EVALUATING THE PERFORMANCE OF NUCLEIC ACID TESTS TO DIAGNOSE HIV IN INFANTS AND YOUNG CHILDREN EXPOSED TO ANTIRETROVIRAL PROPHYLAXIS

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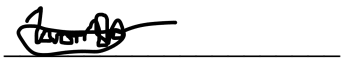
A dissertation submitted to the Faculty of Science, University of Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science

July 2021 in Johannesburg

DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree Master of Science by research only, at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.



Aurélie Mukendi-Isomba

23rd day of July 2021

In memory of my sister

Nadège Mukendi

1985-2017

PRESENTATIONS ARISING FROM THIS STUDY

- Mukendi A, Kufa T, Murray T, Burke M, Strehlau R, Technau KG, Tiemessen CT, Sherman GG, Haeri Mazanderani A. Evaluating the performance of the GeneXpert HIV-1 Qualitative assay as a consecutive test for a new early infant diagnosis algorithm. International Workshop on HIV Pediatrics; 2020 16-17 November Virtual Workshop. Guided Poster Tour and Presentation. (Appendix G)

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- Mukendi A, Kufa T, Sherman GG, Technau KG, Tiemessen CT, Mazanderani AH. Evaluation of the Aptima HIV-1 Quant Dx assay for HIV diagnosis at birth in South Africa. Accepted for publishing in the Journal of Diagnostic Microbiology and Infectious Disease.

COMPLETED ABSTRACTS SUBMITTED FOR CONFERENCES

- Mukendi A, Kufa T, Sherman GG, Technau KG, Tiemessen CT, Mazanderani AH. Evaluation of the Aptima HIV-1 Quant Dx assay for HIV diagnosis at birth in South Africa. Submitted to Pathology Research and Development Congress.

ABSTRACT

Background: As a consequence of a high maternal HIV prevalence, approximately 270,000 HIV exposed infants are born per annum, requiring interventions to prevent mother to child transmission of HIV. However, among infants who do become infected with HIV, the exposure to maternal and infant prophylactic antiretroviral regimens may lead to indeterminate and false-negative HIV PCR results. To address these challenges, inclusion of numerous assays within the national early infant diagnosis (EID) algorithm may need to be considered.

Objectives: The objective was to evaluate the diagnostic performance of the Hologic Aptima HIV-1 Quant Dx assay (Hologic Gen-Probe, Inc., San Diego, CA), in detecting HIV infection in neonates, and the GeneXpert HIV-1 Qualitative Assay (Cepheid, Sunnyvale, CA, USA) as a consecutive test in infants with an HIV-detected screening test.

Methods: Specimens were collected from HIV-exposed neonates for whom birth testing was performed using ethylenediaminetetraacetic acid whole blood samples at four tertiary sites in Gauteng between June 2014 to January 2020. The performance of the Aptima, compared with the Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0 (CAP/CTM), and the performance of the Xpert EID, compared with HIV status on CAP/CTM follow-up testing, were determined as proportions with 95% confidence intervals.

Results: The Aptima assay had a sensitivity of 93.75% (95% CI: 79.19%-99.23%), specificity of 99.4% (95% CI: 97.83%-99.93%), positive predictive value (PPV) of 93.75% (95% CI: 78.98%-98.36%), negative predictive value (NPV) of 99.4% (95% CI: 97.73%-99.84%), and overall accuracy of 98.9% (95% CI: 97.2%-99.7%).

As a consecutive assay, the Xpert EID yielded a sensitivity of 96.5% (95% CI: 92.1%-98.9%), specificity of 100% (95% CI: 54.1%-100%), PPV of 100% (95% CI: 100%), NPV of 54.5% (95% CI: 33.7%-74.1%), and overall accuracy of 96.7% (95% CI: 92.4%-98.9%%).

Conclusion: The Aptima assay and the Xpert EID demonstrated good EID diagnostic performance. The Aptima assay can be used as an alternate assay within South Africa's National Health Laboratory Service. The Xpert EID can be used as a consecutive test for patients with an HIV-detected screening test, and will reduce diagnostic uncertainty and time to final result.

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NOMENCLATURE

Abbott test	Abbott RealTime HIV-1 Test
Aptima	Aptima HIV-1 Quant DX assay
ART	Antiretroviral therapy/treatment
ARV	Antiretroviral drugs
AZT	Zidovudine
BANC	Basic antenatal care
CAP/CTM	Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0
CD4 count	CD4 Cell Count (expressed as 10^6/litre)
CI	Confidence interval
Cobas assay	Roche COBAS AmpliPrep/TaqMan HIV-1 Test v2
Ct	Cycle threshold
DBS	Dried blood spot
DNA	Deoxyribonucleic Acid
DTG	Dolutegravir
EDTA	Ethylenediaminetetraacetic acid
EID	Early infant diagnosis
ELISA	Enzyme-linked immunosorbent assay
eMTCT	Elimination of mother to child transmission
HIV	Human immunodeficiency virus
LTFU	Loss to follow up
MTCT	Mother to child transmission
NAAT	Nucleic acid amplification test
NHLS	National Health Laboratory Service
NPV	Negative predictive value
NSP	National Strategic Plan
NVP	Nevirapine
PCR	Polymerase chain reaction
PMTCT	Prevention of mother to child transmission
POCT	Point of care
PPV	Positive predictive value
RFI	Relative fluorescence intensity
sdNVP	Single dose nevirapine

SOP	Standard operating procedure
UNAIDS	The Joint United Nations Programme on HIV/AIDS
VL	Viral load
WHO	World Health Organization
Xpert EID	GeneXpert HIV-1 Qualitative Assay

CHAPTER 1

1. Literature Review

1.1. Introduction

In 2019, it was estimated that 13.5% of the total population, approximately 7.97 million people, were living with HIV in South Africa (SA). [1] The highest prevalence was found in adults aged 15 to 49 years, estimated to be at 20.6%. Within this estimate, men who have sex with men, transgender women, sex workers, people who inject drugs and pregnant women, presented even higher HIV prevalence (Figure 1.1). The incidence of HIV in women is four times greater than in men. [2]

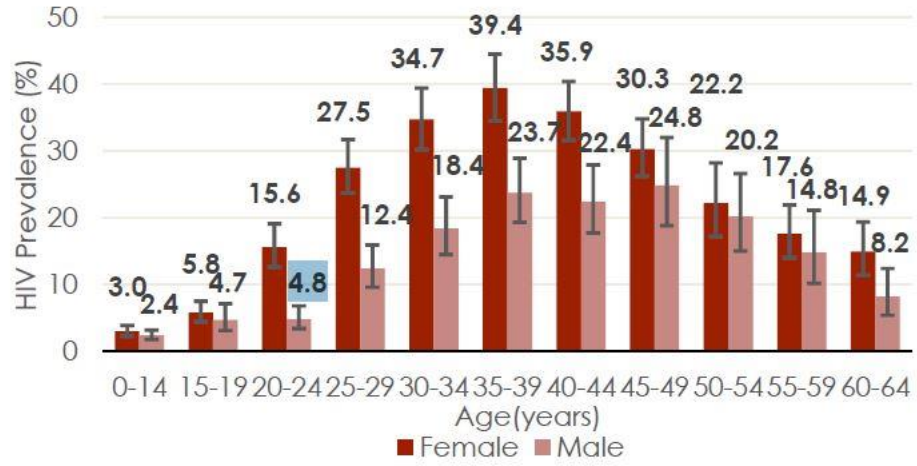


Figure 1.1 HIV prevalence in South Africa for 2019, by age and sex [3]

In an effort to combat the high prevalence of HIV, SA has developed the largest antiretroviral treatment (ART) programme in the world, with nearly 4.1 million people living with HIV retained on treatment as of 2018 [4] (Figure 1.2).

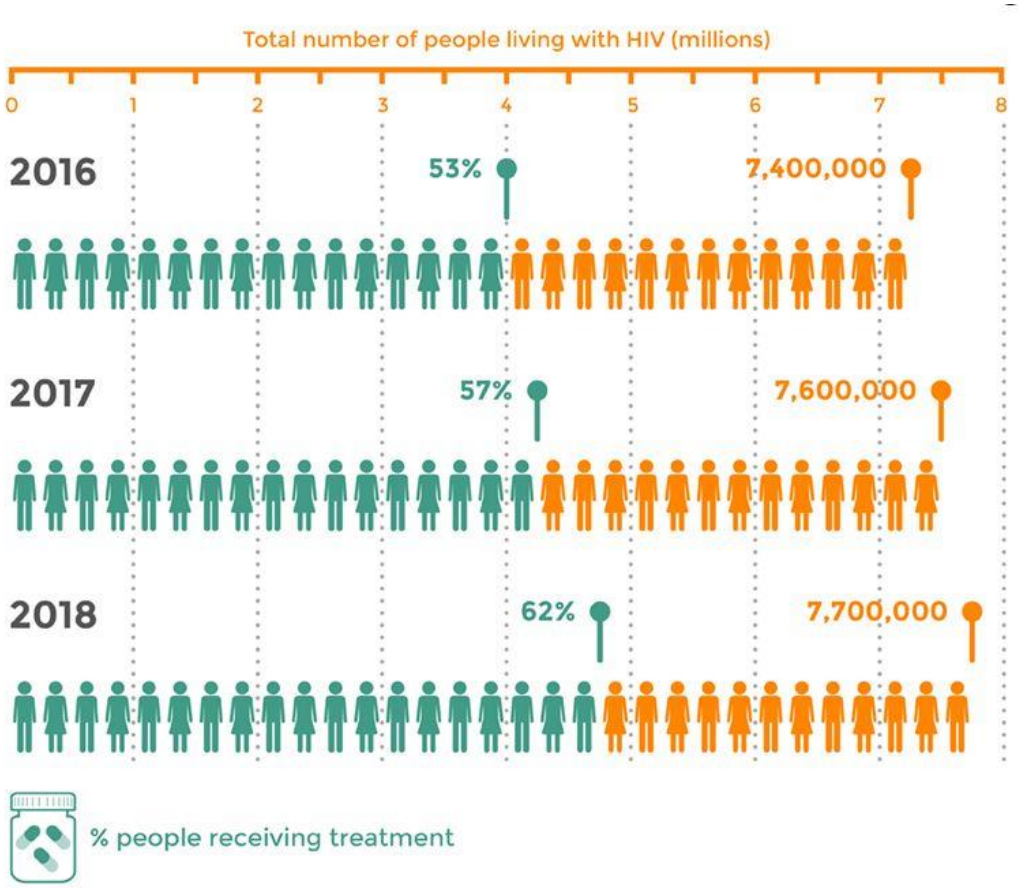


Figure 1.2 People living with HIV in South Africa receiving treatment 2016-2018 [5]

The high maternal prevalence of HIV means that per annum, approximately 270 000 HIV-exposed infants are born. Infants born to women living with HIV require interventions to prevent mother to child transmission (MTCT) of HIV, [6] with accurate and timely HIV testing services being of the utmost importance. Globally mother to child transmission of HIV accounts for approximately 9% of new infections [7], vertical transmission of HIV can occur during pregnancy, labour and delivery, or through breastfeeding. Without early diagnosis and treatment, HIV can have a devastating effect among infants and young children, and can lead to infant mortality, [2] or chronic diseases that greatly shortens life expectancy.

Antenatal HIV prevalence amongst women attending antenatal care clinics in SA, is approximately 30.8% [8-9] (Figure 1.3). Antenatal care clinics allow women living with HIV access to ART, providing an opportunity for mothers to improve their health outcomes. In spite of a persistently high maternal HIV prevalence, the ART programme has proven

particularly successful among pregnant women living with HIV for the prevention of mother to child transmission (PMTCT). Maternal adherence to ART, and infant antiretroviral (ARV) prophylaxis interrupts the cycle of HIV transmission. Among infants who do become infected with HIV, timely access to ART delays detrimental disease progression, thus preventing HIV-related morbidity and mortality. It is estimated that without ARVs, up to 52% of perinatal HIV infections, and 26% of post-natal HIV infections, would result in infant death. [10-11]

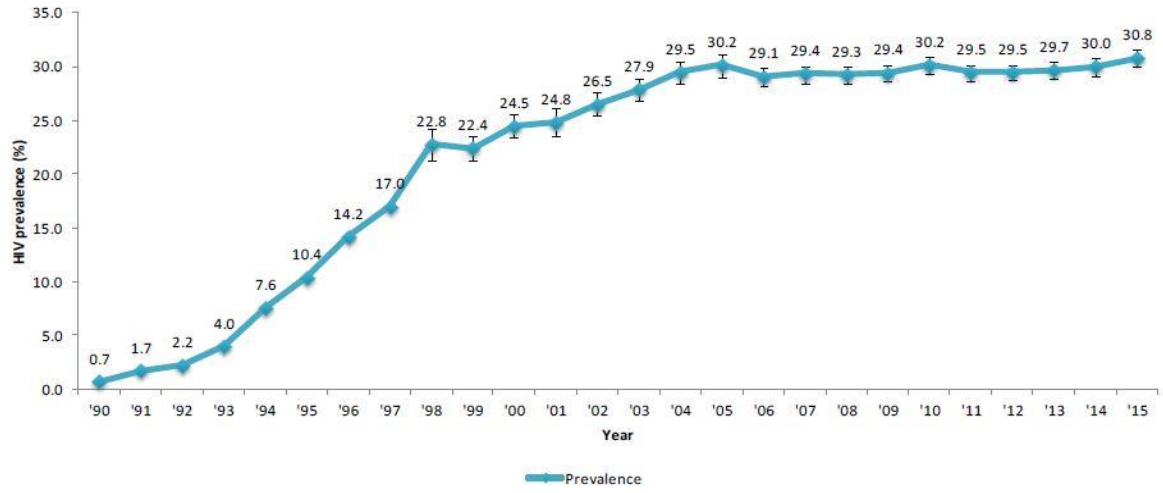


Figure 1.3 National antenatal HIV prevalence in South Africa 1990-2015[10]

Maternal adherence to ART allows for virological suppression to occur, thereby reducing the risk of MTCT of HIV. As such, a critical part of the ART programme extends to pregnant women living with HIV and the interventions needed for PMTCT of HIV. [11]

1.2 Prevention of mother to child transmission programme

The PMTCT programme refers to the interventions implemented to prevent vertical transmission of HIV, from women living with HIV to their infant/s during pregnancy (*in utero*), labour and delivery (intrapartum), and breastfeeding (postnatally). The PMTCT programme enables women to access HIV testing services during routine antenatal appointments. The applied interventions of the PMTCT programme, namely the provision of ART to pregnant women living with HIV and ARV prophylaxis to HIV-exposed infants, has been instrumental in reducing the risk of infant HIV acquisition. This was evident in the significant reduction of the national early infant HIV transmission rate, from >20% to <2% between 2004 and 2015. [12] This translates to roughly 832 HIV-infected infants per 100 000 live births. [12,13] As per World Health Organization (WHO) global targets, SA needs to

achieve 50 or less new paediatric infections per 100 000 live births, and a transmission rate of 5% or less among breastfeeding women, 2% or less among non-breastfeeding women, in order to successfully meet the criteria for elimination of mother to child transmission (eMTCT) of HIV. [14] In order to achieve the eMTCT of HIV targets, the Last Mile Plan was developed. A key focus of the Last Mile Plan is to reduce the number of PCR positive infants at around 10 weeks. The implementation of the Last Mile Plan saw a reduction of PCR positive infants around 10 weeks from 1.35% to 0.9% in 2017. [10] In South Africa, HIV remains the third leading cause of maternal mortality. [15] Therefore, prioritising the health of women living with HIV and preventing MTCT of HIV is crucial in order to ensure positive health outcomes for all women and children living with HIV. The PMTCT program outlines four essential pillars for the elimination of MTCT of HIV. These pillars are outlined in Figure 1.4**.**

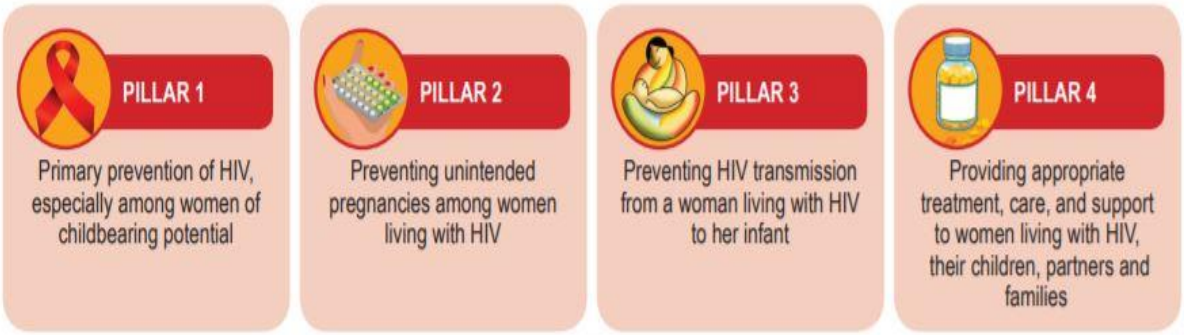


Figure 1.4 The four pillars of PMTCT for HIV [15]

The first iteration of the PMTCT programme in 2004 included the provision of single dose nevirapine (sdNVP) to women living with HIV, and their children within 72 hours. [11, 16-17] In 2010, the guidelines were amended so that infants who were identified as being at low-risk of vertical transmission of HIV, received nevirapine (NVP) for six weeks. In 2015 infants who were deemed to be at high risk of vertical transmission of HIV had prophylaxis extended to twelve weeks, with zidovudine initially started in the first six weeks. In Table 1.1, the risk factors associated with high vertical transmission of HIV in infants are outlined.

Table 1.1 Risk factors associated with vertical transmission of HIV [7]

Maternal Health Risk Factors	Infant Health Risk Factors
HIV diagnosis confirmed after 28 weeks of pregnancy.	A low birthweight, clinically defined as below 2.5 kilograms.
Poor maternal adherence to ART.	Delivered before week 37 of gestation is completed.
Co-infection with other sexually transmitted diseases such as chlamydia, syphilis etc.	
High plasma viral load greater than 1000 copies/ml* at delivery or late pregnancy.	
Short ART history less than 12 weeks *prior* to delivery.	
HIV seroconversion during pregnancy.	

*Low levels of viraemia defined as HIV RNA ≤1000 cps/ml, have been found to be significant in transmission of HIV from women living with HIV to their infant/s. [7] ART, antiretroviral treatment; HIV, human immunodeficiency virus

The WHO PMTCT Option B+ [18] implemented in South Africa in 2015 had as one of its recommendations that all women living with HIV who are pregnant, breastfeeding or are within one-year post-partum, be provided with lifelong ART, regardless of their CD4 cell count or clinical stage. Furthermore, it was recommended that short-term ARV prophylaxis be provided to all infants born to women living with HIV for 6-12 weeks, in order to prevent MTCT of HIV.

In 2019, South Africa updated their PMTCT guidelines with a focus on the prevention of HIV and unintended pregnancies among women of childbearing potential, maternal viral suppression, preventing MTCT during the breastfeeding period, and retention in care of women living with HIV and their children. A summary of the main changes is presented in Table 1.2.

Table 1.2 Summary of the main changes in the SA PMTCT programme, 2019 [19]

Contents	2015 Consolidated HIV Guideline	2019 PMTCT Guideline
Maternal HIV Testing	At first visit, at 32 weeks, delivery and every three months during breastfeeding	At first visit and at every subsequent BANC visit, and three-monthly during breastfeeding.
Maternal HIV VL Monitoring	Guidelines for newly diagnosed mothers, and women living with HIV already on ART.	Additional guidance for previously ART-exposed mothers. VL done at delivery and at six months post-partum for all women, and six monthly during breastfeeding.
Infant HIV testing	HIV PCR test at birth and 10 weeks. 18-week PCR test for high-risk infants who received extended NVP for 12 weeks. HIV test at six weeks' post-cessation of breastfeeding. 18-month rapid test for HIV-exposed infants.	HIV PCR test at birth and 10 weeks remain unchanged 18-week HIV PCR test for high-risk infants removed Six-month HIV PCR test for all HIV-exposed infants introduced. At six months of age, establish the HIV status of all infants not already known to be HIV-exposed by offering an HIV test to the mother. If a maternal HIV test is not feasible, consent should be obtained to perform a rapid HIV test on the child. Age-appropriate HIV testing at six weeks' post-cessation of breastfeeding is retained, with emphasis on testing even if breastfeeding continues for longer than 18 months. 18-month rapid test/ ELISA for all children regardless of HIV exposure (universal testing). HIV PCR test used as a confirmatory test for any HIV-positive result up to age two years
Infant post-exposure prophylaxis	High-risk infants: AZT for six weeks, and NVP prophylaxis for 12 weeks.	AZT for six weeks and NVP prophylaxis for a minimum of 12 weeks. NVP is stopped after 12 weeks only if the maternal VL is proven to be < 1000 copies/mL. If the maternal VL is not suppressed by 12 weeks, continued NVP is given until maternal VL suppression is achieved, or until four weeks after breastfeeding cessation.

ART, antiretroviral treatment; AZT, zidovudine ; BANC, basic antenatal care; ELISA, enzyme-linked immunosorbent assay; HIV, human immunodeficiency virus; NVP, nevirapine; PCR, polymerase chain reaction; VL, viral load

In order to achieve eMTCT, retention in care of all pregnant women living with HIV, and ensuring treatment adherence in all breastfeeding mothers during pregnancy, and postnatally needs to be a priority focus. Furthermore, in spite of PMTCT interventions, there are infants who do become infected with HIV, highlighting the importance of regular and accurate HIV testing services among pregnant and breastfeeding women who test HIV negative.

1.3 The importance of accurate early infant diagnosis services

The WHO recommends that all children who are infected with HIV should be initiated on ART regardless of their clinical and/or immunological status. [20] However, only fifty percent of children living with HIV globally receive ART. [9] HIV infection has a devastating impact on children, particularly among infants. [8] The morbidity and mortality rates are highest among infections that are untreated. A comparison between HIV infected older children with higher CD4 cell counts and infants revealed that there was a higher disease progression rate and higher mortality rate among infants. [8] Early infant diagnosis (EID) and initiation of treatment in infants is crucial in order to achieve virological suppression, significantly reduce the size of the HIV viral reservoir [21], prevent rapid disease progression, and HIV-related infant deaths. [22]

1.4 Early infant diagnosis testing methods

Infants living with HIV have the highest rate of mortality between the ages of 6 weeks to 4 months. Therefore, EID is crucial in order to identify infants that need prompt ART initiation. [23] Early infant diagnosis is defined as testing of infants to establish their HIV status following HIV-exposure during pregnancy (*in utero)*, labour and delivery (intrapartum), and breastfeeding (postnatally). [24] The presence of maternal HIV antibodies in the HIV-exposed infant poses a challenge for accurate diagnosis of HIV in infants and young children using serological tests. For this reason, the diagnosis of HIV among infants and children less than two years of age is achieved by nucleic acid amplification testing (NAAT), with highly sensitive polymerase chain reaction (PCR) testing most commonly used. [6,25]

In South Africa, HIV PCR testing in the public sector is performed within one of eleven designated EID laboratories within the National Health Laboratory Service (NHLS). [26] Prior to 2019, guidelines in SA recommend routine qualitative HIV PCR testing at birth (birth

testing relates to HIV PCR tests performed in the first week of an infant's life), 10 weeks of age, and 6 weeks' post-cessation of breastfeeding (if weaning occurs before 18 months of age) for all HIV-exposed infants. [11] The greatest advantage of birth PCR testing is that it allows clinicians to identify intra-uterine HIV-infected infants and provide such infants with early linkage to care and ART initiation. [6,25] Intrapartum and postnatal HIV infections are not detectable at birth, thus repeat testing is necessary. [25,27] In 2019 HIV PCR testing at 6 months was introduced for all HIV-exposed infants, and serological testing at 18 months of age for all children regardless of HIV exposure history. [19]

South Africa has a comprehensive HIV EID programme using 11 centralized laboratories, all of which use an assay from a single manufacturer. From 2014, the Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA.), referred to hereon as the CAP/CTM, has been used for HIV PCR testing across all NHLS EID laboratories. The CAP/CTM is a dual-target *in vitro* diagnostic real-time PCR assay targeting the long terminal repeat (*LTR*) and Gag region of the HIV-1 genome (Figure 1.5).

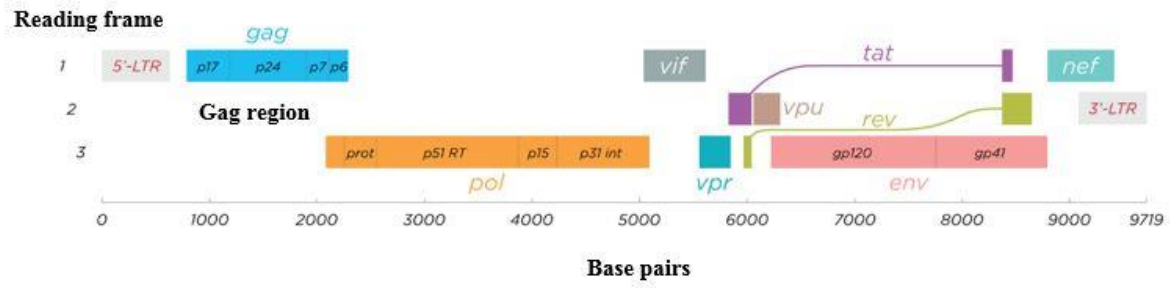


Figure 1.5 Structure of the RNA genome of HIV-1 with *LTR* and Gag region identified [28]

A national standard operating procedure (SOP) is applied across the NHLS with routine testing performed on whole blood, collected either in an ethylenediaminetetraacetic acid (EDTA) tube or on a dried blood spot (DBS) filter paper card. Dried blood spot specimens carry a low biohazard risk, and are easier to collect, store and transport in resource-limited settings. [29-30] In South Africa, DBS specimens are the predominant sample type for EID. The lower limit of detection (LoD) of the CAP/CTM when using DBS specimens is around 300 RNA copies/mL. The reported diagnostic sensitivity of the CAP/CTM using DBS is 100%, with a specificity of 99.9%. [31] Result interpretation on the CAP/CTM as per package insert is shown in Table 1.3.

Table 1.3 Result interpretation on the CAP/CTM as per the manufacturer's package insert [31]

Sample Type	Detection Result	Interpretation
EDTA Plasma	Not Detected	Ct value for HIV-1 above the limit for the assay or no Ct value for HIV-1 obtained. Report results as "HIV-1 not detected".
	Detected	Report results as "HIV-1 detected".
DBS	Not Detected DBS	Ct value for HIV-1 above the limit for the assay or no Ct value for HIV-1 obtained. Report results as "HIV-1 not detected".
	Detected DBS	Report results as "HIV-1 detected".

Ct, cycle threshold; DBS, dried blood spot; EDTA, ethylenediaminetetraacetic acid; HIV, human immunodeficiency virus

The SA guidelines for PMTCT of HIV recommends prompt ART initiation for all infants with a positive HIV-PCR. Furthermore, South African guidelines recommend confirmatory testing on a new sample for any child under two years with a positive HIV-PCR test. At the discretion of clinicians, a viral load (VL) test, instead of a confirmatory PCR test, may be performed which allows confirmation of HIV diagnosis as well as providing a baseline VL which can be used to monitor ART response in the infant or child. The care of HIV-exposed infants regardless of final HIV status is illustrated in Table 1.4.

Table 1.4 Care of HIV-exposed infants up to 6 months of age [15]

3-6 DAYS	6 WEEKS	10 WEEKS	6 MONTHS
Birth PCR is performed. All results are followed-up and managed accordingly. Infants with a detected HIV result should be discussed/referred to a clinician experienced in managing an HIV positive neonate. ART should be initiated even if the infants weighs less than 2,5 kg.	Ensure that birth PCR and mother's VL results were checked, recorded and acted upon correctly.	HIV-PCR test performed for all HIV-exposed infants who previously tested HIV-PCR negative.	**Known HIV-exposed infants:** Perform HIV-PCR test at 6 months in all HIV-exposed infants, except in those who previously tested positive and are on ART. **Infants not known to be HIV-exposed:** • At six months of age, establish the HIV status of all infants not already known to be HIV-exposed • Offer an HIV test to the mother. If she tests HIV negative, no infant test is required. • If the mother is not available, or refuses an HIV test, get consent and do an HIV rapid test on the infant. • All positive infant rapid tests need to be confirmed with an HIV-PCR.

ART, antiretroviral treatment; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; VL, viral load

1.5 Challenges to early infant diagnosis of HIV

Since the implementation of the PMTCT programme, SA has made great strides in EID coverage. [5] The implementation of a National Strategic Plan (NSP), now in its fourth

iteration, has positively impacted EID birth testing coverage [2] which was at 93.5% in 2018. [32] This has been associated with a reduction of intrauterine infected infants diagnosed during the postnatal period. In the 2017/2018 period the national HIV PCR birth positivity rate was 0.9% [33], which was significantly lower than the positivity rate of 1.3% that was projected for the 2018/2019 period in the NSP. [2] Furthermore, a reduction in the MTCT transmission rate of HIV at six weeks from more than 3.5% to ~1.5% [32] was also achieved. These are important milestones as SA continues to move towards its goal of eMTCT. Nevertheless, major challenges to EID testing services still exist. Previous studies have reported on post-analytic challenges relating to result return. Such studies indicated poor maternal return rate for collection of results, as well as linkage of infected infants to care [33] as significant challenges that impact access to EID of HIV. In addition to this, factors related to a decrease in both the diagnostic sensitivity and the positive predictive value (PPV) of tests used for infant diagnosis of HIV are significant challenges for accurate EID of HIV.

As a consequence of the PMTCT programme, increased maternal access to ART combined with a reduced HIV infection rate among children, has led to a reduction in the incidence of paediatric HIV. Infant exposure to maternal ART means that among infants with a true HIV-infected status, HIV RNA may remain undetectable [34] as result of viral suppression. This makes accurate diagnosis of an infant's HIV clinical status harder, particularly in the case of HIV-exposed infants with a true positive HIV clinical status. [35] In addition, there are concerns that infant exposure to ARVs *in utero* and, in the postnatal period via breastmilk may interfere with accurate diagnosis of HIV in infants and young children further compromising the accuracy of tests used for EID. [36] Furthermore while infant ARV prophylaxis reduces the risk of perinatal acquisition of HIV, the exposure to ARV prophylaxis may cause virological suppression. This can result in reduced diagnostic sensitivity in assays used for HIV diagnosis and result in infants receiving false-negative HIV diagnosis. False-negative results may lead to interruption of ART for infants with a true HIV-infected status, which may have detrimental consequences. The performance of tests employed for EID of HIV therefore need to be re-evaluated within the context of infant exposure to ARVs in order to establish an accurate HIV diagnosis. [37]

As the prevalence of paediatric HIV has continued to fall in SA, the PPV of infant diagnostic tests used for HIV has also reduced. [34,38] There is a non-linear relationship between disease prevalence and the PPV of a test. Figure 1.6 illustrates this relationship, whereby the lower

the prevalence of a disease the less likely the person testing positive is to be truly infected. [35,39] A decreased EID PPV means that a greater proportion of HIV-detected PCR results will be false-positives.

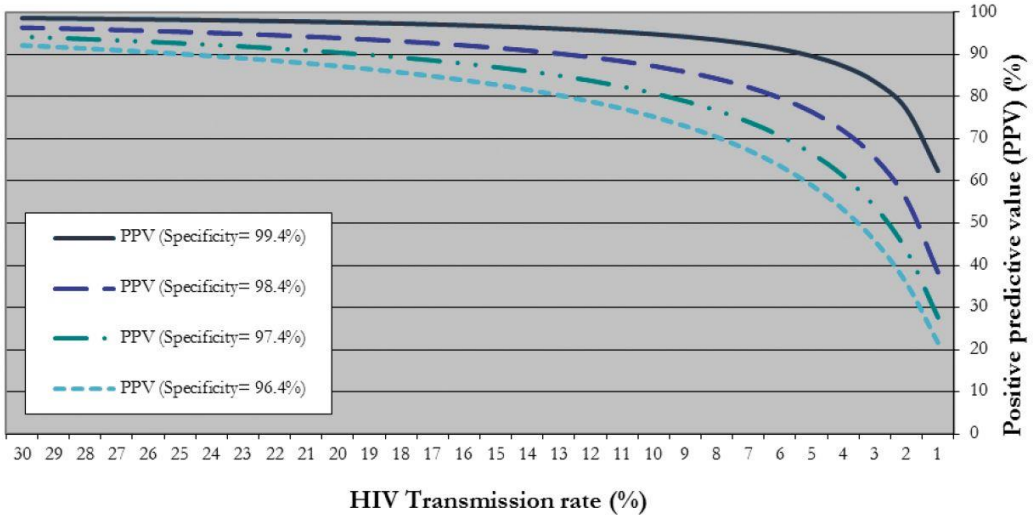


Figure 1.6 HIV transmission rates and the effect on the PPV of NAATs used for EID of HIV [40] Figure 1.6 demonstrates the effect that the HIV transmission rate has on the PPV of diagnostic tests used for infant diagnosis of HIV. When the transmission rate of HIV is high, the PPV remains high, as the transmission rate decreases, so too does the PPV

While reported specificities for EID virological assays are generally good, some ≥99%, [25] not all infants who receive a positive HIV PCR result will be infected with HIV. [18] For infants who are HIV-exposed but uninfected and receive a false-positive diagnosis, the effects of unnecessary treatment initiation are costly and can cause medication related toxicities. Furthermore, psychologically a false-positive HIV diagnosis can be detrimental and subject infants to HIV-associated stigma that unfortunately remain commonplace. [41]

1.6 Indeterminate results and their impact on early infant diagnosis of HIV

Viral suppression has the potential to limit the size of the HIV reservoir in infants, [41] thereby impacting HIV results obtained from diagnostic testing. This can lead to infants receiving indeterminate and false-negative HIV PCR results. [37, 43-44] Indeterminate and false-negative results represent diagnostic challenges within the EID cascade. Such results can cause a significant delay in ART initiation in the HIV-exposed infant, [43] as well as the time to determining final HIV status in the infant.

An indeterminate HIV PCR result is as a valid result, reported by the testing instrument as HIV-detected, but interpreted as being neither clearly positive nor negative i.e. inconclusive. [41,43] The cycle threshold (Ct) value is associated with the amount of PCR product in the reaction - a higher Ct value indicates a low concentration of nucleic acid target material or a false-positive result. [44-45] Within the NHLS, indeterminate results are differentiated from positive results by the observed Ct value, and relative fluorescence intensity (RFI) value. The detectable target emits a low amplified viral signal that can be misinterpreted as a potential false-positive. [45]

In lieu of using multiple assays, the WHO recommends the use of an indeterminate range for nucleic acid–based EID assays to address this anticipated increase in the false-positivity rate, and differentiate potentially false-positive results from clearly positive cases. This is currently estimated to be equivalent to a Ct value of >33 when using the CAP/CTM. [18] Similarly, in SA the NHLS national EID SOP defines an indeterminate as a result with an RFI below 5, and/or Ct value >33. Figure 1.7 shows the amplification curve of two specimens, one with an indeterminate result (a) on the CAP/CTM from a patient who tested negative on repeat testing, and the other with a clear positive HIV PCR result (b).

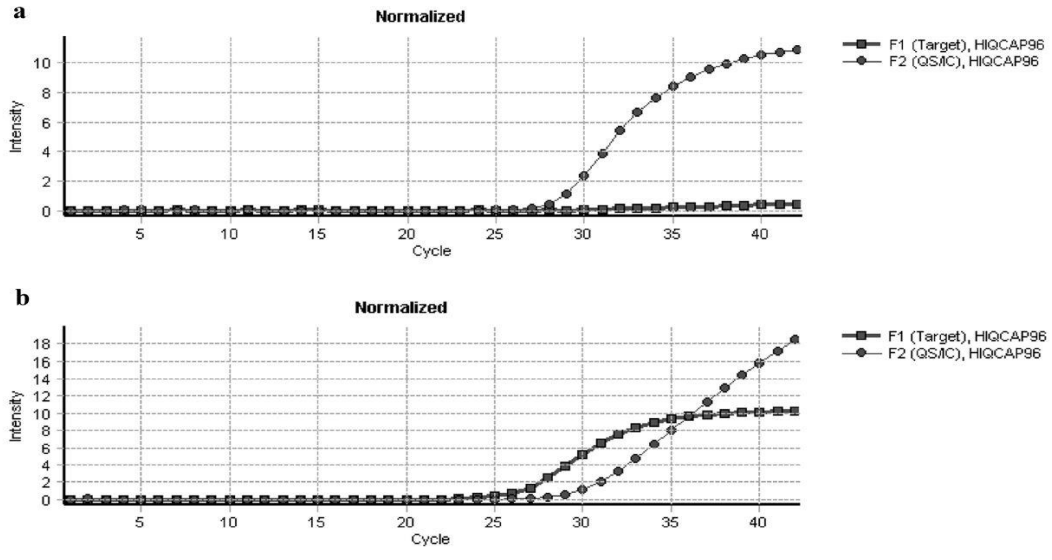
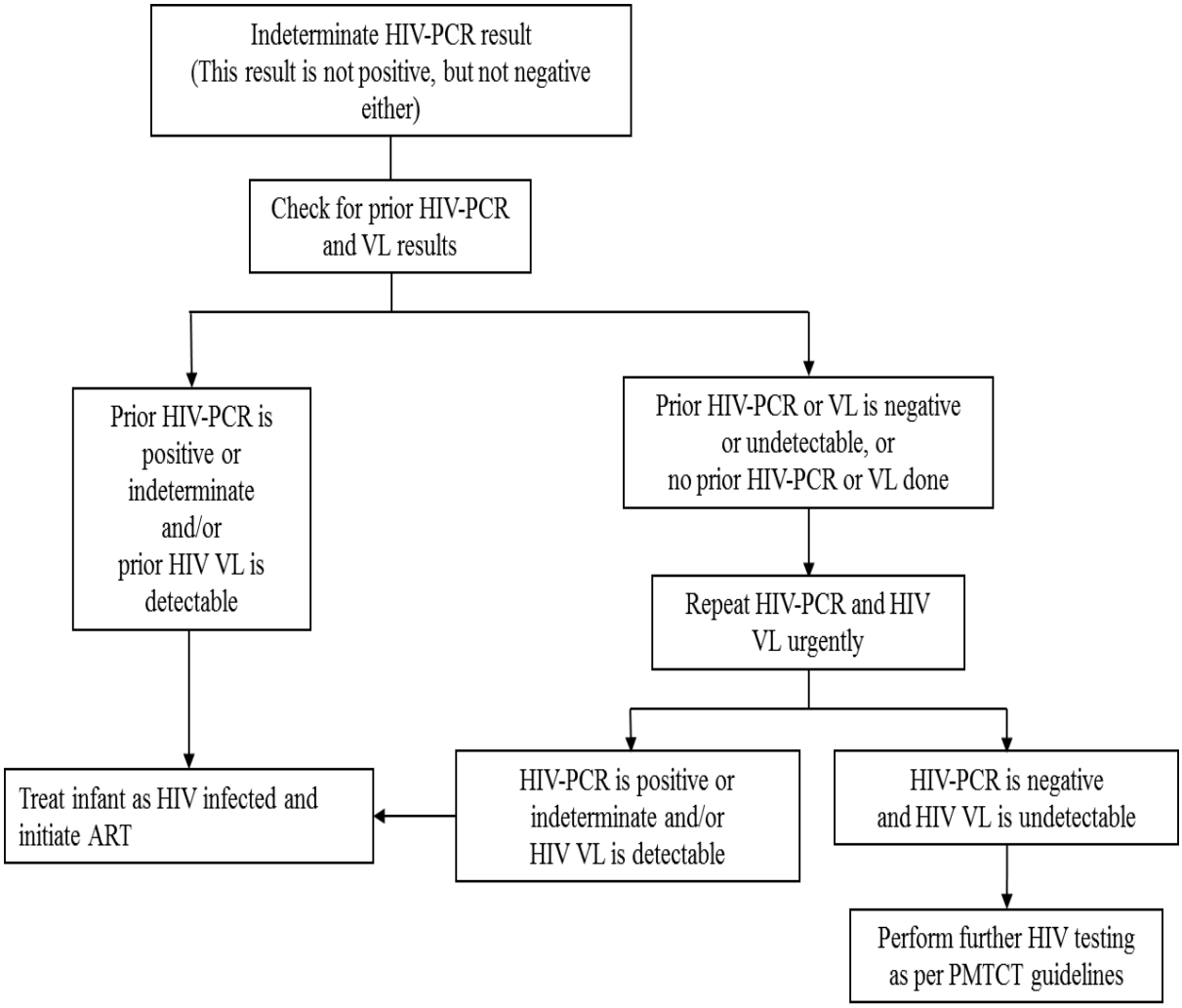


Figure 1.7 Amplification curves obtained from HIV PCR testing on the CAP/CTM. Example of an indeterminate result (a), and a positive result (b) [46] Amplification curves are shown as extracted from the archive of Amplilink® software. Target amplification curves are indicated by squares (F1) and internal control amplification curves by circles (F2). The x axis represents the cycle threshold, while the y axis represents the relative fluorescence intensity

Although, indeterminate results represent less than 1% of all registered specimens within the South African EID programme [45] they account for approximately 3 000 HIV PCR test results per annum. [47] Furthermore, indeterminate results account for more than 16% of all HIV-detected PCR results. [45] The high volume of indeterminate HIV PCR results highlights the need to evaluate the performance of alternate EID assays within the context of ARV-exposure and to determine whether the HIV status of infants who test indeterminate can be resolved by testing the same specimen on alternate assays prior to result verification. Approximately fifty percent of infants with a birth indeterminate result have been demonstrated to be infected with HIV upon follow-up. [43] As such, all infants with an indeterminate result need to be followed-up with confirmatory testing performed as a matter of urgency. [21] With regards to clinicians and carers involved in the personal and clinical management of an infant, an inconclusive HIV result can lead to loss to follow up (LTFU) and infant death in those infants who have not received a conclusive test result that can be acted upon. [43]

The management of infants and children with indeterminate i.e. inconclusive HIV test results poses significant delays in diagnosis regarding a child's final HIV status. Figure 1.8 outlines the updated 2019 guidelines for the management of infants reported as indeterminate designed to alleviate diagnostic dilemmas, whereby it is recommended that infants with repeat indeterminate results be managed as HIV-infected.



ART, antiretroviral treatment; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; PMTCT, prevention of mother to child transmission; VL, viral load

Figure 1.8 The management of indeterminate results in infants [15]

1.7 Justification of the study

The PMTCT programme has been associated with significant gains in improving neonatal and maternal healthcare and survival. However, the diagnostic challenges encountered among infants and young children exposed to maternal ART and infant prophylactic regimens persist. Inaccuracies of EID can also pose significant delays in treatment initiation. [34] The aforementioned diagnostic challenges necessitate a revisiting of the EID testing algorithm in SA to obtain accurate HIV test results from HIV-exposed infants. A combination of EID assays may become of greater importance to improve diagnostic accuracy of assays used for EID, in order to identify those who are most vulnerable and require ART initiation at the earliest opportunity.

In light of this, we evaluated the performance of the Hologic Aptima HIV-1 Quant Dx assay (Hologic Gen-Probe, Inc., San Diego, CA), referred to hereon as the Aptima, as an alternate EID test within South Africa's infant HIV testing programme. Secondly the performance of the GeneXpert HIV-1 Qualitative Assay (Cepheid, Sunnyvale, CA, USA), referred to hereon as the Xpert EID, was evaluated as a consecutive test, among specimens with an HIV-detected screening test, for an alternative EID algorithm to reduce the burden of diagnostic dilemmas within South Africa's EID testing programme.

1.8 Study aims and objectives

The aim of this study was to describe the diagnostic performance of nucleic acid tests for the virological detection of HIV-1 in infants and young children exposed to antiretroviral prophylaxis.

OBJECTIVE A

- To evaluate the diagnostic performance of the Aptima assay, compared with the CAP/CTM assay as the gold standard, on DBS samples from HIV-exposed infants at birth

OBJECTIVE B

- To evaluate the performance of the Xpert EID assay as a consecutive test for infants with an HIV-detected PCR screening test and its ability to differentiate 'true-positive' from 'false-positive' results among infants who tested HIV-detected on CAP/CTM.

CHAPTER 2

2. Materials and Methods

2.1 Study design

OBJECTIVE A

The diagnostic performance of the Aptima was a cross-sectional laboratory-based evaluation of DBS specimens from HIV-exposed infants and children. Standards for the Reporting of Diagnostic Accuracy Studies (STARD) were adhered to.

OBJECTIVE B

The accuracy of the Xpert EID as a second consecutive test for HIV diagnosis in infants was evaluated retrospectively. Standards for the Reporting of Diagnostic Accuracy Studies were adhered to. Results from infants with an HIV-detected CAP/CTM result at birth (i.e. positive and indeterminate results) and a simultaneous Xpert EID birth test, and a confirmed HIV status were included in the study. A confirmed HIV status was determined by additional testing of follow-up blood samples using the CAP/CTM.

2.2 Study setting

Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Chris Hani Baragwanath Academic Hospital (CHBAH), Kalafong Provincial Tertiary Hospital (KPTH), and Rahima Moosa Mother and Child Hospital (RMMCH), were study sites for two separate studies evaluating the implementation of HIV virological POC testing. [48, 49] There are approximately 800 live births per month at CMJAH, and 1335 at CHBAH, 550 at KPTH, and 1000 at RMMCH. The estimated antenatal HIV prevalence is 29% for CMJAH and CHBAH, 25% for KPTH and 23% for RMMCH.

OBJECTIVE A

This evaluation was conducted at RMMCH and KPTH, between March 2018 and January 2020. These hospitals were sites for a study on the implementation of HIV diagnostic point of care (POC) testing whereby whole blood samples were collected at time of birth from HIV-

exposed infants and tested concurrently using CAP/CTM (in a centralized laboratory).

OBJECTIVE B

This evaluation was performed on laboratory data collected from CMJAH, CHBAH, KPTH and RMMCH, between June 2014 and December 2019. The aforementioned hospitals were sites for two separate studies on the implementation of HIV diagnostic POC testing whereby whole blood samples were collected at time of birth from HIV-exposed infants and tested concurrently using CAP/CTM (in a centralized laboratory) and on the Xpert EID (at near POC).

2.3 Study population

The study population for Objectives A and B was limited to HIV-exposed infants and children less than 2 years of age seen at CMJAH, CHBAH, KPTH, and RMMCH between June 2014 and January 2020.

2.4 Sample size calculations

OBJECTIVE A

Assuming a 5% prevalence of EID positivity using CAP/CTM, a minimum total sample size required to measure a sensitivity and specificity of 98% +/- 2% for the Aptima was 97 infant DBS specimens. Hence, specimens tested using CAP/CTM of five positives and 92 negatives were required for this study. Our calculations were informed by guidelines from the WHO stating that EID assays used for EID, should have a sensitivity and specificity more than or equal to 98%.

OBJECTIVE B

The sample size for this objective was limited to results obtained from infants with an HIV-detected CAP/CTM result at birth (i.e. positive and indeterminate results) and a simultaneous Xpert EID birth test, and a confirmed HIV infection status for the duration of the study period for Objective B. This was a total of 172 infants.

2.5 Eligibility criteria

OBJECTIVE A

2.5.1 Inclusion criteria

I. HIV-exposed infants and children <2 years of age (as determined by maternal history of HIV-infection).
II. Stored DBS specimen (≥2 spots available).
III. Valid CAP/CTM HIV-1 PCR result at same time-point of DBS specimen collection.

2.5.2 Exclusion criteria

I. Dried blood spot specimens collected from children ≥ 24 months.
II. Infants with insufficient stored DBS i.e. ≤2 spots available for testing.

OBJECTIVE B

2.5.3 Inclusion criteria

I. HIV-exposed infants with an HIV-detected CAP/CTM result at birth with simultaneous Xpert EID test result, and a confirmed HIV status based on subsequent CAP/CTM testing.

2.5.4 Exclusion criteria

I. Infants who tested indeterminate or positive on the CAP/CTM with no final HIV status.

2.6 Study procedures

2.6.1 Sample collection and preparation

OBJECTIVE A

The DBS specimens were prepared from whole blood ethylenediaminetetraacetic acid (EDTA) samples within 1 week of the blood draw. Whole EDTA blood was spotted on

demarcated circles on a pre-perforated DBS Whatman 903 Neonatal STD EU card (GE Healthcare, Westborough, MA, USA) or on Munktell TFN specimen collection cards (Munktell Filter AB, Falun, Sweden). Each DBS circle accommodates approximately 75-80µl of whole blood. The DBS cards were dried for a minimum of 3 hours and stored in individual Ziploc bags containing desiccant, and a humidity indicator card. Thereafter the specimens were stored at -80ºC. Plasma specimens collected within the NHLS were used for VL testing on the Abbott RealTime HIV-1 Test (Abbott test) and the COBAS® HIV-1 Test (Roche Diagnostics, Basel, Switzerland) referred to hereon as the Cobas assay, while whole blood DBS was used for VL testing on the Aptima assay.

OBJECTIVE B

Laboratory data were extracted from all infants with an HIV-detected PCR test at birth on the CAP/CTM, with concurrent testing performed on the Xpert EID. Confirmatory diagnostic results obtained from subsequent testing on a separate specimen were also extracted. To determine HIV status, clinic records and the NHLS Data Warehouse were searched for follow-up results. As South Africa does not have a unique patient identification system available at birth, the NHLS Data Warehouse was searched using patient demographic details and a validated probabilistic patient-linking algorithm. [11]

2.6.2 Laboratory testing method

OBJECTIVE A

Testing using the Aptima was performed at the National Institute for Communicable Diseases (NICD) in Johannesburg. The Aptima is a dual-target total nucleic acid amplification test for in vitro diagnostic use [50] which relies on transcription-mediated amplification (TMA) to amplify the *pol* (polymerase) and *LTR* regions of the HIV genome. The Aptima, which reports both a qualitative and quantitative result (in RNA copies per millilitre without a Ct value), is processed on Hologic's Panther system that allows full automation of molecular testing. The Aptima assay has been validated for both Munktell TFN and Whatman 903 DBS cards.

The diagnostic sensitivity of the Aptima when using DBS specimens is reported by the manufacturer as 99.6%, with a specificity of 99.2%, and a LoD using DBS specimens of 873

copies/mL. [51] The Panther system reports quantitative VL measurements in copies/mL and $\log_{10}$ copies/mL based on the DBS conversion factor of 29.1 for DBS specimens. This conversion factor is designed to prevent overestimation of quantitative measurements. Specimens with results listed as "Not Detected" are interpreted as a negative HIV result i.e. HIV-undetected, while specimens with results listed as "<873 detected" or with a quantified VL within the linear range of the Aptima, indicate a positive HIV result i.e. HIV-detected.

All specimens were processed according to the Aptima package insert. For the purposes of this study, all specimens that tested positive on the Aptima were repeated as follows: the first DBS circle was tested twice using the test order function on the Panther system, a second DBS circle was excised and placed in a separate testing tube, and tested once as per package insert. This means that all specimens with an HIV-detected result on the Aptima had three unique test results. Specimens with discordant results between spots, as well as specimens that resulted in an error, were retested by excising an additional spot from the DBS card and processed according to the package insert. The first valid Aptima result was used for evaluation of assay performance.

Qualitative HIV results obtained from testing performed on the CAP/CTM within accredited NHLS laboratories, were used as the gold standard from which test performance of the Aptima was calculated. The CAP/CTM is a dual-target in vitro diagnostic real-time PCR assay targeting the *LTR* and Gag region of the HIV-1 genome. The LoD of the CAP/CTM when using DBS specimens is 300 copies/mL. The reported diagnostic sensitivity of the CAP/CTM is 100%, with a specificity of 99.8%. [31] The pre-analytical steps in the DBS work-flow for the CAP/CTM is shown in Figure 2.1.

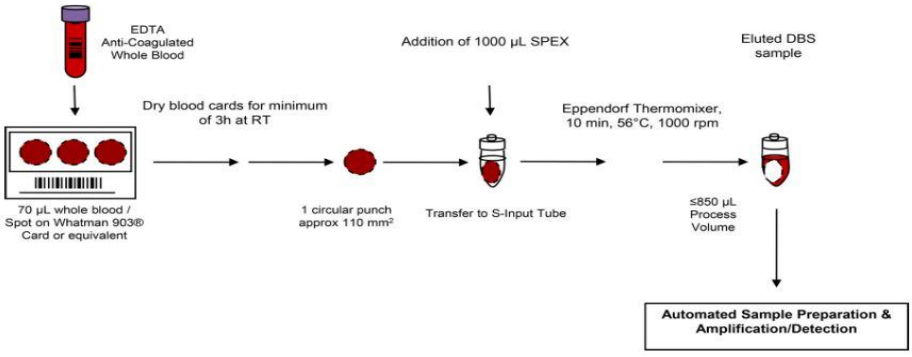


Figure 2.1 DBS pre-analytical steps on the CAP/CTM [52]

Viral load data resulted from quantitative testing of plasma on the Abbott and the Cobas assays were compared with VL data from testing DBS specimens on the Aptima. The Abbott test is a real-time in vitro quantitative test, targeting the integrase region of the pol gene. The LoD is 40 copies/mL. The reported diagnostic specificity of the Abbott test is 100%. [53] The Cobas assay is a dual-target real-time in vitro quantitative test, targeting the *LTR* and Gag region of the HIV-1 genome. The LoD is 20 copies/mL. The reported diagnostic specificity of the Cobas assay is 100%. [31]

OBJECTIVE B

The GeneXpert POC technology is a fully integrated and automated platform that detects and identifies specific HIV nucleic acid sequences using real-time reverse transcription PCR. The Xpert EID is a total nucleic acid based test that detects HIV-1 total nucleic acids (viral RNA and proviral DNA) targeting the highly conserved 3'end of 5' *LTR* region of the HIV-1 genome. In whole blood specimens collected from infants younger than 18 months, the Xpert EID has a reported sensitivity of 98.69% and specificity of 100%. [54] The LoD in EDTA whole blood is estimated to be 350 copies/mL, and 634 copies/mL when using DBS specimens. [54]

Testing was performed using venous EDTA whole blood routinely collected from HIV-exposed infants at time of birth and subsequent clinic visits. The samples for CAP/CTM testing were collected in 500µl EDTA tubes and sent to an accredited NHLS laboratory where testing was performed according to the national EID SOP. [13] At the same time, an additional 500µl EDTA sample was collected for birth PCR testing on the Xpert EID assay which was performed on site. Testing on the Xpert EID was conducted according to manufacturer's instructions. Patients with an HIV-detected result were traced and a second sample tested where possible for determination of HIV status. This second sample was either sent to the NHLS or Bio Analytical Research Corporation South Africa (BARC) for confirmation of HIV status. All results were returned to patients, regardless of whether they were study participants or not. Clinicians and health care providers acted upon results as per national guidelines at the time. [11]

2.7 Ethics

OBJECTIVE A

Permission was granted to use stored leftover DBS whole blood specimens from HIV-exposed infants who had EID testing performed (Protocol number M140955- Appendix A) as part of two separate POC implementation studies. The first study was conducted at RMMCH between June 2014 and April 2016 (Protocol number M170778- Appendix B), and the second study was conducted between March 2018 and January 2020 at RMMCH and KPTH (Protocol number M1711115- Appendix C). Objective A was conducted as per ethical clearance obtained from the University of the Witwatersrand Human Research Ethics Committee, Johannesburg, South Africa (Protocol number M190645- Appendix D). All patient information was kept confidential as per routine practice. The co-principle investigator of the point-of-care study (Protocol number M1711115- Appendix C) granted permission for retrieval and use of leftover blood samples and access to de-identified data from the point-of care study on condition of ethics approval (Appendix E).

OBJECTIVE B

A submission was made to the Human Research Ethics Committee (HREC) at the University of the Witwatersrand to report on laboratory data collected as part of a prospective cross-sectional EID POC implementation study between March 2018 and January 2020 at CMJAH, CHBAH, KPTH and RMMCH (Protocol number M1711115- Appendix C). The co-principle investigator of the aforementioned POC study granted permission for access to de-identified data from the POC study (Appendix E). All patient information was kept confidential as per routine practice.

2.8 Classification of HIV virological status

OBJECTIVE A

The first valid Aptima result was used to calculate the diagnostic performance of the Aptima.

OBJECTIVE B

Infants with a positive HIV status were defined by two HIV-detected virological results from samples taken at two different time points using the CAP/CTM. Infants with a negative HIV

status were defined as those having an isolated HIV-detected virological result followed by at least two subsequent valid HIV-not detected PCR results using the CAP/CTM.

2.9 Measurable outcomes

In this study, the assay performance for Objectives A and B was determined as it relates to assay sensitivity, specificity, PPV, negative predictive value (NPV), and accuracy.

2.10 Data management

The data obtained for Objective A and Objective B were electronically entered using Microsoft Excel. The patient data in the database was de-identified prior to analysis. The data were password protected and accessible only to the student, supervisors and designated research staff. Patient study identifiers and specimen NHLS barcodes were used as patient identifiers in lieu of patient name, surname or date of birth in order to protect patient confidentiality at time of analysis (Appendix F).

2.11 Statistical analysis

OBJECTIVE A

Date and site of specimen collection, NHLS barcode, hospital number, date of birth, and HIV virological result including available VL data, were collected for each sample. These data were exported into Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) for further analysis. Descriptive statistical analysis was performed with median and interquartile ranges used to describe age at DBS testing as well inter-assay and intra-assay variability of HIV VL $\log_{10}$ values. A two-by-two table was used to calculate the performance of the Aptima in comparison to the CAP/CTM, using the first valid Aptima result. Sensitivity, specificity, PPV, NPV and accuracy are reported as proportions with 95% confidence intervals (CI). Bland-Altman analysis was performed using Stata® 14.2 (StataCorp, Texas, USA) to evaluate differences and limits of agreement between VL results on the Aptima compared with Abbott or Cobas results. Bland-Altman analysis was also performed to determine the agreement of VL results obtained from repeat testing using a separate spot from the same DBS card on the Aptima assay.

OBJECTIVE B

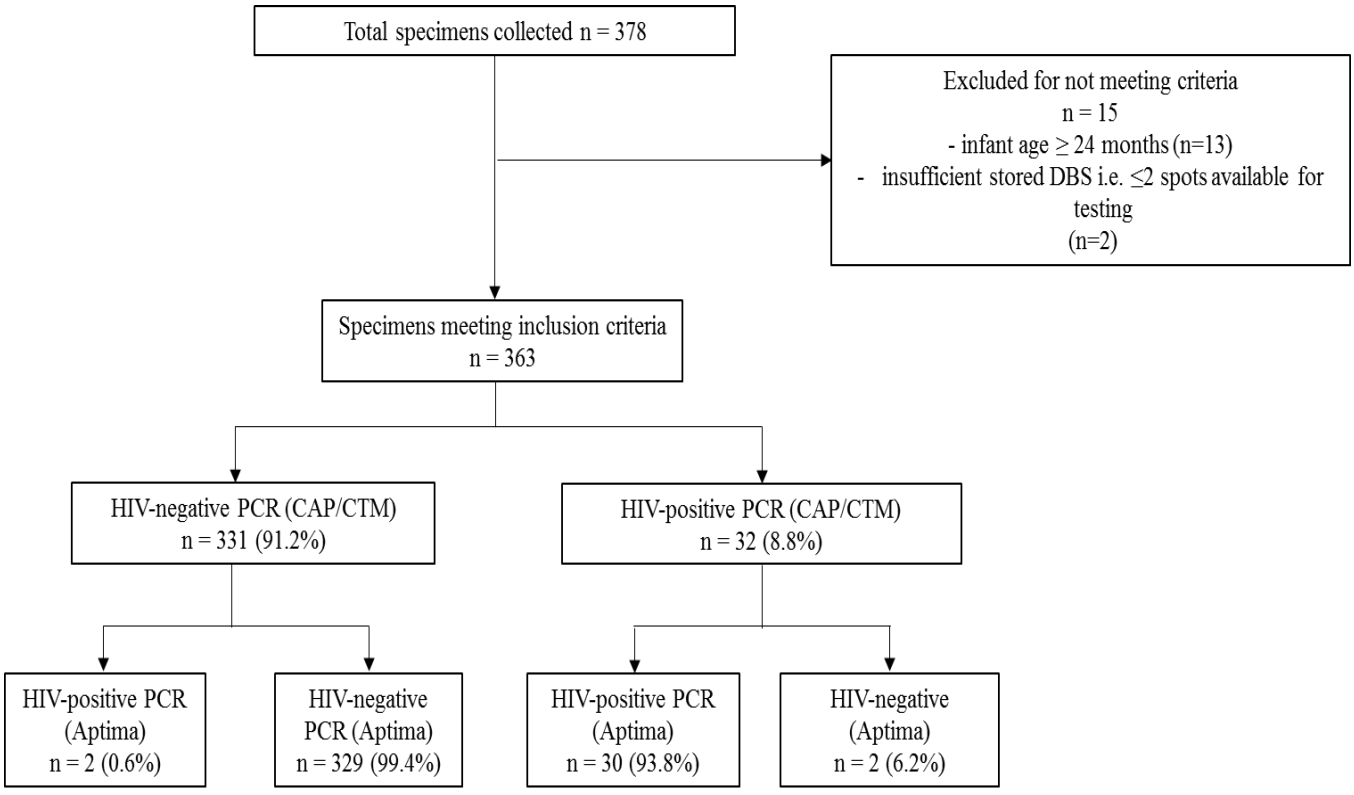
Patient data, including demographic details, birth test (both CAP/CTM and Xpert EID) and follow-up HIV virological results (CAP/CTM), were exported from REDCap databases (The REDCap Consortium, Vanderbilt) [55] of the two separate POC implementation studies, and exported into Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) for descriptive analysis. A two by two table was used to evaluate overall performance, determined as proportions with 95% confidence intervals, calculated using Stata® 14.2 (StataCorp, Texas, USA), of the Xpert EID assay in predicting status amongst infants with an HIV-detected CAP/CTM screening test at birth. A novel approach to verifying indeterminate results was evaluated whereby discordant CAP/CTM/ Xpert EID results were interpreted as indeterminate. This approach was compared with CAP/CTM indeterminate results as per NHLS verification SOP using Ct/ RFI cut-off, to describe the rate of indeterminate and false positive results of the two approaches.

CHAPTER 3

3. Results

3.1 OBJECTIVE A

A total of 378 samples were collected from HIV-exposed infants of which 15 did not meet the inclusion criteria. Of the 363 samples that met the inclusion criteria, 32 (8.8%) were HIV-detected on CAP/CTM, with two of these samples classified as indeterminate as per NHLS verification. There were 331 (91.1%) samples with a negative HIV test result on the CAP/CTM. Figure 3.1 provides the study flow with inclusion and exclusion criteria. The median age of patients at sample collection was one day (interquartile range [IQR]:1- 2).



Aptima, Hologic Aptima HIV-1 Quant Dx assay; CAP/CTM, Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0; HIV, human immunodeficiency virus; PCR, polymerase chain reaction

Figure 3.1 Study flow with inclusion and exclusion criteria and initial HIV status of specimens.

Among 32 samples with an HIV-detected result on the CAP/CTM, 30 (93.8%) had an HIV-detected result on the Aptima. The two samples with an HIV-detected result on CAP/CTM, were HIV-undetected on the Aptima. These two samples were both reported as indeterminate on CAP/CTM as per NHLS verification (Table 4.1). Of the 331 samples with an HIV-undetected result on CAP/CTM, 329 (99.4%) were also HIV-undetected on the Aptima. The two samples with an HIV-undetected result on the CAP/CTM were HIV- detected on the Aptima. These two results were reported as having an HIV VL below the lower limit of quantification (i.e. <873 copies/ml) on initial testing on the Aptima. Both yielded a positive result <873 copies/ml on replicate testing of the same spot and negative HIV results on repeat testing when using a separate spot. Compared to the CAP/CTM, the Aptima had a sensitivity of 93.75% (95% CI: 79.19%-99.23%), specificity of 99.4% (95% CI: 97.83%-99.93%), PPV of 93.75% (95% CI: 78.98%- 98.36%), NPV of 99.4% (95% CI: 97.73%-99.84%), and overall accuracy of 98.9% (95% CI: 97.2%-99.7%).

Table 3.1 HIV results using the Aptima versus CAP/CTM assay

HIV status	CAP/CTM screening test result as per NHLS verification	Aptima test result
HIV positive (n=32)	PCR positive (n=30)	HIV- detected (n=30)
	PCR indeterminate (n=2)	HIV-undetected (n=2)
HIV negative (n=331)	PCR positive (n=0)	HIV-detected (n=0)
		HIV-undetected (n=0)
	PCR negative (n=331)	HIV-detected (n=2)
		HIV-undetected (n=329)

Aptima, Hologic Aptima HIV-1 Quant Dx assay; CAP/CTM, Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0; HIV, human immunodeficiency virus; NHLS, National Health Laboratory Service; PCR, polymerase chain reaction

There were 37 errors (10.2%) which occurred (Figure 3.2) on the Aptima. Twenty-four (64.9%) errors occurred due to an internal assay processing error that invalidated the controls. This type of error results in all associated samples with that run being invalidated. Twenty-

three (95.8%) of these errors were resolved by retesting of specimens. One specimen resulted in an error when retested. The remaining 13 (35.1%) errors that occurred were due to an internal hardware error. In order for this error to be resolved, a hardware update was performed. Of these errors, 12 (92.3%) were resolved by retesting of specimens. One sample could not be retested due to an insufficient number of stored DBS spots.

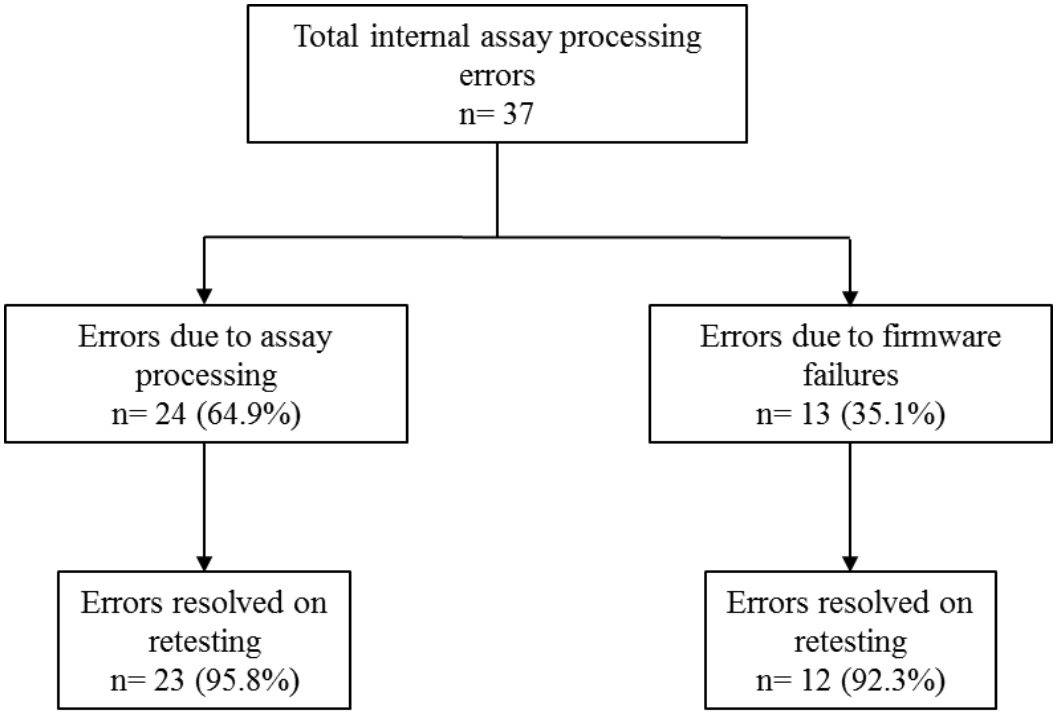


Figure 3.2 Internal assay processing errors on the Aptima

Viral load comparability

The Aptima reports a quantitative VL result for all samples that have an HIV-detected result. Among 32 samples with an HIV-detected result on the CAP/CTM, 30 had an HIV-detected result on the Aptima. Of these 30, 20 (62.5%) had routine laboratory VL test data available for comparison from samples taken on the same day as the DBS. Testing was performed on the Abbott (90%) and the Cobas (10%) assays. The median time between sampling to when the VL was resulted was one day (IQR: 0-9) on the Abbott assay and 0 days (IQR: 0-1) on the Cobas assay.

Bland-Altman analysis performed (Figure 3.3) showed that the limits of agreement (LoA) between the Aptima and the CAP/CTM were wide (LoA [$\log_{10}$VL]: -2.039 to 2.551) with moderate correlation (r=0.555) suggested by Pearson's correlation coefficient. The relative

mean difference was 0.256 (CI -0.281 to 0.793). Pitman's Test of variance showed that the difference in variance was not significant (p = 0.360). The absolute median log_{10} difference between VL measurements on the Aptima, and the Cobas/Abbott was 0.66 (IQR: 0.36-1.71). Of the 20 samples that had routine laboratory VL test data available, 13 (65%) of these had an absolute log_{10} difference greater than 0.5 on the Aptima when compared to the Cobas/Abbott assay.

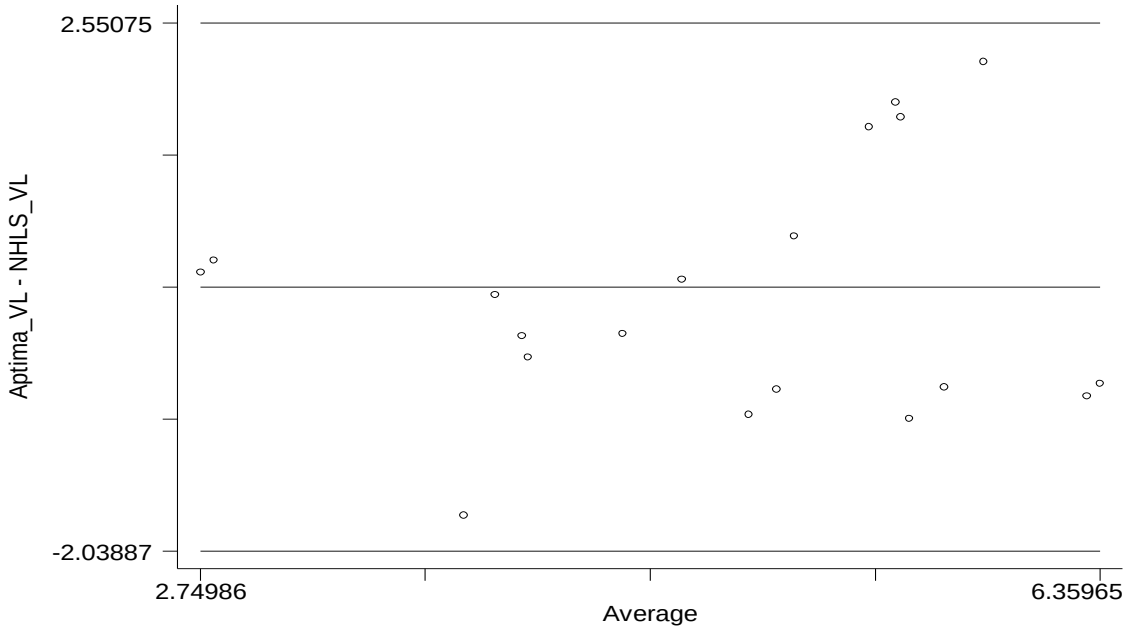


Figure 3.3 Bland-Altman plot of agreement between VL measurements on Aptima and Abbott/Cobas assays

Repeat testing of samples

Samples with an HIV-detected result on the Aptima were tested an additional two times on the Aptima to determine assay reliability of results. The initial test refers to testing performed on the same DBS spot using the 'replicate' function on the Aptima, the repeat test refers to testing performed using a separate DBS spot without the 'replicate' function. Thirty-two samples had an HIV-detected result on the CAP/CTM, with two samples reported as indeterminate (as per NHLS verification practice). Two of the 32 specimens produced an HIV-undetected result on initial testing when using the Aptima.

In Figure 3.4a results obtained from initial testing on the first DBS spot are compared with results obtained from repeating the first DBS spot (i.e. using the 'replicate' test order function on the Panther system). The Bland-Altman plot showed that the LoA between VL

measurements obtained on initial testing, and VL measurements obtained from the repeated result of the same DBS sample showed variance (LoA [log_{10}VL] = -1.011 to 0.761). However, Pitman's Test showed that the variance in VL measurements obtained when using the replicate function on the Aptima (p = 0.529) was not significant. The absolute median log_{10} difference between VL measurements obtained from initial testing on the Aptima, and repeat testing was 0.286 (IQR: 0.176-0.4).

In Figure 3.4b, results obtained from the first result obtained from initial testing of a DBS spot on the Aptima are compared with results obtained from testing a separate DBS spot from the same DBS card at a different time-point. The LoA between the VL measurements was wide (LoA [log_{10}VL] = -0.801 to 0.503). However, Pitman's Test showed that there was no significant variance in VL measurements obtained from initial testing, and subsequent testing performed 24 hours later (p = 0.674). The absolute median log_{10} difference between VL measurements was 0.155 (IQR: 0.04-0.36).

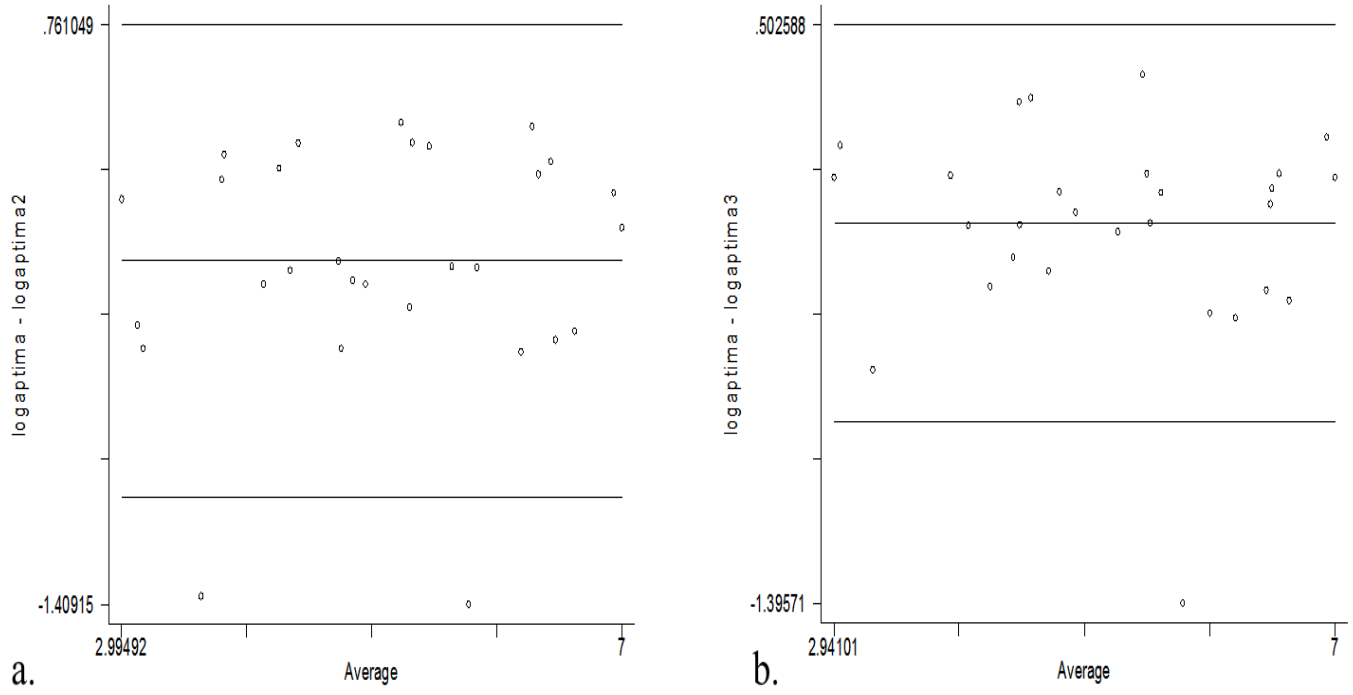


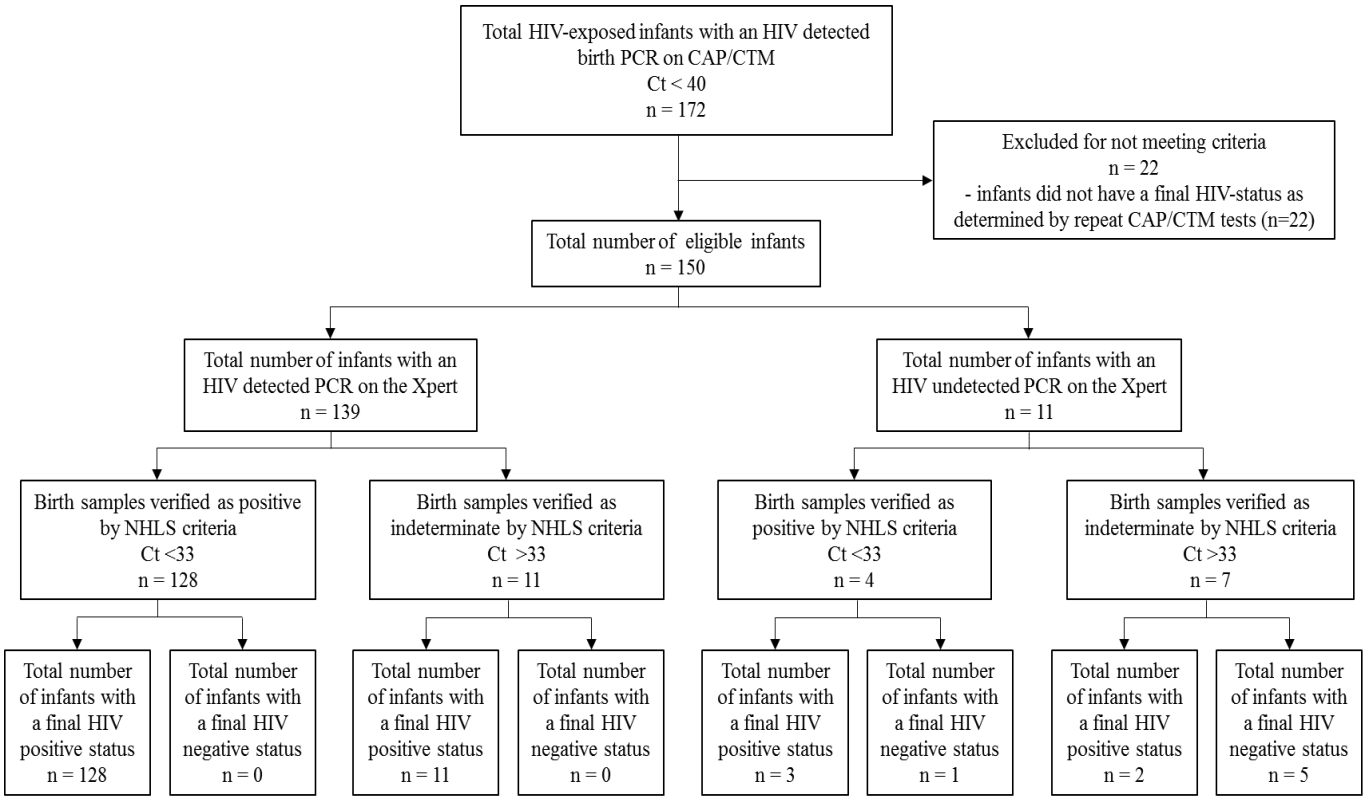
Figure 3.4 Bland-Altman plot of agreement on VL measurements on the Aptima. Comparison of VL measurements obtained on the Aptima when using the 'replicate' function on a single spot from the DBS card **(a)**. Comparison of initial VL results on the Aptima, compared to VL results obtained from testing a separate spot from the DBS card 24 hours later on the Aptima **(b)**.

CHAPTER 4

4. Results

4.1 OBJECTIVE B

A total of 172 HIV-exposed infants had an initial HIV-detected HIV PCR birth result on the CAP/CTM, and had a simultaneous Xpert EID test. Figure 4.1 provides the study flow with inclusion and exclusion criteria. Of these infants, 150 met the inclusion criteria - an HIV-detected CAP/CTM birth test, a simultaneous test result obtained on the Xpert EID assay (including both HIV-undetected and HIV-detected results) and a confirmed HIV status as determined by subsequent virological testing performed on the CAP/CTM (see Figure 4.1). The median age at time of birth testing was one day (interquartile range (IQR): 0-1). The median age at time of final HIV status was one day (IQR: 1-3) for infants with an HIV positive status and 20 days (IQR: 7-43) for infants with an HIV negative status.



CAP/CTM, Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0; HIV, human immunodeficiency virus; PCR, polymerase chain reaction; Xpert EID, GeneXpert HIV-1 Qualitative assay

Figure 4.1 Study flow showing eligible participants with final HIV status

Among results of 150 infants with an initial HIV-detected CAP/CTM screening test, 144 (96%) were found to have an HIV-detected status on follow-up (Table 4.1) of which 139 (97%) had an HIV-detected result on Xpert at birth. Of the six (4%) infants with an HIV uninfected status on follow-up, six (100%) tested HIV-negative on Xpert EID at birth. There was a total of five (3.3%) infants where the Xpert EID result at birth was discordant to HIV-status on follow-up. All five infants had a false-negative Xpert EID result. In this study, there was an infant who received a positive result on the Xpert EID as well as a positive birth PCR result obtained from testing performed on the CAP/CTM. Upon subsequent testing, results obtained from this infant on both assays were negative. Three months later further testing was performed with an indeterminate test result received. Additional blood collected from this infant was tested on a single copy assay and resulted as <3 copies/1.5 million cells.

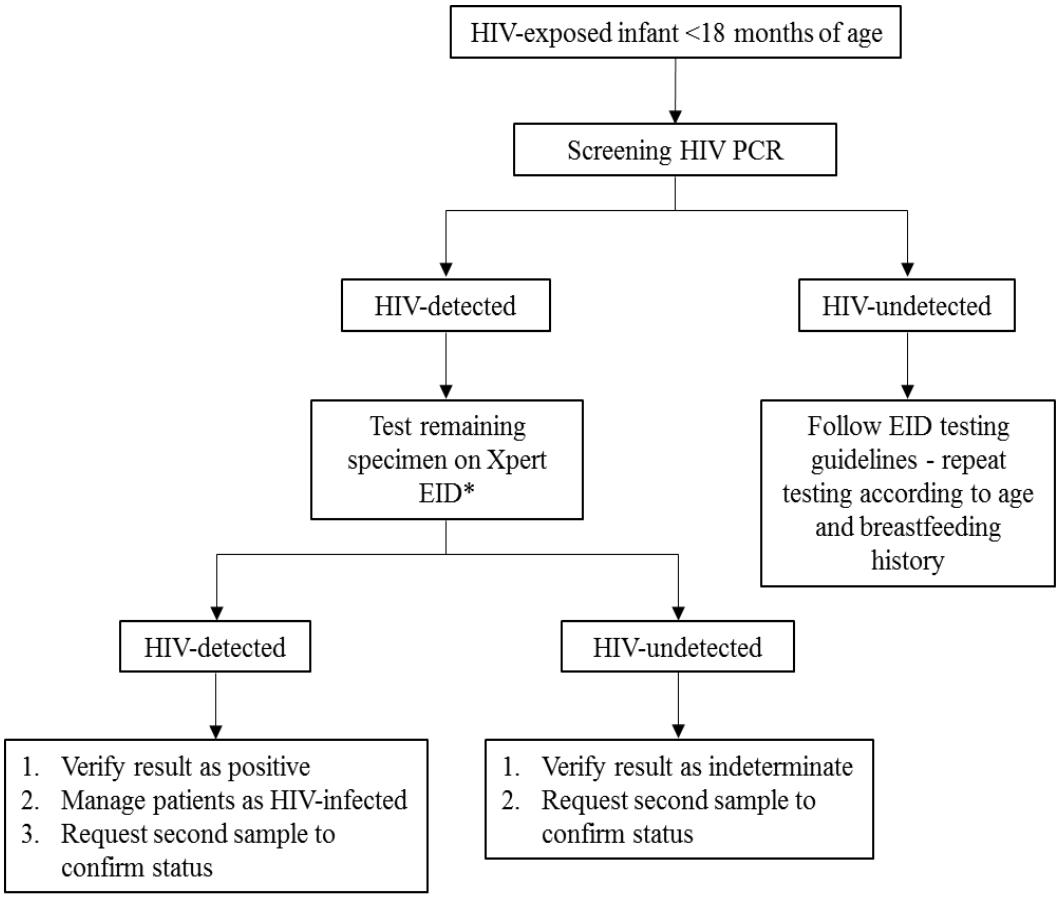
As a second consecutive assay, the Xpert EID yielded a sensitivity of 96.53 (95% CI: 92.1% to 98.9%), specificity of 100% (95% CI: 54.1% to 100%), PPV of 100% (95% CI: 100%), NPV of 54.5% (95% CI: 33.7% to 74.1%), and overall accuracy of 96.1% (95% CI: 91.5%-98.5%).

Table 4.1 Comparison of initial HIV-1 result on the Xpert EID, with HIV result on CAP/CTM

Final HIV status	CAP/CTM screening test result as per NHLS verification	Xpert EID consecutive test result
HIV positive (n= 144)	PCR positive (n= 131)	HIV-detected (n= 128)
		HIV-undetected (n= 3)
	PCR indeterminate (n= 13)	HIV-detected (n= 11)
		HIV-undetected (n= 2)
HIV negative (n= 6)	PCR positive (n= 1)	HIV-detected (n= 0)
		HIV-undetected (n= 1)
	PCR indeterminate (n= 5)	HIV-detected (n= 0)
		HIV-undetected (n= 5)

CAP/CTM, Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0; HIV, human immunodeficiency virus; NHLS, National Health Laboratory Service; PCR, polymerase chain reaction; Xpert EID, GeneXpert HIV-1 Qualitative assay

Eighteen (12%) infants had an indeterminate HIV PCR screening result on CAP/CTM at birth as verified by the NHLS. Upon subsequent testing on the CAP/CTM, five (27.8%) infants were found to have an HIV negative status. All of these infants had tested HIV-undetected on Xpert EID at birth. The remaining thirteen infants (72.2%) had an HIV-detected status of which 11 (84.6%) had an HIV-detected result on Xpert EID at birth. Using alternate criteria for verifying indeterminate results whereby specimens with discordant CAP/CTM/ Xpert EID results were interpreted as indeterminate and concordant HIV-detected CAP/CTM and Xpert EID results (regardless of Ct/ RFI value) interpreted as HIV-detected, there would have been 11 (7.3%) infants with an indeterminate birth result of which six out of 11 (54.5%) would have been found to have an HIV positive status on follow-up testing. In addition, if verification practice was informed by concordant or discordant qualitative CAP/CTM and Xpert EID results regardless of Ct/ RFI value (Figure 4.2), a drop in the indeterminate rate by 42.1% would have been achieved without increasing the rate of false-positive results.



*If sample is insufficient, interpret result according to Ct to differentiate positive from indeterminate results. EID, early infant diagnosis; HIV, human immunodeficiency virus; PCR, polymerase chain reaction

Figure 4.2 Proposed new EID algorithm

CHAPTER 5

5. Discussion and conclusion

5.1 Outcome of results obtained

In this study, the Aptima showed good diagnostic performance as a qualitative test when compared to the CAP/CTM. However, HIV VL quantification on DBS specimens cannot be considered equivalent to plasma VL and should not be used for virological monitoring purposes. The diagnostic performance of the Xpert EID demonstrated excellent PPV and overall accuracy as a consecutive test for specimens with a positive HIV-detected screening result on the CAP/CTM. The addition of the Xpert EID has the potential to improve the PPV and reduce the indeterminate rate, thereby reducing diagnostic challenges and time to final status, within South Africa's EID programme.

5.2 Performance of the Aptima

The Aptima demonstrated excellent diagnostic performance as a qualitative assay for HIV diagnosis in infants. The results obtained closely correlated with HIV results obtained from testing on the CAP/CTM, and provided accurate classification of clinical samples evaluated in this study.

We evaluated the Aptima within a defined population of HIV-exposed infants tested at birth. While previous studies have evaluated the performance of the Aptima using DBS, [56-57] this study was restricted to specimens obtained at birth. The results obtained demonstrated good diagnostic performance of the Aptima in comparison with CAP/CTM assay as a qualitative EID assay at birth. A possible explanation for this could be that both tests utilize dual target detection. In addition to the diagnostic performance of the Aptima, we also found good intra-assay reliability of Aptima HIV VL results. However, VL results using DBS specimens processed on Aptima were much higher than plasma VL results performed using Abbott or Cobas assays. In general, VL performed on DBS specimens yield higher results than VL performed on plasma, likely due to the presence of intracellular viral DNA and RNA. When using DBS specimens, the Aptima applies a DBS conversion factor designed to prevent overestimation of quantitative viral load measurements. In the specimens that were tested in

this study, the viral loads obtained from DBS specimens on the Aptima were much higher than paired plasma specimens taken at the same time and tested using alternate assays. The inter-assay reliability between the Aptima and Abbott/Cobas assays was found to be poor. Although the sample size of eligible specimens for VL comparison was small, our findings suggest that DBS specimens tested on Aptima should not be used for HIV VL monitoring. However, the intra-assay HIV VL variability of the Aptima was within an acceptable range.

The Panther system on which the Aptima is processed, allows users to define the number of tests that they wish to apply to a specimen. This means that a single DBS circle of roughly 70µl can be tested more than once. Furthermore, testing performed on the Aptima provides an opportunity for clinicians to receive an initial HIV diagnostic result, alongside a baseline VL. In a high burden setting such as SA, this may prove beneficial in resolving diagnostic challenges by providing accurate EID of HIV.

One of the challenges in this study was the high error rate experienced. The majority of errors that occurred were due to internal assay processing errors that required the assistance of a technician to resolve. Errors that occur on the Aptima were quick to resolve in cases where the error was due to failures in assay processing. Utilising the 'replicate' function means that should the result from initial testing (i.e. 'first replicate') yield an error, valid results can still be obtained without requiring additional sample, provided that specimens are stored appropriately. However, in cases where an error is due to a firmware failure i.e. instrument hardware updates and failure of controls, invalidation of all specimens associated with that run will occur. Such errors may prove to be inconvenient, particularly in cases where it is not feasible to obtain additional specimen/s for testing. In this study we excluded one specimen due to the fact that all DBS spots for this specimen were depleted on account of the errors experienced. In addition to this, repeat testing including using the replicate function, requires additional reagents as well as consumables involved in processing of specimens. In the case of our study, the errors that occurred consumed the equivalent of one Aptima test kit (i.e. 100 tests). This meant that an additional kit had to be purchased to accommodate testing of all collected eligible specimens in this study.

5.3 Performance of the Xpert EID

The Xpert EID has been associated with good performance with a reported diagnostic sensitivity of 100% when used on HIV-infected neonates [58] and has previously been described as an attractive alternative to laboratory based testing (LABT), particularly in resource-limited settings. [58]

In this evaluation, the Xpert EID demonstrated excellent PPV and overall accuracy as a second consecutive test for specimens that screened HIV-detected on CAP/CTM at birth. An overwhelming percentage of results obtained from testing were comparable to results obtained from diagnostic testing on the gold standard i.e. the CAP/CTM. The Xpert EID result had a reduced sensitivity, when compared to the CAP/CTM, and was discordant with final status in only five cases, all of which (3.3%) had a negative HIV status. In the case of infants who receive a first positive HIV result, the WHO and South African guidelines recommend that all infants or young children that yield a positive HIV test result have a second specimen drawn, and repeated/confirmed on a second HIV virological assay. [59]

However, uptake of confirmatory testing has been poor within EID testing programmes. In a computer simulated model of paediatric HIV infection, diagnosis and treatment, it was found that testing strategies where consecutive testing was employed were cost-saving when compared to cases without confirmatory testing. [34] In spite of no difference being observed in the life expectancy of infants/young children living with HIV, confirmatory testing successfully averted the high costs associated with HIV care in uninfected infants. In this same study, it was found that consecutive testing can help in increasing the PPV of diagnostic assays used for EID, as well as resolving false-positive HIV diagnoses.

Hence, the addition of the Xpert EID as a consecutive test provides an opportunity to improve the diagnostic accuracy and PPV of EID algorithms. The implementation of the Xpert EID has the potential to reduce the indeterminate rate and volume of diagnostic dilemmas in South Africa. This is especially important at birth as HIV-infected infants increasingly demonstrate low-level viraemia and loss of detection at time of confirmatory testing on account of exposure to antiretroviral prophylaxis and maternal ART. By reducing the indeterminate rate and providing prompt diagnosis, the volume of diagnostic dilemmas and time to ART initiation among infected infants will be reduced. The short analytical turnaround time of 90

minutes, when using the Xpert EID in the centralized EID laboratories for consecutive testing of all HIV detected screening results (e.g. using CAP/CTM) would result in minimal delays in result verification whilst potentially improving linkage to care, and reducing loss to follow-up. [60] Furthermore, South Africa already has an extensive footprint of Xpert instruments, placed both in centralized and near point of care laboratories, as part of the national TB programme. Approximately 15 000 specimens yield an HIV-detected result on EID screening per annum and would therefore require a confirmatory Xpert EID test. With capacity to spare, the NHLS Xpert instruments can be leveraged as a consecutive test by the eleven NHLS EID laboratories with minimal disruption to the TB programme (Prof G Sherman, National Institute for Communicable Diseases - personal communication, August 2020).

5.4 Study limitations

A number of important limitations need to be considered with regards to these findings. In both objectives, testing was limited to HIV-exposed infants tested at birth from four facilities in Gauteng. Hence, the results obtained from these evaluations may not be representative of the whole EID programme. In both objectives, the number of infants reported as indeterminate as per NHLS verification practices was less than expected. The low number of indeterminate results may be reflective of the small number of infants that were enrolled in this study, as well as the challenges experienced in linking birth results to follow-up results.

OBJECTIVE A

The clinical specimens used in this study were prepared from collected whole blood samples within one week of the blood draw at study sites as per the study protocol. Delay in testing of stored blood specimens may be associated with cell-lysis which can potentially result in over-quantification when testing is performed on plasma. Conversely, delay in testing may be associated with degradation of HIV RNA, which can lead to under-quantification of total nucleic acid or loss of detection. However, there is local data to suggest viral RNA is stable in EDTA specimens stored for up to a week, even under suboptimal storage conditions. [61] Testing on the Aptima was performed using whole blood DBS specimens, while EDTA whole blood specimens were used for qualitative testing on the CAP/CTM and plasma from EDTA specimens used for quantitative testing on Abbott and Cobas assays. Regarding the accuracy of VL results on the Aptima, our findings suggest DBS specimens will likely yield significant over quantification of VL compared with plasma specimens. However, on account

of our small sample size of eligible specimens we are unable to conclusively determine whether DBS specimens can be used for HIV VL monitoring. The diagnostic performance of the Aptima was evaluated according to HIV test results obtained on the CAP/CTM rather than the true HIV status of the study population. Thus it cannot be determined whether the diagnostic performance of the Aptima meets WHO recommendations which state that assays used for EID have a sensitivity and specificity more than or equal to 98%.

Discordant EID results were evenly distributed between the Aptima and the CAP/CTM assays. Two infants with a negative HIV result on the CAP/CTM had HIV-detected results on the Aptima, albeit irreproducible. The Aptima VL of these two results was below the lower limit of quantification of the Aptima assay, which is 873 copies/mL. Furthermore, two infants with an HIV-detected result on the CAP/CTM, both of which were classified as indeterminate as per NHLS verification practices, had an HIV negative result on Aptima. Due to challenges with linking birth results to follow up results, we were unable to ascertain whether these four infants were true positives or false positives. As such we cannot conclude whether performance of the Aptima assay would be better than the CAP/CTM for resolving diagnostic dilemmas such as indeterminate results. However, our findings suggest that HIV-detected results below the limit of quantification (i.e. <873 copies/ml) and irreproducible results on Aptima should be interpreted with caution, in keeping with findings on the CAP/CTM assay. [13]

OBJECTIVE B

Amongst the small number of eligible infants for Objective B, a subset of these infants (13%) had to be excluded from final analysis due to an inability to follow-up on subsequent test results. Furthermore, the data analysed in this study is limited to testing performed on the CAP/CTM. Intrauterine HIV-infected infants tested at birth have been found to have lower level viraemia compared to older age groups, as well as the highest rate of indeterminate HIV PCR results [44]. It is therefore likely that the Xpert EID would yield fewer false-negative results among older infants. Additionally, in this study testing was performed using 100µl EDTA, whereas 70µl dried blood spot (DBS) specimens are the predominant specimen type used within the NHLS for EID. The Xpert EID assay has, however, been validated for DBS use. Processing of DBS specimens using the Xpert EID assay is simplified, aside from routine laboratory consumables, the only additional equipment needed is a ThermoMixer C. As the Xpert EID is already situated in NHLS labs, access to additional equipment is unlikely

to be an issue.

5.5 Conclusion

5.5.1 Overall diagnostic performance

Currently the majority of data on EID of HIV is on the CAP/CTM. As a result, of a well-developed PMTCT programme the prevalence of HIV in infants has continued to decrease. The consequence of this is a reduced PPV. Thus South Africa will have to revisit current testing algorithms to explore whether a combined usage of different assays will prove effective to improve the PPV and diagnostic sensitivity of current diagnostic assays employed.

From the results obtained in this study it was concluded that the diagnostic performance of the Aptima is comparable to that of the CAP/CTM. The Aptima can therefore be utilised as an alternate EID assay within the NHLS. A benefit of the Aptima is that the assay provides a quantitative result i.e. viral load, which may prove useful for viral load monitoring if testing is performed using plasma. In this study while we evaluated VL data, these data were obtained from DBS specimens tested on the Aptima. As such we are unable to comment on whether the Aptima can be leveraged to provide clinicians with a baseline viral load for initiation of ARV treatment, and VL monitoring in resource limited settings. Furthermore, our findings suggest that DBS cannot be considered as an alternate sample type for VL monitoring.

An assay such as the Xpert EID has the benefit of providing a clinical HIV result with minimal LTFU without compromising the accuracy of the test. [62] As a consecutive test the Xpert EID demonstrated excellent PPV and overall accuracy for specimens that were HIV-detected on the CAP/CTM. Incorporating the Xpert EID as a consecutive assay has the potential to enhance EID accuracy by bringing down the rate of indeterminate results without increasing the false-positive rate, with minimal cost and delay in turn-around time.

While access to EID testing services remains a cornerstone for the eMTCT of HIV, the importance of prompt linkage to care for these infants should not be forgotten. There are delays in obtaining a final HIV clinical status in infants or young children who have access to

testing, with only 30% of perinatally infected infants effectively linked to EID testing services and initiated on ART. [22] Novel approaches to EID, such as the implementation of consecutive testing of samples that yield HIV-detected results in screening have the potential to improve rapid identification and treatment initiation among infants and young children. There also exists the opportunity to measure the improvement of other outcomes such as LTFU, linkage to care, and final time to diagnosis for all HIV-exposed infants, and young children. Future studies should therefore explore the implementation of the Aptima, and the Xpert EID within the current EID testing algorithm and how these assays can be utilized to resolve diagnostic dilemmas particularly in infants exposed to ARV prophylaxis. The implementation of the Aptima and the Xpert EID has the potential to improve overall result return rates, and most importantly allow for earlier ART initiation in infants with a true HIV infection. Earlier and more accurate identification of HIV has the potential to alleviate costs associated with factors such as ART initiation in infants who receive a false positive HIV result, and costs associated with multiple repeat HIV tests. Future studies are required to determine whether the implementation of the Aptima and the Xpert EID as additional resources for EID testing services is worth the additional costs that could be incurred.

964 **Appendix A: Human Research Ethics Certificate M140955**

R14/49 Prof Adrian Puren

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M140955

NAME: (Principal Investigator)	Prof Adrian Puren
DEPARTMENT:	Virology and Communicable Diseases National Institute for Communicable Disease National Health Laboratory Services
PROJECT TITLE:	The Research and Development Programme: Development of Diagnostic Assays for Disease Monitoring with a Special Focus on HIV/AIDS in Surveillance Settings
DATE CONSIDERED:	03/10/2014
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Professor P Cleaton-Jones, Chairperson, HREC (Medical)
DATE OF APPROVAL:	06/02/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

965

Appendix B: Human Research Ethics Certificate M170778

RENEWAL

R14/49 Dr KG Technau

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M170778

NAME: (Principal Investigator)	Dr KG Technau
DEPARTMENT:	School of Clinical Medicine Division of Paediatrics Empilweni Services and Research Unit Rahima Moosa Mother and Child Hospital
PROJECT TITLE:	Cohort study of HIV-infected and exposed children receiving care at Rahima Moosa Mother and Child Hospital Supported by Empilweni Services and Research Unit
DATE CONSIDERED:	25/05/2009
DECISION:	Approved unconditionally
CONDITIONS:	Renewal of M140760 and M090501
SUPERVISOR:	Not applicable
APPROVED BY:	Professor CB Penny, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	04/09/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and ONE COPY returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **May** and will therefore be due in the month of **May** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 5/9/2017

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

968 **Appendix C: Human Research Ethics Certificate M1711115**

969

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr S Carmona et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M1711115

NAME: (Principal Investigator)	Dr S Carmona et al
DEPARTMENT:	School of Pathology Department of Haematology and Molecular Medicine NHLS
PROJECT TITLE:	Evaluating implementation of HIV virological point-of-care testing with remote quality assurance monitoring, in high-prevalance clinical settings
DATE CONSIDERED:	24/11/2017
DECISION:	Approved conditionally
CONDITIONS:	This approval applies to Charlotte Maxege, Chris Hani Baragwanath & Rahima Moosa M&C Hospitals Any further letters of approval to be sent to the HREC
SUPERVISOR:	Not applicable
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	16/05/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **November** and will therefore be due in the month of **November** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

Appendix D: Human Research Ethics Certificate M190645

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Aurélie Mukendi

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M190645

NAME: (Principal Investigator)	Miss Aurélie Mukendi
DEPARTMENT:	School of Pathology Centre for HIV and STIs National Institute for Communicable Diseases
PROJECT TITLE:	Evaluating the performance of nucleic acid tests to diagnose HIV in infants and young children exposed to antiretroviral prophylaxis
DATE CONSIDERED:	28/06/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof G. Sherman, C. Tiemessen and A.H. Mazanderani
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	19/07/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **June** and will therefore be due in the month of **June** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix E: Permission to retrieve and use leftover blood samples and access to de-identified clinical data

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

Centre for HIV and Sexually Transmitted Infections
1 Modderfontein Road, Sandringham, 2031

06/06/2019

Re: Permission to retrieve and use left-over blood samples and access to de-identified clinical data

To whom it may concern,

This is to confirm that I am the co-principle investigator of the study, 'Evaluating implementation of HIV virological point-of-care testing with remote quality assurance monitoring in high-prevalence clinical settings,' (referred to as the point-of-care study) approved by the Human Research Ethics Committee of the University of the Witwatersrand (M1711115) and Faculty of Health Sciences Research Ethics Committee of the University of Pretoria (50/2018).

I am aware of the proposed research study, 'Evaluating the performance of nucleic acid tests to diagnose HIV in infants and young children exposed to antiretroviral prophylaxis' of which Aurélie Mukendi is principle-investigator. On condition of ethics approval, I grant permission for retrieval and use of left-over blood samples and access to de-identified data from the point-of-care study.

Sincerely,

Ahmad Haeri Mazanderani
Pathologist (Clinical Virology)
Centre for HIV and STIs
National Institute for Communicable Diseases
Division of National Health Laboratory Service
1 Modderfontein Road, Sandringham
Tel: +27 11 555 0484 | Cell: +27 82 642 8609
ahmadh@nicd.ac.za | www.nicd.ac.za

Appendix F: List of variables to be extracted

Study ID

NHLS Barcode

Hospital Number

Facility

Date of Birth

Date Sample Taken

HIV virological result (PCR real-time PCR parameters e.g. Ct values)

Maternal ART History

Maternal Viral Load

Infant ARV History

Infant Feeding History

Final HIV Clinical Status

Appendix G: International Workshop on HIV Pediatrics 2020

Poster 34

Evaluating the performance of the GeneXpert HIV-1 Qualitative assay as a consecutive test for a new early infant diagnosis algorithm in South Africa.

Aurélie Mukendi[1,2], Tendesayi Kufa[2,3], Tanya Murray[2,4], Megan Burke[5], Renate Strehlau[5], Karl-Günter Technau[5], Caroline T Tiemessen[1,2], Gayle G Sherman[2,5], Ahmad Haeri Mazanderani[2]

1. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. 2. Centre for HIV and STIs, National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, South Africa. 3. School of Public Health, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa. 4. Paediatric HIV Diagnostics, Wits Health Consortium, Johannesburg, South Africa 5. Empilweni Services and Research Unit, Rahima Moosa Mother and Child Hospital, Department of Paediatrics and Child Health, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

*Contact details: aureliem@nicd.ac.za

Background

- Due to South Africa's comprehensive prevention of mother-to-child transmission (PMTCT) programme, the proportion of HIV-exposed infants and young children infected with HIV has markedly declined over the past decade.
- This in turn has reduced the positive predictive value (PPV) of diagnostic assays, necessitating review of early infant diagnosis (EID) algorithms to ensure improved accuracy.
- We describe the performance of the GeneXpert HIV-1 Qualitative (Xpert EID) assay as a consecutive test for infants with an HIV-detected PCR screening test.

Methods

- Retrospective analysis of a longitudinal cohort of HIV-exposed infants for whom birth testing was performed at four tertiary sites in Gauteng between June 2014 - December 2019.
- All infants with a Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0 (CAP/CTM v2.0) HIV-detected screening test, concurrent Xpert EID test and subsequent confirmatory CAP/CTM v2.0 test on a separate specimen were included.
- Performance of the Xpert EID in predicting final HIV status was determined as proportions with 95% confidence intervals.
- A comparison of indeterminate CAP/CTM v2.0 results, as per National Health Laboratory Service (NHLS) verification practice, with discordant CAP/CTM v2.0/ Xpert EID results was performed.

Results

- A total of 150 infants met the inclusion criteria (**Figure 1**) of which 6 (3.9%) had an Xpert result discordant with final HIV status: 5 (3.3%) false-negatives and one (0.7%) false-positive.
- As a consecutive assay, the Xpert EID yielded a sensitivity of 96.5% (95% CI 92%-98.9%), specificity of 85.7% (95% CI 42.1%- 99.6%), PPV of 99.3% (95% CI 95.7%-99.9%), negative predictive value of 54.5% (95% CI 32.5%- 74.9%), and overall accuracy of 96.1% (95% CI 91.5%-98.5%).
- Eighteen (12.6%) infants had an indeterminate HIV PCR screening result on CAP/CTM v2.0 at birth as verified by the NHLS (**Table 1.**) Upon subsequent testing on the CAP/CTM v2.0, five (27.8%) infants were found to have an HIV negative status. All of these infants had tested HIV-undetected on Xpert EID at birth.
- The remaining thirteen infants (72.2%) had an HIV-infected status of which 11 had an HIV-detected result on Xpert EID at birth.
- If verification practice was informed by concordant or discordant qualitative CAP/CTM v2.0 and Xpert EID results regardless of Ct/ RFI value (**Figure 2**), the number of indeterminate screening results would have reduced by 42.1%, from 18 (12.6%) to 11 (7.2%), without increasing the false-positive rate.

Table 1. Comparison of initial HIV-1 result on the Xpert assay, with HIV result on CAP/CTM v2.0

CAP/CTM v2.0, Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0; HIV, human immunodeficiency virus; NHLS, National Health Laboratory Service; PCR, polymerase chain reaction; Xpert EID, GeneXpert HIV-1 Qualitative assay.

Final HIV status	CAP/CTM v2.0 screening test result as per NHLS verification	Xpert EID consecutive test result
HIV positive (n= 143)	PCR positive (n= 130)	HIV-detected (n= 127)
		HIV-undetected (n= 3)
	PCR indeterminate (n= 13)	HIV-detected (n= 11)
		HIV-undetected (n= 2)
HIV negative (n= 7)	PCR positive (n= 2)	HIV-detected (n= 1)
		HIV-undetected (n= 1)
	PCR indeterminate (n= 5)	HIV-detected (n= 0)
		HIV-undetected (n= 5)

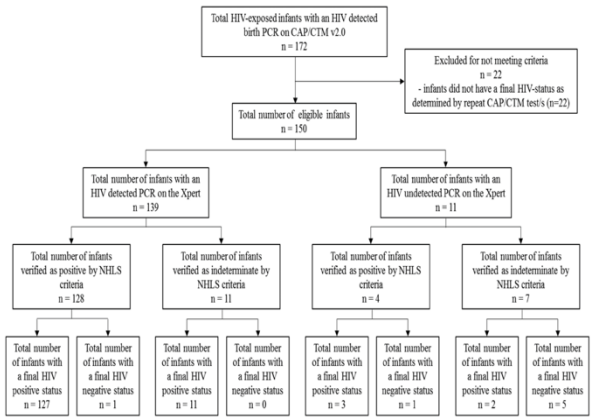
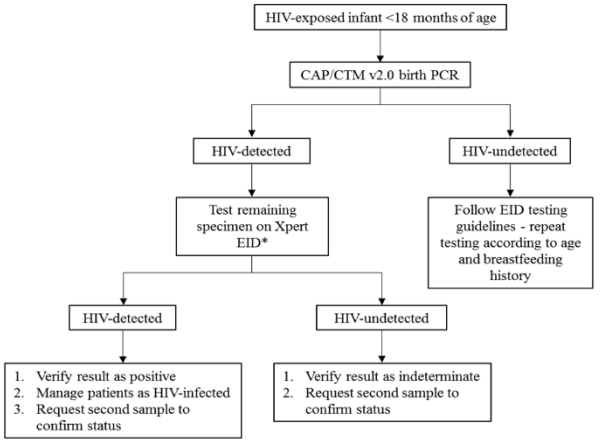


Figure 1. Study flow showing eligible participants with final HIV status



*If sample is insufficient, interpret result according to Ct to differentiate positive from indeterminate results. CAP/CTM v2.0, Cobas® AmpliPrep/Cobas® TaqMan HIV-1 Qualitative Test v2.0; EID, early infant diagnosis; HIV, human immunodeficiency virus; PCR, polymerase chain reaction

Figure 2: Proposed new EID algorithm.

Conclusion

- The addition of the Xpert EID as a consecutive test for specimens with an HIV-detected PCR screening result has the potential to improve the PPV and reduce the indeterminate rate, thereby reducing diagnostic challenges and time to final status, within South Africa's EID programme.

Acknowledgements

- The authors would like to thank Professor Louise Kuhn behalf of the LEOPARD study group, for allowing the authors access to HIV birth testing PCR data.
- This research was partly funded by the Clinton Health Access Initiative (CHAI)/UNITAID (Project ID CHSOAFHLSTP3).

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