standard laboratory plasma-based testing. At a threshold of 1000 copies/mL, PoC has a higher

sensitivity compared to DBS (95.0% vs 78.2%). Therefore, a DBS approach would result in more false negatives (people who were actual unsuppressed but regarded as supressed) than the PoC VL. These false negatives would not be identified as potential treatment failures and thus increase the risk of transmission and resistance to treatment. This has been seen in many other studies where the sensitivity of DBS ranged from 78-100% compared to plasma testing at a threshold of 1000 copies/mL (32–34). The superior performance of PoC over DBS at this threshold will be an important consideration for policy makers as they make allocation decisions regarding how tiered laboratory networks should best reach patients outside the immediate referral area of plasma-based laboratories.

The scaling up of new PoC technologies requires proper attention to operational challenges (35,36). PoC testing for CD4 and EID has been scaled up in many countries and challenges have been addressed for successful implementation. These considerations include how to operationalize highly decentralized quality assurance programs, how to manage data with connectivity systems, and how to optimize the PoC supply chain, operator trainings and site-level patient flow. For m-Pima PoC VL, one such operational challenge is the need for centrifugation of plasma before testing. Centrifugation is generally perceived to be a laboratory-based activity. However, this study proved that this technology can be impactful when deployed to clinical consultation rooms. The provision of very small benchtop mini-centrifuges were easily operated by nurses in this study, and did not hinder testing.

We also compared the agreement of capillary blood and venous blood results and it has a strong correlation. Although the sample size was small, this can give an opportunity for implementation in settings where venous blood collection could be challenging, such as for small babies, children or in large community-based testing. The capillary blood draw option constitutes another advantage for simplifying PoC trainings especially for nurse operator cohorts who already have pre-service training and experience conducting finger pricks for lateral flow rapid tests within their consultation environment.

The direct comparison between the gold standard and the m-Pima PoC VL is challenging. Both quantify different biomarkers and have different LOD. The LOD of m-PIMA is much higher than the gold standard (800 vs 20, respectively). This interferes in the visual presentation of the graphs 2A and 2B. Currently the cut off for treatment failure is 1000 copies/mL and both LOD fall below that threshold. However, if that cut-off is changed to a lower value, the manufactures of m-PIMA

may need to adjust the assay. This would not be the only case for m-PIMA but for other technologies and sample types (DBS) as well. PoC testing, similar to other rapid testing programs, usually involves trade-offs: the gains in better testing access and in more timely clinical action are at the expense of slightly lower technical performance than the gold standard. Our study shows that the mPIMA PoC VL assay is a strong option for decentralizing viral load testing programs and preforms adequately at current global guidelines for clinical thresholds. While the suppression thresholds are important, the purpose of VL monitoring is not solely to detect above/below thresholds. More clinically relevant is to establish longitudinal understanding of a patient's viral load and make appropriate changes to treatment or social packages to keep the value as low as possible, even as global guidelines on thresholds evolve.

Study 2 demonstrates that PoC VL testing for women at maternity wards is feasible when performed by nurses in low-resource primary healthcare clinics. More than 90% of mothers received their result on the same day when using PoC VL, enabling subsequent clinical action if the viral load was above 1,000 copies/mL before they leave the maternity ward. The task shifting from technicians in laboratories to nurses in maternity wards is achievable in routine care with minimal training and supervision support. This is an important finding as it allows policy makers to consider different health facility service points for PoC VL testing, instead of focusing solely on the laboratory where bottlenecks can occur.

Overall, 21.9% of mothers had a viral load greater than 1,000 copies/mL at delivery. This is similar to global data available after one year on treatment (37). As elevated viral load around the time of delivery is associated with increased risk of mother-to-child transmission, this is a strong indication of the need to strengthen ART treatment and adherence at ANC to reduce vertical transmission.

Since the same device was used for both EID and VL at the health facilities, prioritization was made to first process EID. Nonetheless, routine EID testing, confirmation of positive EID results, baseline paediatric viral load testing, and maternal viral load was all possible on same day. Less than 10% of mothers left the health facility before having their final result PoC VL result. This shows that multiplexing EID and VL on the same mPIMA device is feasible and does not compromise priority EID testing. When additional testing is done on PoC devices it increases their utilization rate, a key metric affecting their cost-effectiveness. Increasing volumes of PoC devices

often also lowers the unit costs by reducing the apportioned equipment cost per test. Together, this helps contribute to a more affordable PoC network.

The study further showed that the PoC VL intervention did not result in meaningful difference of post-partum viral suppression rate between arms at week 12 and subsequently did not have an impact on MTCT rates. This result was different from what the STREAM Study from South Africa had shown. The STREAM study was an open-label, non-inferiority, randomized controlled trial. The study included 390 participants and the intervention Arm did PoC VL testing and task shifting to nurses compared to control Arm that did centralized laboratory-based testing. The study showed that 93% of participants in the intervention group had viral suppression at month 12 compared with 83% in the standard-of-care group (difference 10·3%, 3·9–16·8; p=0·0025)(20). The main difference between our study and STREAM study that might have affected the results was the fact the STREAM study included non-pregnant adult patients from the general population and the sample size was much lower than this study. Another difference is that the suppression rate was measured at month 12 compared to our study that was at month 3.

However, our study demonstrated that high proportions of mothers in both arms with a suppressed viral load at baseline maintained viral suppression at 12 weeks (intervention Arm: 91.6% and control Arm 84.3%). Receiving a same-day result of virally suppressed at the delivery may suggest that merely knowing this could provide motivation to continue with good treatment adherence (intervention Arm), however, a positive effect of study participation on adherence to treatment even without knowing the viral load result at delivery (control arm) cannot be overlooked. The former has been advocated by WHO and other partners to reinforce the need of having viral load testing scaled up in the countries.

Another reason for there being no significant effect on suppression rates at week 12 might be the lack of a comprehensive adherence counseling package that should ideally take into consideration psychosocial characteristics and individual barriers for viral suppression of post-partum women. Other studies have shown that there is a high risk of viral rebound during the post-partum period and that comprehensive support from the health system is needed (38–41). PoC testing is always done inside of broader systems. While PoC brings many benefits and shows good technical performance, the way results are acted upon, as with any laboratory test result, will determine the ultimate impact of the investment in diagnostic technologies.

Another factor that could have had a negative effect on suppression rates is that the clinical identification of potential failures that required a switch of treatment regimen to second line was not closely monitored. In the STREAM Study a high viral load was repeated after 2 months to know if the change to second line was needed. In our study, the follow-up was done only after 3 months and that might have delayed any action toward addressing adherence issues. In the routine care of many LIMC's, switching treatment lines is difficult for clinicians due to lack of availability of second line treatment and/or lengthy switching process. Furthermore, in the presence of drug resistance, enhanced counseling only has limited effect on viral suppression if switching is impossible (42–46). These delays in ART optimization based on virological confirmed treatment failure are especially detrimental in the case of breastfeeding mothers, as high maternal viral loads are the key risk factor for post-partum mother-to child HIV transmission. Our findings thus indicate the need to integrate improved diagnostic services such as PoC VL into an improved overall frame of HIV care and treatment services, which implies services at the local health care level as well as guideline aspects within national HIV programs.

Both studies had limitations. First is that the performance and feasibility of m-Pima was assessed within the PMTCT cascade with the aim of reducing the vertical transmission. But the results may not be generalized in other settings where different operator cadre and target population is used.
Another limitation of both studies was related to the technology used. The lower limit of detection for m-PIMA is 800 copies/mL, so establishing a cut-off of 50 copies/mL was not possible to describe the entire population. As per the new WHO treatment monitoring algorithm, the subgroup of participants that have a viral load between 50 -1000 copies/mL requires special attention and enhanced counselling.

For study 2, there was a significant difference between the proportion of suppressed (<1000 copies/mL) in Arm A compared to Arm B. The randomization should have balanced these characteristics across arms but this imbalance had to be taken into the consideration for some analysis.
Another limitation of the study 2 was the fact that the counselling process and reasons for lack of adherence was not collected by study forms. Thus, the quality of all process could not be assed and we cannot know that could have impacted the effect of PoC VL in suppressing the viral load.

Besides these limitations, both studies have their strengths. Both studies make an important contribution on the role of PoC VL in preventing mother-to-child transmission in Africa. There are no

studies available that show the impact on this particular group. The large sample size of study 2 is far more than what we have seen in other studies.

User acceptability and cost-effectiveness studies are also needed to better understand the possibility of scaling up PoC VL.

5. Conclusion

Both studies concluded that PoC VL testing is feasible and accurate when performed by nurses in a resource limited setting. PoC devices for VL testing could be set up in both ANC consultation rooms or maternity wards. The main operational challenge of deploying the m-PIMA PoC VL was the plasma separation in the clinics, but this was resolved in both studies by using appropriate mini-centrifuges. Additionally, PoC VL can be operationalized on existing PoC EID programs using the m-PIMA device. PoC VL testing does not compromise EID testing, and the use of m-PIMA along the MCH or PMTCT cascade can accommodate the priority testing needs of both mothers and children.

At the treatment failure threshold of 1000 copies/mL, the diagnostic accuracy of m-PIMA is good, but at the detectable threshold the accuracy is low. With the assay's current LOD it is not possible to categorize participants with viral load below 50 copies/mL which now defines viral suppression and undetectability according to the WHO.

PoC VL may be an important tool to identify high risk mothers that might benefit from immediate clinical intervention including reinforced counselling. Although this study did not show any clinical impact of PoC VL in reduction of maternal viral loads and consecutively in transmission rates at week 12, it does show that maintaining viral suppression was effective within a POC VL arm.

PoC VL on its own is not a solution to achieving the 3rd 95 of the 95-95-95 targets set for 2030, which requires strong clinical and psychosocial involvement. Implementing PoC VL at birth will require a comprehensive set of interventions, including effective counselling packages, effective social support and availability of second line treatments. The integration of diagnostic services and overall HIV care and treatment services, requires improvements not only at local health care services but also in national and global guidelines of HIV programs.

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7. Appendix

7.1 Published Manuscript

IMPLEMENTATION SCIENCE

OPEN

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

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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

(*J Acquir Immune Defic Syndr* 2021;87:693–699)

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.[1] 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.[2] Indeed, long turnaround times for HIV VL results have been documented across sub-Saharan Africa, with a 2016 study[3] 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.[4] 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.[5]

Point-of-care (POC) testing for HIV diagnosis and disease monitoring has been implemented with success in many resource-limited settings, with accurate performance,[6–8] patient impact,[9–11] and cost-effectiveness.[11,12] 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).[13]

Several evaluations have already established the good performance of POC and near-POC technologies for HIV VL

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assays.[14–16] 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 1º 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-naive 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 K_2 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 K_2 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

TABLE 1. Summary of Type of Sample, Operator, and Platform for Viral Load Testing Used in the Study

Quantity and Sample Type	Operator/ Placement	Platform	Procedure
50 μL of venous plasma	Nurse/ consultation room	m-PIMA	500 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 RealTime 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)[17] 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 r^2 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 r^2 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 r^2 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.

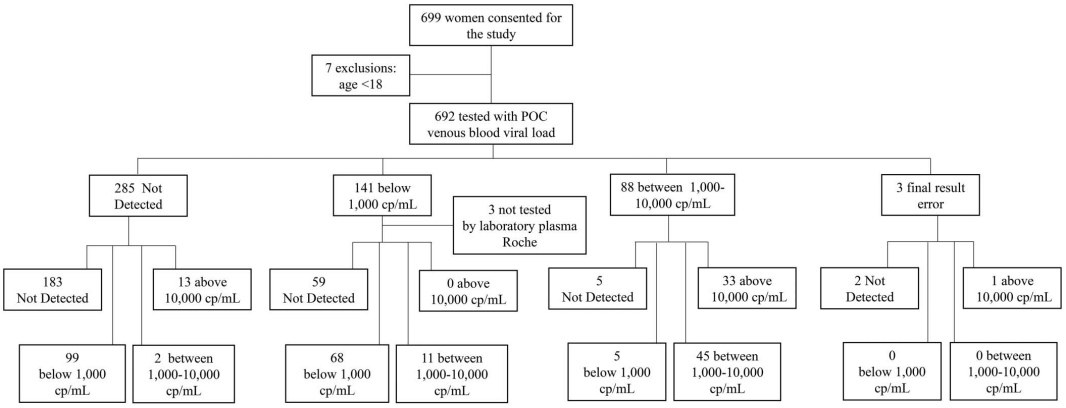

FIGURE 1. Flow diagram of participants of the study. POC, Point-of-Care; cp/mL, copies per milliliter.

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,

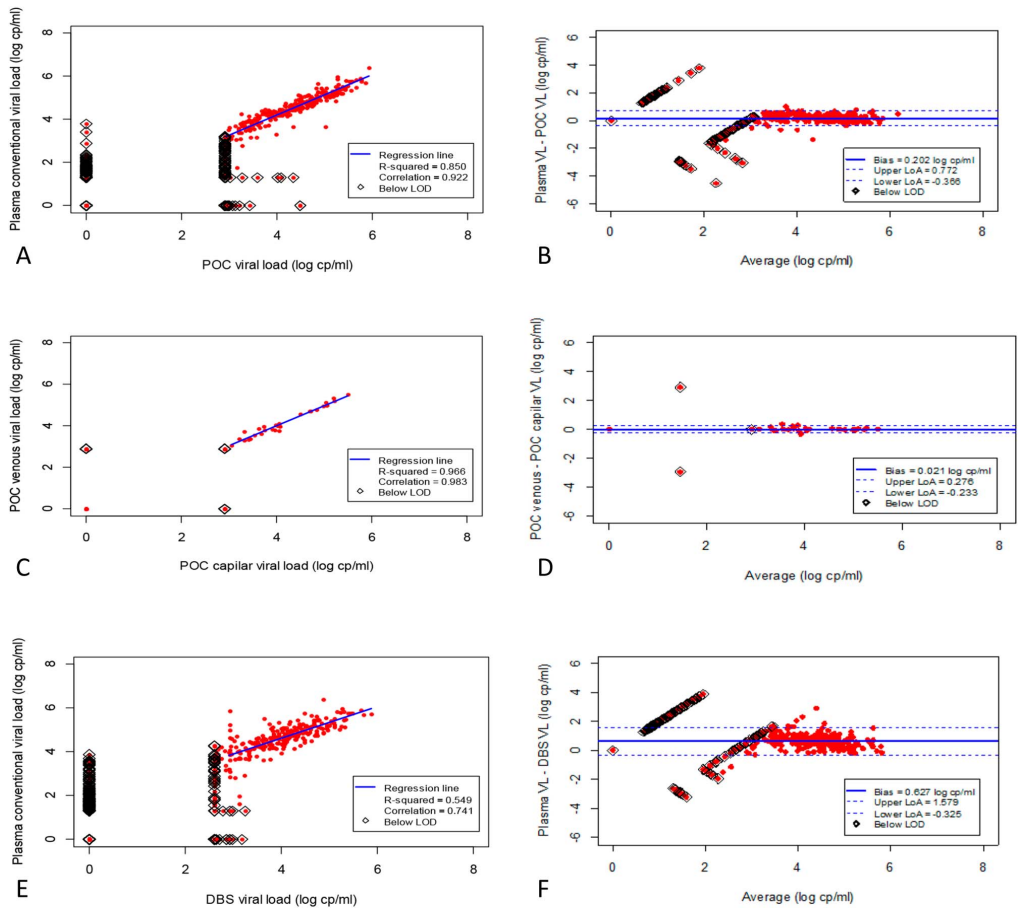

FIGURE 2. Agreement analysis viral load technologies: Linear regressions and Bland–Altman. Paired venous blood plasma m-PIMA and conventional plasma samples (A, B). Paired venous blood and capillary plasma m-PIMA (C, D). Paired DBS and conventional plasma samples (E, F).

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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7.2 Statement on Pre-release and Contribution

One article resulting from this project has been published:

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

Submission: Yes (published) | **Journal:** J Acquir Immune Defic Syndr
Aim: To evaluate the diagnostic performance of the novel m-PIMA HIV-1/2 VL assay (Abbott, Chicago, IL) in Mozambique
Role of PhD candidate: Conception and design of the study, data acquisition, analyses and interpretation of data, drafting of manuscript.

Additionally, 1 is being written from the data collected from this project:

2. Feasibility of maternal HIV POC viral load monitoring at birth and impact on post-partum viral load suppression and MTCT rates in primary healthcare maternity wards in Mozambique and Tanzania

Submission: No (Under internal review) | **Journal:** To be determined
Aim: The aim is to describe the feasibility and usability of POC VL at maternity wards in primary healthcare facilities in Mozambique and Tanzania
Role of PhD candidate: Conception and design of the study, data acquisition, analyses and interpretation of data, drafting of manuscript.

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During the writing of this thesis I have received great support and guidance in different areas that made possible for me to reach this milestone.

First and foremost, I would like to thank God, who has granted countless blessing, knowledge, and opportunity to accomplish the thesis. And a special thanks two very special people of my life who are with GOD guiding me all these years: my beloved mother and father.

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Dr. Michel Pletschette: for the guidance and knowledge and availability provided

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7.5 Study Materials

7.5.1 Study 1 Informed Consent

INFORMED CONSENT SHEET

EVALUATION OF POINT-OF-CARE TESTING ASSAYS FOR THE QUANTITATIVE MEASUREMENT OF HIV VIRAL LOAD (v 4.1)

Dear participant:
We would like to invite you to participate in a study to evaluate the performance and benefit of the new Point-of-care (POC) tests for Viral Load Diagnosis in HIV-positive individuals compared to the conventional laboratory tests used for this diagnosis.

The Diagnosis of viral load in HIV-positive individuals aims to know if the individual is in therapeutic failure of the Anti-Retroviral Treatment (ART). At present, this test is done in sophisticated laboratories that are few in the country and that makes the result take time to reach the hospital. This is called conventional testing.

POC Technologies are simple diagnostic forms that can be done in a short time and in places that do not require a lot of technology.
You were selected as candidate for this study because you meet the criteria as participant:
• Individual with HIV
• has no condition that would disrupt the evaluation results or make testing procedures dangerous to the patient.

For the purpose of this evaluation, participants will be asked to provide blood samples for Diagnosis of viral load in HIV-positive individuals using Conventional Technology and POC Technology. For tests to be performed with conventional laboratory testing instruments, the participant will be pricked into the vein to collect 10mL (two tablespoons) of blood that will be placed in a vacuum tube with EDTA anticoagulant.

For tests to be performed on POC Technology devices, a second sample of finger prick will be required, depending on the specific technology being used. 4 to 5 drops of blood will be collected. These blood collection techniques will cause temporary minor discomfort.

After blood collection, participants will be required to remain in the waiting room of the facility for a period of time until a valid result in the POCC Technology is obtained. If for some reason the POC Technology test is invalid, a sample of capillary blood may be required to repeat the test.

The POC Technology test result will not be released to you as the technique is still being evaluated. The participant will receive the result of the conventional test when you come for your next visit. The study will be important because it will allow increased access to viral load testing, which is currently done exclusively in laboratory settings, particularly to patients in rural populations without access to transportation and electricity. However, researchers can not promise or guarantee that the participant will receive any benefit directly from the study.

Any information obtained in this evaluation of participants will be kept completely confidential. In any publication in which the results of the study appear, the information will be disseminated in such a way that the participant is not identified.

By disseminating the results of this study, researchers will be able to make available the new POC Technologies currently under evaluation in health units in rural areas in Mozambique.

The decision not to participate in this study will in no way prejudice the future relationship between the participant, the health center and the Instituto Nacional de Saude. By agreeing to participate in the study, the participant is free to terminate his / her participation or tutoring (a) at any time and without any consequence.

We investigators encourage you to ask any questions about the study you may have at this time. If you has any questions in the future, the study staff will be available to respond.
A copy of this form will be given to you.

Any individual with questions or complaints about the conduct of this research should contact Dr. Bindiya Instituto Nacional de Saúde, Ministry of Health, Mozambique at (21) 309 317 (Phone).
Any individual with questions or complaints about the conduct of this research should contact the Secretary of the National Bioethics Committee for Health, Ministry of Health, Mozambique at (21) 430 814 (Telephone) or (21) 426 547 (Fax).

CONSENT
I declare that I have read the voluntary information document on this study and all my questions have been properly answered.

_____
Nome of partipant

_____ _____ _____
Signiture of the participant Date Time

_____
Name do witness (if required)

_____ _____ _____
Signiture do witness (if required) Data Time

_____
Name of the person asking consent

_____ _____ _____
Signiture of the person asking consent Data Time

Finger Print (if required)

7.5.2 Study 2 Information sheet and Consent Form

Information Sheet (English)

Version Number: 2.0, 22 February 2019 **Protocol Number:** LMU-LIFE

Protocol Title:

Neonatal HIV early infant diagnosis (EID) versus standard of care EID – Long term Impact on inFant hEalth: a feasibility study of point-of care testing at birth versus at 6 weeks of age,

on the uptake of ART and infant prophylaxis, and on rates of infant survival, morbidity and retention in care.

Principal Investigator Mozambique: Dr. Ilesh Jani
Principal Investigator Tanzania: Dr. Issa Sabi
Sponsor Medical Expert Germany: Dr. Arne Kroidl

Dear mother,
You and your newborn baby are being invited to take part in a research study with the short title "LIFE Study" which is organized by the Instituto Nacional de Saude (INS) in Mozambique, the National Institute for Medical Research - Mbeya Medical Research Centre (NIMR-MMRC) in Tanzania, and the sponsor Division of Infectious Diseases and Tropical Medicine, University Hospital, LMU Munich in Germany.

It is important for you to understand why this study is being conducted and what it will involve. This document should help you to make an informed decision as to whether or not you wish to participate in this study together with your newborn baby. Please take your time to read the following information carefully (or to have it read to you) and discuss it with others if you wish. Please ask if there is anything that is not clear to you or if you feel you need additional information.

Participation in this study is voluntary and if you decide not to take part, clinical care for yourself and your baby will not be affected. Both of you will receive the normal care and treatment at your Health Centre. If you decide for yourself and your newborn to take part in this study you will be asked to date and sign an informed consent form before any study specific procedures are performed. You will receive a copy of both this information sheet and the signed informed consent form. The father of the infant is invited to actively take part in all decision making concerning the newborn's study participation and of course you can bring any relative of yours to discuss the implication of this study.

This research project is funded by the European and Developing Countries Clinical Trials Partnership (EDCTP) and the German Ministry for Education and Research.

Background of the LIFE Study

HIV can be transmitted during pregnancy, delivery and breastfeeding from an HIV-infected mother to her baby. To reduce this risk of transmission, HIV-infected pregnant mothers should take HIV treatment during their pregnancy and continue this treatment life-long after delivery. The risk for HIV transmission is especially high if mothers who are not taking HIV treatment, have just recently started to take antiretroviral treatment, or when the HIV virus is not suppressed because of treatment failure. To further avoid HIV transmission all newborns of HIV infected mothers should receive HIV drugs for at least 6 weeks in Tanzania and even 12 weeks in Mozambique. It is not clear yet if 6 or 12 weeks of prophylactic treatment for all infants is better. A longer prophylactic treatment duration might better prevent HIV transmission during breastfeeding, a shorter treatment duration might be better to avoid drug toxicity and complications to give the drugs to the baby. But if there is a high risk for HIV transmission all infants should receive HIV drugs for 12 weeks. To further find out if there is a high risk for HIV transmission, the amount of virus can be measured in the blood of the mother. This requires that blood samples are sent to a specialized laboratory and the results are then received later. There is now a new test that can measure the virus within 2 hours at the health facility, this test is called point-of-care (PoC) viral load (VL).

Still, HIV transmission cannot be fully avoided, even if mothers take their HIV treatment. It is very important to find out if a baby is HIV-infected and then start as soon as possible HIV treatment before the baby gets sick. Currently, HIV testing in babies is performed around 6 weeks after birth. This is done, because HIV transmission can occur during birth, but the HIV in the baby can then only be detected after some weeks. It is sometimes not easy to find out if a baby is really HIV infected. HIV testing in infants requires tests that are done at specialized laboratories and the results are then received later. Again, there is a new test that can measure the virus in babies within 2 hours at the health facility; this test is called point-of-care (PoC) "Early Infant Diagnosis" (EID). It is not clear if HIV testing at week 6 is sufficient or if babies should also be tested already at the time of birth as some babies get already infected during pregnancy. The advantage of birth testing is that an HIV infection can be detected earlier and treatment can be started straight away. The disadvantage is that babies need to be pricked already at birth, that two times testing needs to be affordable for the health system, and that mothers sometime do not come back for the week 6 testing.

If a baby has a positive HIV test result it should be confirmed that this test truly indicates that the baby is HIV infected – and is not false positive. Therefore, positive HIV PoC tests should always be repeated by a second HIV PoC test.

If a baby has a positive HIV test, HIV treatment should be started immediately before the infant get sick. HIV treatment for infants is more complicated than for adults, there are fewer drugs options available for infants, the doses of HIV treatments need to be adapted as the infant grows, and it can be difficult to give the treatment to the baby. There is a need to improve HIV treatments for babies, especially if they need to start treatment as early as birth. Lopinavir/r is already given to babies in Tanzania and Mozambique, but there is a new formulation that is called Lopinavir/r granules which can be mixed with food and is better tolerated by infants. All the HIV treatments are recommended by WHO for infant treatment in Africa, but some are not yet available in your country and therefore needs to be provided by the study.

What is the purpose of the LIFE Study?

The LIFE Study is conducted at 28 health facilities in Tanzania and Mozambique where mothers deliver their babies.

- Half of the health facilities perform the PoC-EID testing in infants at birth and week 6, and the PoC VL testing for the mother during delivery. If the PoC-EID test in a baby is positive, HIV treatment will be started immediately, even as early as birth.
- The other half of the health facilities will test mothers and babies according to the current standard practice in each country. That is they will perform the PoC-EID testing in infants at week 6. Mothers will not receive VL testing at delivery. If the PoC-EID test in a baby is positive, HIV treatment should be started immediately at week 6.

The study wants to compare if HIV PoC-EID testing for infants at birth and HIV PoC-VL testing in mothers at delivery has an advantage or disadvantage.

1. Do HIV-infected babies get less often sick or even die?
2. Is HIV less often transmitted from the mother to the baby during breastfeeding?
3. Are the HIV treatments given to HIV-infected infants well tolerated and do not induce great toxicity?
4. Are HIV treatments in HIV-infected infants effective? This will be tested by measuring the viral load in regular intervals until the infant is 18 months of age
5. Is birth testing cost-effective and should therefore be afforded by the health systems

In addition, we want to perform specialized tests from blood and mother milk samples collected from mothers and blood collected from babies for science. We want to measures how the transmitted HIV viruses from mothers change in the babies, if the HIV virus is resistant against certain

HIV drugs, and how much the HIV has already infected blood cells in the baby. These tests cannot be performed in Tanzania and Mozambique, therefore blood samples will be sent to a specialized laboratory in Portugal.

If a health facilities involved in this study will provide HIV PoC testing for the infants and PoC VL testing for mothers already at birth, or provide HIV PoC testing starting from week 6 will be determined before the study starts by a process called randomization. This means that the assignment to either intervention at a health facility is by chance and all mothers and infants at this health facility will receive the same HIV testing procedures. It is therefore also by chance if you deliver your baby at a health facility that performs birth testing or not.

Who can participate in the study, who cannot participate?
Mothers and their newborn babies can participate in the study if

1. The mother or legal guardian has signed an informed consent that she agrees study participation for herself and her baby/babies
2. The mother is 18 years of age or older
3. The mother is HIV infected
4. Willingness to consent to HIV testing of the child and the mother
5. Willingness to consent to active tracing including home tracing

Study Participation is not possible for

1. Prisoners
2. Women or infants with an emergency requiring immediate medical care
3. Mothers having delivered more than 72h ago
4. Stillbirths, infants with severe malformation or very low birth weight
5. If the investigator or the doctor thinks that study participation would possibly add non acceptable risk or burden to the mother and/or infant
6. If you or the investigator have doubts that you can follow the study procedures as planned

What are the study procedures?
You and your infant(s) will start study procedures at the time of delivery; follow-up visits will take place at 6 weeks and 12 weeks after delivery.
During the first visit around birth, we might decide that you cannot participate in the study because you are not eligible according to the criteria outlined above. The following procedures will be performed during the first visit.

- Information on your age, when your HIV-infection was diagnosed, since when you are taking HIV treatment, if you have taken HIV treatments regularly, about your last CD4 count and viral load results. By this we also want to find out if there is a greater risk for HIV transmission to the baby.
- Details about your current pregnancy (e.g. day of last menstruation period), delivery (e.g. caesarian section or vaginal delivery) and the birth outcome of your child (weight, height, is your child healthy). For this information, we might ask you to allow us to also have a look into your hospital charts.
- We will take blood from you for viral load testing (only if PoC VL test is performed), CD4 count and blood samples for science (18 mL, about 4 teaspoon). If you received PoC VL testing and your viral load is too high we will advise you what you should do.

For your baby we will assess at birth:

- If is born healthy, of if we find evidence of disease
- We will take a few drops of blood by pricking the heel of the baby. This blood will be used either for HIV testing at the clinic using the PoC test, or be stored at a specialized laboratory to find out if your baby was already infected at birth when HIV is diagnosed at a later time-point.
- All babies who have not been diagnosed with HIV infection should take prophylactic treatment for at least 6 weeks, some for 12 weeks, depending if you live in Mozambique or Tanzania, or if we find that there is a higher risk for HIV transmission.

During the visits at week 6 and 12 all babies will receive PoC HIV testing at the clinic from heel blood, and a small amount of heel blood will again be send to the specialized laboratory in case we need to confirm if an HIV infection has really happened.

If a baby has a positive HIV test, HIV treatment should be started as soon as possible, even the same day and even as early as birth. We will also provide HIV treatments that are not yet available in your country, however, that are recommended by the WHO to be used for infant treatment as early as birth in African countries (e.g. lopinavir granules). We expect that these treatment are easier to take and more potent to suppressed HIV. You will receive detailed explanation how to give the HIV treatment to your baby and how to dose the treatment. We will also ask you questions

how easy or difficult it is for you to give the HIV drugs to you infants. Before HIV treatment is started we would need to take blood from the vein of your baby for the following reasons:

- To again confirm if the baby is really HIV infected (blood is sent to the specialized laboratory)
- To check if we find any abnormalities (e.g. anaemia, disease of the liver of kidney) that could complicate HIV treatment.
- Some blood will be used for science

The amount of blood has been carefully discussed and will be about 8 mL (about 2 teaspoons).

The same amount of blood needs to be again taken from HIV-infected babies from the vein at the following visits (after 6 and 12 weeks) to check if the HIV treatment is well tolerated and if the HIV is suppressed. Again, we will use some blood for science. All mothers will also receive a blood draw (3 ml, about 1 teaspoon) at week 12 to check if the HIV is suppressed in the blood.

If a baby is found to be HIV-infected at week 6 or 12 we also want to once take some blood from the mother (18 mL, about 4 teaspoons) again, plus collect a small amount of mother milk. From these samples we will check if the HIV is suppressed, if HIV drugs are effective to suppress the HIV, and some samples will be used for science.

After 12 weeks the study will be over for most mothers and their infants who are not HIV-infected by this time-point. Babies who are diagnosed HIV positive during the first 12 weeks will however be further followed-up at 6, 12 and 18 months after birth. During these visits we will again take the same amount of blood from the veins of the babies to check if the HIV treatment is tolerated, works against the HIV and perform science. Most important, at each visit we want to find out if your HIV-infected babies has become sick, was even hospitalized or even died. Therefore, it is very important for the study – and certainly your baby – that any time when your baby becomes sick you will report this and seek for care. Mothers will be asked at each visit if they are still taking HIV treatments, how they feed their babies, or if there are problems with feeding.

In addition, some mothers with babies who are not HIV-infected until week 12 will be asked to continue in the study at months 9 and 18 after birth. During this visits we want again to check from heel blood samples if the baby has become HIV-infected, using the HIV PoC test. If we find that a baby is HIV infected we will once perform the same procedures as mentioned above. After this care and treatment will be continued at your health clinic.

What happens if the mother or the baby get(s) sick?

The study wants to find out if birth testing for babies is better, as babies might less often become sick. Therefore, it is very important that we know if your baby gets sick. All procedures or treatments during the study have been used in babies and mothers before. If you or your baby gets sick your local health clinic will manage the disease, your baby might even be transferred to a larger health clinic if necessary. There is a possibility that when the health status of your baby is reviewed we may see an abnormality that we did not expect to see in this study. It is the duty of your health facility to inform you of any abnormalities related to your or your infant's health that becomes aware during the study. The study is able to provide advice, there are local paediatricians affiliated to this study who will look after sick babies if your health clinic cannot handle the problem. To some degree the study will also help if special investigations for sick infants are needed, but in general the study is not responsible to cover costs for health care. However, as we think that infant HIV testing at birth is cost effective, we will ask you about costs that you have in the case that your baby becomes sick or event needs to be hospitalized.

What will happen to the information provided and my and my infant's blood samples?

To maintain confidentiality your nor your infant's personal data and study information will not be identifiable by your names but only by a specific study number. Blood samples that are taken during the course of the study will be used to assess the HIV and HIV associated health status only. All test results obtained that are important for your and your infant's medical management or treatment decisions (viral loads, haematology, liver or kidney tests) will be provided to your doctor. Samples will be analysed at the laboratories of your health clinic or at specialized laboratories in your country (e.g. the NIMR-MMRC and Mbeya Zonal Referral Hospital in Mbeya, Tanzania, or the Beira Ponta-Gêa laboratory and Beira Central Hospital or the Central laboratory of the Chimoio Hospital Provencial in Mozambique). The study will perform further tests from your and your infant's stored blood samples to further characterize the HIV (e.g. how similar is the HIV between mothers and infants, how much are blood cells infected with the virus). These investigations are mainly of scientific interest and are usually not important for health and treatment management. Most of these scientific analyses cannot be performed in Tanzania or Mozambique**.** Blood and mother milk samples will be stored in Tanzania at the NIMR-MMRC and in Mozambique at the INS in Maputo. Some samples will be shipped to a central laboratory in Portugal, or and might then further be sent to specialized laboratories in Europe or the United States of America. All samples will not be used for commercial purposes (e.g. they will not be sold) and only

tests approved by the ethic committees in Tanzania, Mozambique and Germany will be performed. If samples will be used for other investigations that are not mentioned by this study, the ethics committees will be asked for permission before any further investigation. You will be asked throughout your signature, that samples from you or your child can be used for science.

Confidentiality and Data Protection Rights

The Principal Investigator and his designees will maintain medical records of you and your infant(s) taking part in this study. You have the right to obtain access to your personal data and to receive a copy free of charge. On your request you may even request that your personal data will be deleted, and the final decision if this is possible would be done by the ethics committee. All medical records will be held confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. By signing the consent form, you hereby grant permission for original medical records about you and your infant to be made available to authorized representatives of health authorities, ethics committees, and the sponsor. The review of these medical records may be in respect to this study and any further research that may be conducted in relation to it. The information collected will be checked by specialized data protection officers in Tanzania, Mozambique and Germany if they provide confidentiality. If you have any doubts or issues about data protection you should contact your study site which is able to also directly link you to independent data protection officers for advice.

Potential Risks – Potential Benefits

Participation in the study is thought to only add minimal risk to you and your infant. Potential risks are mainly associated with obtaining the blood samples where a short discomfort or in rare cases inflammation at the injection site can occur.

You will be informed about your babies HIV test result immediately during the study visit. A positive HIV PoC test result will trigger immediate further blood analyses in order to confirm your infant's HIV status. All confirmatory HIV test results will once available be communicated to you and your doctor and may confirm effective, beneficial HIV treatment in your infant. However, one also has to consider that false positive HIV test results can happen. In detail, this means that a positive HIV POC test result may be communicated to you but the positive result might not be confirmed by established/repeated HIV tests. You might be put under unnecessary mental stress for the time span between the HIV test result and the confirmatory HIV test result communication.

In very complicated cases the study will ask a panel of paediatric HIV experts how to best confirm that your baby is really HIV infected or not.

HIV treatment in infants starting at birth is performed in the US and Europe, in Africa most experiences are from South Africa. In general HIV treatments in infants are well tolerated, mostly better than in adults. Some HIV drugs should not be taken at birth (e.g. lopinavir), but can be started at week 2 of age. Common side effects are vomiting – mainly because infants do not like the taste of the treatments. In rare cases there is a rash because of treatments which then sometimes need to be stopped or switched. Also all infants should receive a blood draw after 4-6 weeks to find out about very rare liver or kidney toxicities, or if the child becomes anaemic. The main risk is under dosing (the virus will not be adequately suppressed) or over dosing (increased risk for toxicity) of treatments as neonates quickly gain weight and treatment dosing needs to be adjusted regularly. You will receive detailed explanation and charts how to dose and administer the HIV treatments (for HIV-infected infants) or prophylactic treatments (for not HIV-infected infants) by your nurse. If your child needs to start HIV treatment at birth a doctor from your local HIV clinic will confirm that treatment should be started, and the type of drugs and the doses are correct.

It may happen that you and your child will not directly benefit from study participation; however, you might contribute to increase the knowledge regarding HIV detection in newborns leading to benefit for others. Nonetheless, you may benefit from additional tests performed like HIV viral load testing as well as from focused medical advice.

Can I stop the study?

During the course of the study you may change your mind about being in the study. In case information becomes available that may be relevant to your willingness to continue participation in the trial you will be informed by your investigator in a timely manner. You may stop at any time, without giving a reason.

You must inform your study personnel of this decision immediately otherwise the study team will try to contact you. The decision on your part to terminate the study will not influence the availability of future medical care or other benefits to which you are entitled outside of this study. An end of study visit will be requested if you decide to withdraw.

If you decide to take part in the study your investigator has the right to stop your participation in the study at any time, with or without your consent, if he/she feels that this is in your and your infant's best interest. If you are withdrawn you may be asked to have end of study laboratory tests and evaluations.

The study or parts of the study may be stopped anytime at the discretion of the sponsor, health authorities, or the ethics committees, who review the study for the proper conduct and the rights and welfare of the participants.

My responsibility

As a participant on a research study it is your responsibility to return together with your infant to the scheduled study visits. If you cannot come, please let us know, otherwise the study team will try to contact you to understand what has happened. For this purpose we will ask you to provide us with contact details how we can best reach you.

Who makes sure that this study is done correctly?

The ethics committees in Tanzanian, Mozambique and Germany, as well as regulatory authorities (food and drug agencies) in Tanzania and Mozambique have approved this study. The study was also discussed and approved by an independent ethic advisor from South Africa and independent experts from Africa and Europe. Furthermore, the study coordinating centre in Munich, Germany will regularly visit your health facility. These institutions and people watch over this study to see that your and the rights of your infant are protected and that the researchers are following the study plan in accordance with internationally accepted standards of conducting clinical studies. In addition, the sponsor ensures that experts from Africa and Europe will monitor the proper conduct of this study as well as the safety and the well-being of the study participants.

What will happen to the results of the study?

The study results will be available once the study is completed and will be submitted for publication in relevant medical journals. Should you wish to see the results, or the publication, please ask your study personnel. You will not be identified in any report or publication.

Compensation for time and inconvenience

You will receive 10.000 Tsh for Tanzania and 250 MT for Mozambique per study visit to cover your travel expenses and to compensate the time you spend with this research.

Persons to contact in case of problems or questions

Questions about the study, study procedures or any other questions can be addressed to the institution or persons listed below. The same addressees should be contacted in case you are injured as a result of participation in this study.

Mbeya Medical Research Programme at MMRC
P.O. Box 2410 Mbeya
Tel: +255 (25) 2503364
Fax: +255 (25) 2503134

Name	Mobile Tel No:	Responsibility
Dr. Issa Sabi	+255 713 558722 +255 767 578722	Principal Investigator
Dr Ombeni Eliud Chimbe	+255 25 250 3364	Research Coordinator

Instituto Nacional De Saúde
Tel: +258 21430814/427131
Fax: +258 21426547

Name	Mobile Tel No:	Responsibility
Dr. Ilesh V. Jani	+258 843012208	Principal Investigator
Dr Arlete Mahumane	+255 826425947	Research Coordinator

Contact with Ethics Committees
You may also wish to communicate with the ethics committees regarding this study by contacting the following:

Mbeya Medical Research and Ethics Committee Dr. Godlove Mbwanji Mbeya Zonal Referral Hospital P.O. Box 419 Mbeya Phone: +255-25-2503456 or 2503351 Fax: +255-25-2503577	**Chairman** **National Ethics Committee** **National Institute for Medical Research (NIMR)** Ocean Road, P.O. Box 9653, Dar es Salaam Phone: +255-22-2121400 Fax: +255-22- 2121360

Comité Institucional de Bioetica para Saúde do INS Vila de Marracuene Estrada Nacional N°1, Parcela N°3943 Província de Maputo – Moçambique Telefone: +258 21430814/427131 Fax: +258 21426547	**Comité Nacional de Bioetica para Saúde de Moçambique** Av. Eduardo Mondlane/Salvador Allende R/C , Maputo, Moçambique Caixa Postal 264 Telefone: +258-21427131/4. Mobile: +258-843012211 ou +258-843012212

Contact with Data Protection Officer
If you have concerns regarding the use of your data you may also ask the data protection officer in Germany responsible for the conduct of the study.

Authorised Data Protection Officer Medical Center of the University of Munich (LMU) Pettenkoferstr. 8, 80336 Munich, Germany Email: datenschutz@med.uni-muenchen.de	**Bavarian State Authority for Data Protection (BayLfD)** Postal: P.O. Box 22 12 19, 80502 Munich, Germany Phone.: +49-89 212672-0; Fax: +49-89 212672-50

Thank you for taking the time to read this information leaflet and for listening to the explanation about this study.

Informed Consent Form (English)

Version Number: 2.0, 22 February 2019 **Protocol Number:** LMU-LIFE

Protocol title:
Neonatal HIV early infant diagnosis (EID) versus standard of care EID – Long term Impact on inFant hEalth: a feasibility study of point-of care testing at birth versus at 6 weeks of age, on the uptake of ART and infant prophylaxis, and on rates of infant survival, morbidity and retention in care.

Principal Investigator Mozambique: Dr. Ilesh Jani
Principal Investigator Tanzania: Dr. Issa Sabi
Sponsor Medical Expert Germany: Dr. Arne Kroidl

I, the undersigned: _____declare that I have read and/or had this information sheet explained to me, I have had the opportunity to ask all the questions I wanted to _____.

I have had a period of reflection and, by the present, I agree to participate in the study, knowingly and freely, by signing this form.

I understand the objectives, constraints, risks and benefits related to my and my infant's participation in this aforementioned study.

I accept that the study team might try to contact me including home visits in the case of problems or missing visit schedules.

I accept that my and my infant's personal data and study information collected during this trial may be subjected to computerized processing. To ensure confidentiality these data will not be identifiable for the study by our names but only by a specific number (pseudonymous data)

I accept that all nurses, doctors, and scientists involved in this study as well as the representatives of the health authorities and ethic committees have access to information in the strict respect of confidentiality.

I can, at any time, if I so wish, withdraw my or my infant's participation without having to justify my decision, but I will do my best to inform _____. The termination will not have any effect on the quality of subsequent care for me and my infant.

I have been informed that by signing this informed consent form, I do not renounce to any of my rights and do not, in any way, release the investigators or hospitals where this study is taking place from their legal and professional responsibilities.

I have been told that I will receive a dated and signed copy of this form and the information sheet.

I authorize that blood samples will be stored in order to conduct further tests including virus characterisation in Tanzania and Mozambique or collaborating institutions outside Tanzania and Mozambique:

□ YES □ NO

I hereby agree to participate together with my newborn infant(s) in the LIFE Study.

_____
(Participant's / Legal guardian's full name)

Date: ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Signature or thumb print

Optionally: I, the father of the newborn infant, agree that my infant participates in the LIFE Study.

_____
(Father's full name)

Date: ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Signature or thumb print

Only applicable for illiterate participants: By signing the consent, I attest that the information in the consent form and any other written information was accurately explained to, and apparently understood by the subject and that informed consent was freely given by the subject.

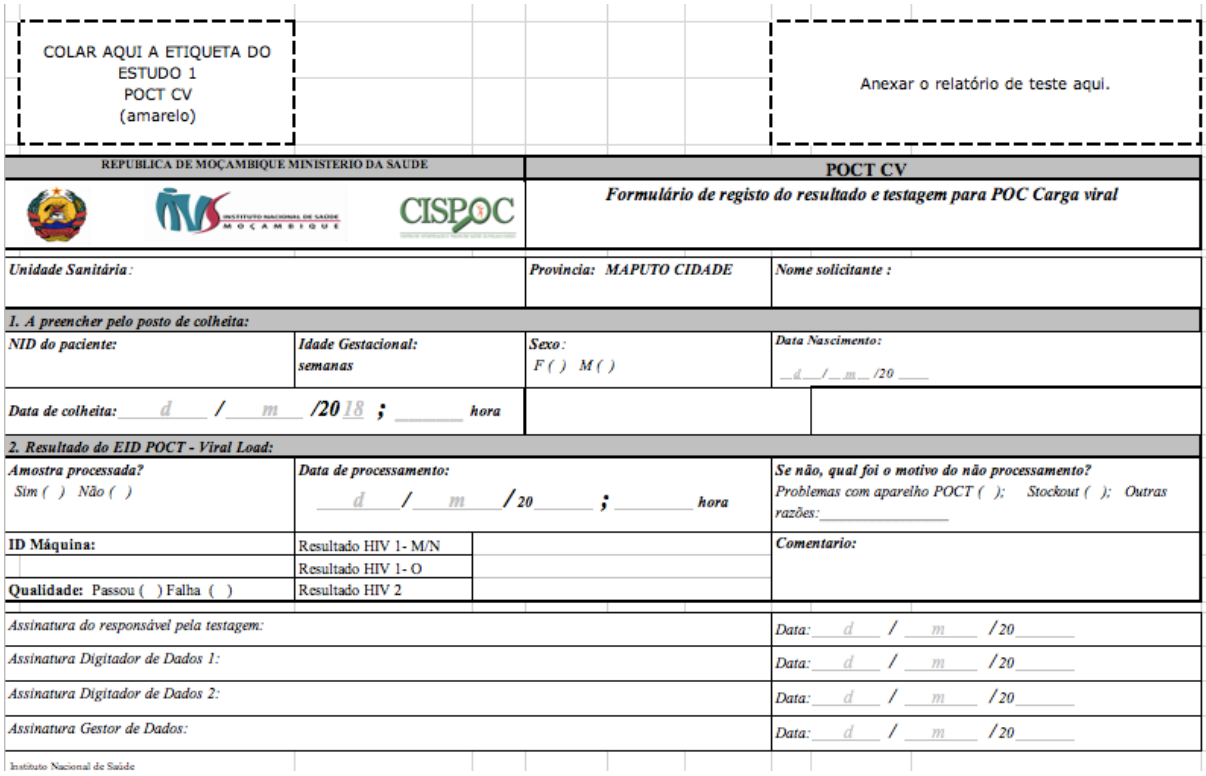
_____
Witness' last name and first name (only applicable for illiterate participants)

Date: ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Signature

I, the undersigned **study personnel**, _____ certify that I have clearly explained the objective, duration, risks and benefits of this protocol to the participant.

Date: ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋ ⌊_⌋⌊_⌋⌊_⌋⌊_⌋ Signature

7.5.3 Study 1 Data Collection Form



COLAR AQUI A ETIQUETA DO ESTUDO 1 POCT CV (amarelo)		Anexar o relatório de teste aqui.

REPUBLICA DE MOÇAMBIQUE MINISTERIO DA SAUDE		POCT CV
		Formulário de registo do resultado e testagem para POC Carga viral

Unidade Sanitária:	*Provincia: MAPUTO CIDADE*	*Nome solicitante :*
1. A preencher pelo posto de colheita:		
NID do paciente:	***Idade Gestacional:*** *semanas*	***Sexo:*** *F ( ) M ( )*
Data Nascimento: *__d__/__m__/20____*		
Data de colheita: *__d__ / __m__ /20 18 ;* _____ ***hora***		
2. Resultado do EID POCT - Viral Load:		
Amostra processada? *Sim ( ) Não ( )*	***Data de processamento:*** *__d__ / __m__ /20____ ;* _____ ***hora***	***Se não, qual foi o motivo do não processamento?*** *Problemas com aparelho POCT ( ); Stockout ( ); Outras razões:*_____
ID Máquina:	Resultado HIV 1- M/N	***Comentario:***
	Resultado HIV 1- O	
Qualidade: Passou ( ) Falha ( )	Resultado HIV 2	

Assinatura do responsável pela testagem:	*Data: __d__ / __m__ /20____*
Assinatura Digitador de Dados 1:	*Data: __d__ / __m__ /20____*
Assinatura Digitador de Dados 2:	*Data: __d__ / __m__ /20____*
Assinatura Gestor de Dados:	*Data: __d__ / __m__ /20____*

Instituto Nacional de Saúde

7.5.4 Study 2 Data Collection Form

7.5.4.1 Birth Data Collection Form

Data Collection Form	DCF-1 Delivery (VISIT 1)	Page 1 of 5
LIFE Study	PID: |__| - |__|__|__|__|.|__| - |__|	MOTHER

0.	Date of Visit	|__|__| . |__|__|__| . |__|__|__|__| D D M M M Y Y Y Y
1. Inclusion criteria – to be assessed before any study specific procedure is performed		
1.1.	Voluntary and informed consent of the mother and her child for study participation	☐1 Yes ☐2 No → *mother/infant pair not eligible*
1.2.	Is the mother and, if applicable, the legal guardian, 18 years or older?	☐1 Yes ☐2 No → *mother/infant pair not eligible*
1.3.	Documented maternal HIV infection	☐1 Yes ☐2 No → *mother/infant pair not eligible*
1.4.	Consent to HIV testing for mother (if mother participates) and child included in the study	☐1 Yes ☐2 No → *mother/infant pair not eligible*
1.5.	Consent to active tracing inclusive home tracing	☐1 Yes ☐2 No → *mother/infant pair not eligible*
2. Exclusion Criteria – to be assessed before any study specific procedure is performed		
2.1.	Deficiency in the mother for herself or the infant to take part in the study or understand the information provide (including alcoholism, drug dependency, psychiatric illnesses, suicidal tendencies or any other inability)	☐1 Yes → *mother/infant pair not eligible* ☐2 No
2.2.	Having delivered more than 72 hours ago	☐1 Yes → *mother/infant pair not eligible* ☐2 No
2.3.	Prisoners	☐1 Yes → *mother/infant pair not eligible* ☐2 No
2.4.	Women presenting with an emergency requiring immediate medical assistance if not resolved at study inclusion	☐1 Yes → *mother/infant pair not eligible* ☐2 No
2.5.	Study adds within the discretion of the investigator or the involved gynaecologist not acceptable risk or burden to the mother.	☐1 Yes → *mother/infant pair not eligible* ☐2 No
2.6.	Mother/legal guardian is unlikely to comply with protocol as judged by the investigator or his designee	☐1 Yes → *mother/infant pair not eligible* ☐2 No
3. Eligibility and study participation		
3.1.	Is the MOTHER eligible for study participation?	☐1 Yes, and infant is eligible/enrolled → *Complete mother DCF-1 & fill DCF-21 (Participant Entry)* ☐2 Yes, but infant is not eligible/enrolled → *Stop mother DCF-1 & fill DCF-21 (Participant Entry)* ☐3 No, but infant is eligible/enrolled → *Stop mother DCF-1 & fill DCF-21 (Participant Entry)*
4. Demographics		
4.1.	Date of maternal birth	|__|__| . |__|__|__| . |__|__|__|__| D D M M M Y Y Y Y
4.2.	Education	☐1 None ☐2 None, but able to read and write ☐3 Primary school ☐4 Secondary school ☐5 Post secondary

5. HIV history and treatment		
5.1.	Date of maternal HIV diagnosis	|__|__| . |__|__|__| . |__|__|__|__| or ☐ Not known D D M M M Y Y Y Y
5.2.	Has the mother disclosed her HIV status to her family	☐1 Yes ☐2 No
5.3.	Is the mother currently on antiretroviral treatment?	☐1 Yes ☐2 No → If **Yes**, go to **5.4** If **No**, go to **5.5**
5.4.	Antiretroviral medication currently taken by the mother (one answer possible): ☐1 Tenofovir + Lamivudine + Efavirenz ☐2 Tenofovir + Emtricitabine + Efavirenz ☐3 Zidovudine + Lamivudine + Efavirenz ☐4 Zidovudine + Lamivudine + Nevirapine ☐5 Tenofovir + Lamivudine + Nevirapine ☐6 Tenofovir + Emtricitabine + Nevirapine ☐7 Zidovudine + Lamivudine +Lopinavir/ritonavir ☐8 Tenofovir + Lamivudine + Lopinavir/ritonavir ☐9 monotherapy Zidovudine ☐10 other, specify_____ _____ Start Date of above treatment: |__|__| . |__|__|__| . |__|__|__|__| → *Go to* **5.6** D D M M M Y Y Y Y	
5.5.	The mother is currently not on antiretroviral treatment. → was there an antiretroviral treatment taken ever before ? ☐1 Yes ☐2 No If Yes indicate stop date of previous ART: |__|__| . |__|__|__| . |__|__|__|__| ☐ Not known D D M M M Y Y Y	
5.6.	Last CD4-count	|__|__|__|__| cells/µl or ☐ not known
5.7.	Date of last CD4-count	|__|__| . |__|__|__| . |__|__|__|__| or ☐ not known D D M M M Y Y Y Y
5.8.	Was a viral load assessment performed during the last 4 weeks?	☐1 Yes ☐2 No If yes, what was the outcome: ☐1 Not detected/below detection limit ☐2 |__|__|__|__|__|__| copies/mL ☐3 don't know
6. Pregnancy and delivery		
6.1.	Attendance of Antenatal Care (ANC):	☐1 Yes ☐2 No — first trimester (week 1 to 12) ☐1 Yes ☐2 No — second trimester (week 13 to 27) ☐1 Yes ☐2 No — third trimester (week 28 to 42)
6.2.	Gravida (number of pregnancies inclusive the current one):	|__|__|

6.3.	Para (number of births without current):	|__|__|
6.4.	First day of last menstrual period:	|__|__| . |__|__|__| . |__|__|__|__| or ☐ not known D D M M M Y Y Y Y
6.5.	Gestational age at delivery in weeks	|__|__| weeks or ☐ not known
6.6.	Date of delivery:	|__|__| . |__|__|__| . |__|__|__|__| D D M M M Y Y Y Y
6.7.	Time of delivery:	|__|__| : |__|__| H H M M (24 hours clock)
6.8.	Number of infants delivered:	☐1 Single ☐2 Twins ☐3 Triplets or more
6.9.	Was there a premature rapture of the membrane?	☐1 Yes ☐2 No ☐3 Don't know
6.10.	Mode of delivery:	☐1 normal vaginal, at home ☐2 normal vaginal, at hospital ☐3 assisted vaginal, at home ☐4 assisted vaginal, at hospital ☐5 elective caesarian section ☐6 emergency caesarian section ☐7 other, specify:_____
6.11.	Complications during delivery?	☐1 Yes ☐2 No If Yes, specify

☐1 Yes ☐2 No	antepartum haemorrhage
☐1 Yes ☐2 No	postpartum haemorrhage
☐1 Yes ☐2 No	prolonged labour
☐1 Yes ☐2 No	features of chorioamnionitis (e.g. fever, PROM, foul smelling fluid)
☐1 Yes ☐2 No	malpresentation of the child (e.g. obligue, transverse lie)
☐1 Yes ☐2 No	obstructed labour (e.g. cephalopelvic disproportion, shoulder dystocia)
☐1 Yes ☐2 No	poor uterine contraction strength
☐1 Yes ☐2 No	abnormal presentation of the placenta (e.g. placenta praevia)
☐1 Yes ☐2 No	abnormal umbilical cord presentation (e.g. cord around the neck)
☐1 Yes ☐2 No	Others, specify _____

6.12.	Did the mother die before discharge	☐1 Yes ☐2 No ***→ if Yes fill DCF-15 (Medical Events) and Reportable Event Form***

7. Point-of-Care (PoC) VL testing		
7.1.	Was PoC VL testing performed?	☐1 Yes ☐2 No, indicate reason:_____ ☐3 N/A (Study Arm B) → if **No or N/A**, go to section **8**
7.2.	Date PoC VL blood sample was taken	|__|__| . |__|__|__| . |__|__|__|__| D D M M M Y Y Y Y
7.3.	Time PoC VL blood sample was taken	|__|__| : |__|__| H H M M (24 hours clock)
7.4.	Final PoC VL result	☐1 Not detected ☐2 Below detection limit ☐3 |__|__|__|__|__|__| copies/mL ☐4 error/invalid
7.5.	Was the PoC VL repeated?	☐1 Yes ☐2 No → if yes, indicate reason ☐1 First test error/invalid ☐4 Did not trust first result ☐2 Power-cut ☐5 Other ,specify ☐3 Not enough blood _____
7.6.	Date PoC VL test result was obtained	|__|__| . |__|__|__| . |__|__|__|__| D D M M M Y Y Y Y
7.7.	Time PoC VL test result was obtained	|__|__| : |__|__| H H M M (24 hours clock)
7.8.	Any comments about PoC test handling, performance, etc.?	☐1 Yes ☐2 No If yes, specify _____
7.9.	Date PoC VL test result was communicated to mother	|__|__| . |__|__|__| . |__|__|__|__| D D M M M Y Y Y Y
7.10.	Time PoC VL test result was communicated to mother	|__|__| : |__|__| H H M M (24 hours clock)
7.11.	Was high VL counselling performed?	☐1 Yes ☐2 No, please indicate reason:_____ ☐3 No, VL was <1000 copies/mL
8. Other sampling – laboratory tests performed		
8.1.	Blood draws for	☐1 Yes ☐2 No — CD4 count → Fill results DCF-20 (CD4-count) ☐1 Yes ☐2 No — Viral load → Fill results DCF-17 (Viral load) ☐1 Yes ☐2 No — Blood biorepository **Note:** Request VL only if this is indicated and not done by PoC at the clinic!

9. *High-risk criteria for HIV mother-to-child transmission (HR-MTCT)*			
9.1.	Do any of the following HR-MTCT criteria apply	☐1 Yes ☐2 No/Not done	VL ≥1000 copies/mL at delivery if performed
		☐1 Yes ☐2 No	Known VL ≥1000 copies/mL during past 4 weeks and VL at delivery not known
		☐1 Yes ☐2 No	Mother not on ART at delivery
		☐1 Yes ☐2 No	Mother HIV diagnosed at delivery for the first time
		☐1 Yes ☐2 No	Mother on ART <4 weeks at delivery and VL at delivery not known or VL not <1000 copies/mL
		☐1 Yes ☐2 No	Clinical reasons, specifiy:_____

→ if any of the above criteria is "Yes" this should be considered high-risk for HIV mother-to child-transmission and the infant should receive enhanced infant prophylactic treatment!

→ all mothers without ART or new HIV-diagnosis should start ART as soon as possible!

→ all mothers on ART with VL≥1000 copies/mL should receive enhanced ART counselling and should be referred for repeated VL testing or treatment switch!

DCF filled by: _____	*Signature Date:* |__|__| |__|__|__| |__|__|__|__| *Day Month Year*
DCF checked by: _____	*Signature Date:* |__|__| |__|__|__| |__|__|__|__| *Day Month Year*
1st data entry by: _____ ___	*Entry Date:* |__|__| |__|__|__| |__|__|__|__|
2nd data entry by: _____	*Entry Date:* |__|__| |__|__|__| |__|__|__|__| *Day Month Year*

7.5.4.2 Week 12 Data Collection Form

Data Collection Form	DCF-3 Week 6 (VISIT 2)	Page 1 of 2
LIFE Study	PID: |__| - |__|__|__|__|.|__| - |__|	MOTHER

0.	Date of Visit	|__|__| . |__|__|__| . |__|__|__|__| (D D M M M Y Y Y Y)
1. Medical Update		
1.1.	Did Mother attend the visit?	☐1 Yes ☐2 No → *If **No**, specify **(only one answer possible)*** ☐1 Not attended visit, tracing initiated or ongoing ☐2 Withdrawn study participation → ***Fill DCF-22 (Participant Exit)*** ☐3 Died → ***Fill DCF-15 (Medical Events), Reportable Event Form, DCF-22 (Participant Exit)*** ☐4 Hospitalized → ***Fill DCF-15 (Medical Events), Reportable Event Form*** ☐5 Other specify, _____ → *If **No**, end of CRF*
1.2.	Was tracing (telephone or home visit) needed for mother to attend the visit?	☐1 Yes ☐2 No
1.3.	Any AE (medical or social event related to the study) since the last visit?	☐1 Yes ☐2 No → ***if Yes fill DCF-15 (Medical Events)***
1.4.	Any SAE (serious medical or social event related to the study) since the last visit?	☐1 Yes ☐2 No → ***if Yes fill DCF-15 (Medical Events) and Reportable Event Form***
1.5.	Any hospitalization since the last visit?	☐1 Yes ☐2 No → ***if Yes fill DCF-15 (Medical Events) and Reportable Event Form***
2. HIV Treatment		
2.1.	Any changes in HIV treatment since last visit?	☐1 Yes ☐2 No → ***if No go to section 3*** If Yes, ☐1 HIV treatment was started → ***go to question 2.3*** ☐2 HIV treatment was changed → ***go to question 2.3*** ☐3 HIV treatment was stopped → ***go to question 2.2***
2.2.	The mother has stopped HIV treatment - indicate stop date?	|__|__| . |__|__|__| . |__|__|__|__| (D D M M M Y Y Y Y) → ***go to section 3***
2.3.	HIV medication mother was started or switched to (one answer possible): ☐1 Tenofovir + Lamivudine + Efavirenz ☐2 Tenofovir + Emtricitabine + Efavirenz ☐3 Zidovudine + Lamivudine + Efavirenz ☐4 Zidovudine + Lamivudine + Nevirapine ☐5 Tenofovir + Lamivudine + Nevirapine ☐6 Tenofovir + Emtricitabine + Nevirapine ☐7 Zidovudine + Lamivudine +Lopinavir/r ☐8 Tenofovir + Lamivudine + Lopinavir/r ☐9 other, specify_____ Start Date of above treatment: |__|__| . |__|__|__| . |__|__|__|__| (D D M M M Y Y Y Y)	

3. COMPLIANCE to HIV treatment		
3.1.	Over the course of the last week, on how many occasions did you forgot take part or all of your HIV treatment? ***(only one answer possible)***	☐1 Never ☐2 1-2 times ☐3 2-4 time ☐4 More than 4 times ☐5 Do not take HIV treatment
3.2.	Over the course of the last month, was the anti-HIV treatment interrupted? ***(only one answer possible)***	☐1 Never ☐2 For 1 day or more but less than a week ☐3 For 1 to 2 weeks ☐4 For more than two weeks ☐5 Do not take HIV treatment
4. Breastfeeding information		
4.1.	Is the mother breastfeeding?	☐1 Yes ☐2 No ***→ if No go to section 5***
4.2.	Have you experience and or the following breast problems since the last visit?	☐1 Yes ☐2 No If Yes, specify
		☐1 Yes ☐2 No — Swelling
		☐1 Yes ☐2 No — Warmth
		☐1 Yes ☐2 No — Erythema
		☐1 Yes ☐2 No — Pain
		☐1 Yes ☐2 No — Purulent discharges
		☐1 Yes ☐2 No — Fissures/ulcerations
		☐1 Yes ☐2 No — Others, specify _____
5. Infant HIV status & maternal sampling		
5.1.	Was an infant of this mother newly diagnosed HIV-positive at this visit	☐1 Yes ☐2 No ***→ if No skip question 5.2***
5.2.	Were the following samples collected?	☐1 Yes ☐2 No — CD4 count → Fill results DCF-20 (CD4-count)
		☐1 Yes ☐2 No — Viral load → Fill results DCF-17 (Viral load), PoC or TaqMan
		☐1 Yes ☐2 No — Blood biorepository
		☐1 Yes ☐2 No — Mother milk samples

DCF filled by: _____	*Signature Date:* |__|__| |__|__|__| |__|__|__|__| *Day Month Year*
DCF checked by: _____	*Signature Date:* |__|__| |__|__|__| |__|__|__|__| *Day Month Year*
1st data entry by: _____ ___	*Entry Date:* |__|__| |__|__|__| |__|__|__|__|
2nd data entry by: _____	*Entry Date:* |__|__| |__|__|__| |__|__|__|__| *Day Month Year*