UNIVERSITY OF CAPE TOWN

Impact of Introducing an HIV-PCR Test at Birth to Attendance at Follow-Up Early Infant Diagnosis (EID) Services for HIV-Exposed Infants in Cape Town

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15th August 2016

DECLARATION OF PLAGIARISM

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ABSTRACT

Introduction: PCR testing at birth ('birth-testing') is conditionally advised by new World Health Organization guidelines for rapid diagnosis of infants infected with HIV *in utero*. Prompt diagnosis and early introduction of antiretroviral therapy (ART) can dramatically reduce mortality in HIV-infected infants. However, a negative result at birth must be followed by engagement in subsequent routine early infant diagnosis (EID) services (recommended at 6-10 weeks of age) to rule out *intra-partum* infection. There are few data on the implementation of this approach in sub-Saharan Africa and whether birth-testing affects the uptake of subsequent routine EID testing is unknown.

Methods: We conducted a retrospective cohort study using routine clinical and laboratory data from a large obstetric hospital in Cape Town. All infants suspected to be at high risk of HIV-transmission, underwent birth-testing between July 2013-August 2015. Infants with a negative birth-test were matched to HIV-exposed infants who did not receive birth-testing. Maternal antenatal and obstetric characteristics of neonates were abstracted via folder review. Primary outcome was any subsequent HIV-PCR test before the end of follow-up, ascertained from the National Health Laboratory Service database. Data were analyzed using logistic regression models, examining independent predictors of presentation at follow-up EID testing.

Results: Overall, 575 neonates underwent birth-testing, with 22 positive (3.8%) and 551 negative results. At follow-up EID presentation (n=871), 4 infected infants were identified (0.4%). Fewer infants who underwent birth-testing presented for later EID compared to infants who did not receive a birth-test (73% vs 85%), (Odds Ratio, 0.46; 95% confidence interval, 0.34-0.62). Infants who underwent birth-testing, also presented for later EID at a significantly older age (mean age 60 days vs.50 days, $p<0.001$). The significantly lower rate of EID presentation among birth-tested children persisted in multivariable analyses adjusting for maternal age, nadir CD4 cell count, ART use during pregnancy, gestation, infant sex, birthweight and infant feeding modality (Adjusted odds ratio, 0.60 95% confidence interval, 0.40-0.88).

Conclusions: Neonates undergoing HIV testing at birth may be less likely to present for subsequent EID testing than those not birth-tested. Emphasis must be placed on appropriate counseling provided to caregivers on the need for further HIV testing after negative birth-test results.

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ACRONYMS

AIDS	Autoimmune deficiency syndrome
ANC	Antenatal Care
ART	Antiretroviral therapy
cARP	Combined antiretroviral prophylaxis
CD4	(Cluster of differentiation 4) T helper cells
CI	Confidence intervals
C/S	Cesarean section
DBS	Dried blood spot
DNA	Deoxyribonucleic acid
EID	Early infant diagnosis
HIV	Human immunodeficiency virus
IP	Intra-partum
IU	In utero
LTFU	Loss to follow up
MMH	Mowbray Maternity Hospital
MTCT	Mother to child transmission
NAAT	Nucleic acid amplification test
NHLS	National Health Laboratory Service
NVD	Natural vaginal delivery
PCR	Polymerase chain reaction
PMTCT	Prevention of mother to child transmission
PP	Post-partum
UNAIDS	United Nations Programme on HIV/AIDS
VL	Viral load
WHO	World Health Organization

PART A: RESEARCH PROTOCOL

STUDY SYNOPSIS

Protocol for epidemiological study of the impact of introducing an HIV-PCR test at birth for HIV-exposed infants in Cape Town.

Study Design:	Retrospective cohort study of HIV-exposed infants born at Mowbray Maternity Hospital, Cape Town.
Duration:	9 months to completion: 5 months data collection, 2 months data analysis, 2 months dissemination of findings.
Sample Size:	Cohort will be assembled retrospectively and include 1102 HIV-exposed infants.
Population:	Infants born at Mowbray Maternity Hospital, Cape Town identified as HIV-exposed by either maternal self-identification with recorded diagnosis on their Road to Health Card or confirmed rapid antibody test.
Primary Study Objective:	Describe attendance at routine EID services stratified by receipt of an HIV-PCR test at birth, and to investigate possible predictors of non-attendance in this population.
Secondary Study Objective:	Examine the yield of positive results at each HIV-PCR test and potential linkage to care for infected infants.
Data collection:	Data will be collected retrospectively from routine medical service records of HIV-exposed infants and their HIV-infected mothers. The National Health Laboratory Service database will be searched for HIV-PCR results.
Informed Consent:	No direct contact with participants, no informed consent will be administered. Multiple steps will be taken to minimize the risk of any loss of confidentiality throughout study design and conduction of the research.
Compensation:	There is no individual compensation for participation in this study.

BACKGROUND

Global HIV/AIDS Epidemic

According to UNAIDS there are an estimated 37 million people living with Human Immunodeficiency Virus (HIV) globally, of whom, 2.6 million are children under 15 years of age (1). HIV infections in infants are predominantly the result of mother-to-child-transmission (MTCT), with those infected *in utero* at highest risk of rapid disease progression, and mortality (2,3). Prevention programmes involving the scale-up of treatment for pregnant and post-partum women have averted a potential 1.3 million infections among children since 2000 (4). Today the majority of HIV-infected women are receiving lifelong antiretroviral therapy (ART) during pregnancy, preventing transmission of HIV to both the unborn child, and uninfected partners (4,5). MTCT rates have fallen below 1% in Europe and North America, with several low resource settings (Cuba & Thailand) also reaching unprecedented lows in 2016 (6,7). However, prevention of mother-to-child transmission (PMTCT) coverage is not yet at 100%, and there are significant concerns regarding adherence during pregnancy, meaning vertical transmissions continue to occur (5,8,9). In sub-Saharan Africa, an estimated 200,000 infants were infected with HIV in 2014, almost 90% of the global total (4). For infants who do acquire the infection perinatally, mortality levels are extremely high between the postneonatal period and 6 months of age (10,2). In South Africa, 70% of maternal deaths and 50% deaths for children under five years of age were associated with HIV (11,12).

Research from the South African CHER trail demonstrated that prompt diagnosis and early introduction of ART can dramatically reduce mortality in children infected with HIV. However, diagnosis of paediatric HIV is a complex process, requiring multiple postpartum visits for mother infant dyads. The World Health Organization (WHO) recommends that all HIV-exposed infants are tested by 6 weeks of age, and those who return a positive result are immediately referred for ART initiation (13). However, there is recognition that loss to follow-up (LTFU) during the early infant diagnosis (EID) pathway is a major barrier to linking infected infants to care and improving early childhood survival in high burden settings (14).

Barriers to the EID cascade

The requirement for virological assays in paediatric HIV diagnosis rather than cheap serological assays, used for HIV diagnosis in adults, has presented both cost and acceptability concerns for EID programmes (14,15), but

dried blood spot (DBS) specimen collection techniques for nucleic acid amplification tests (NAAT) (or polymerase chain reaction (PCR) assays) have been shown to be sensitive and specific for diagnosis of paediatric HIV (16,17). DBS removes the need for phlebotomy and cold chain specimen storage permitting decentralized testing and expanding access in many high burden countries (18,19). The South African 6 week EID test has been scheduled so as to coincide with early childhood immunization timetables. Vaccination visits in South Africa have a coverage of 94%, and by incorporating EID testing into an established programme, implementation costs are reduced whilst maximizing access for HIV-exposed infants (10). However, in 2014, only half of those in need presented for a virological test. Access to EID does not ensure an infected infant will receive their result as LTFU occurs throughout the cascade (4).

The EID cascade is made up of multiple steps (Figure 1); specimen collection, transport and processing, and result return, presenting multiple points at which mother-infant dyads can be lost from care. Attrition between delivery and presentation to testing has been widely studied, for example, a recent study from Johannesburg, South Africa, found that 20% of HIV-infected infants had died or become lost to follow up by 6 weeks of age (20). Studies from Botswana and Malawi indicate that despite known maternal status many HIV-exposed infants attending well child visits do not receive a virological test and therefore never enter the EID pathway (21,22). Even after presentation for testing, loss to follow up is known to continue throughout the cascade. Research carried out in Tanzania found that only 74% of HIV-PCR results were returned to the clinic and only 34% returned to the caregiver (23). In high burden rural settings there can be major delays in specimen processing. Nuwagaba-Biribonwoha et al in Tanzania and Hassan et al. in Kenya found result return to caregivers could take up to 3 months after the initial specimen collection (24,25). It is argued that this waiting time results in operational delays to initiating infants on ART and causes anxiety and worry for caregivers (14).

Research into barriers of retention within PMTCT services have identified not only health system constraints but also many individual and socioeconomic factors. As caregivers bear ultimate responsibility for EID presentation it is important to consider all possible influences on LTFU within the cascade. Relationships, family or community influences could all affect EID presentation (26–29). A recent study from South Africa suggests that fear of discrimination is often a cause of dissociation from care (28). Further research is needed in this area, but it is clear that novel interventions are required to identify infected infants and link them to care.

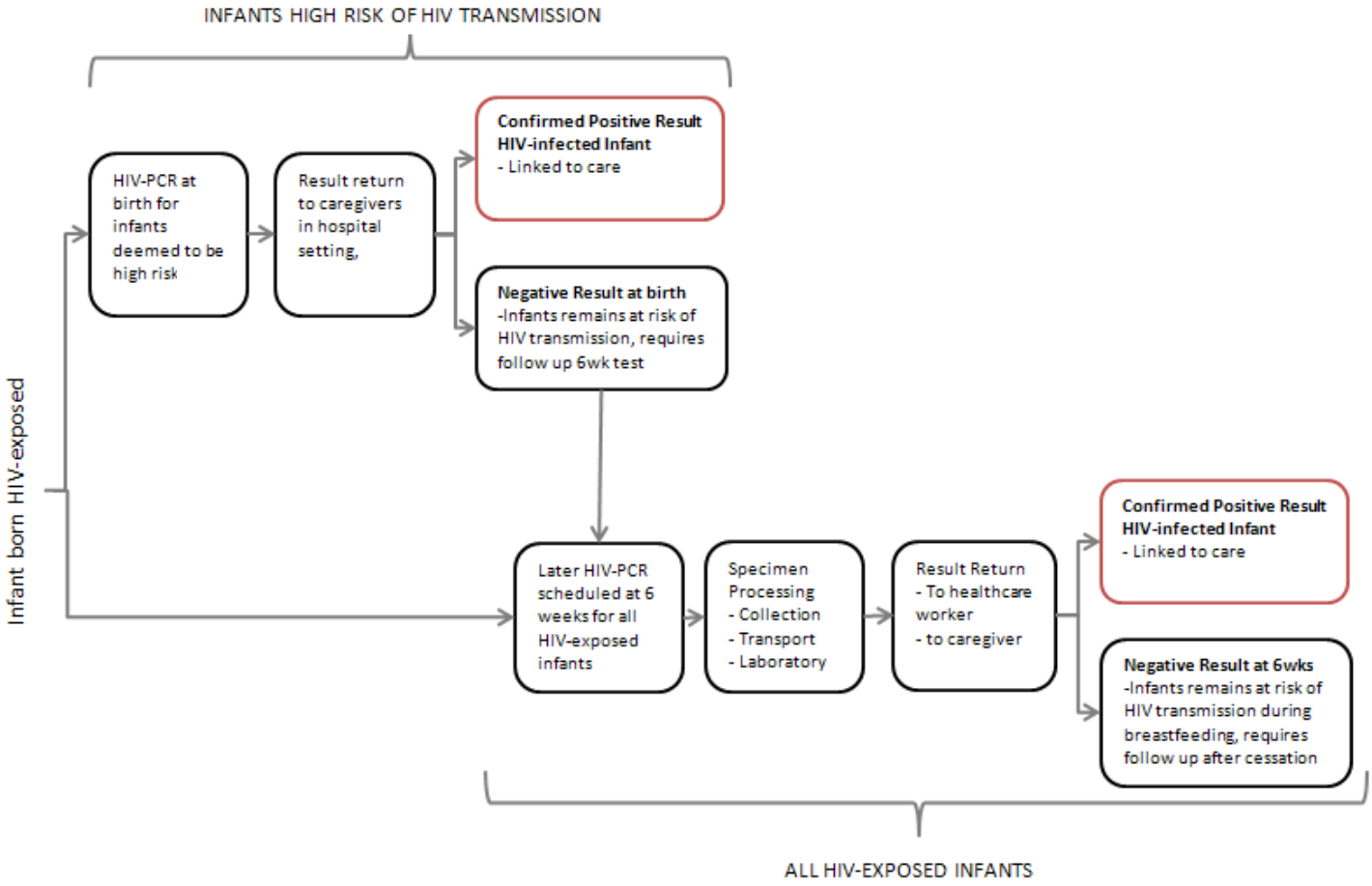
HIV-PCR Testing at Birth

In a setting with high coverage of maternal and infant prophylaxis, Lilian et al. demonstrated that by using more sensitive HIV assays 76% of all early vertical transmissions were detectable at birth (30). It is reasoned that using HIV-PCR testing at birth could overcome many of the logistical delays in the initiation of treatment for infected infants. By returning EID results in the post-partum period prior to discharge, LTFU could be reduced (31). The availability of on-site laboratories at higher level facilities prevent delays in result return, but primary care still encounter many logistical challenges around transportation of samples to central laboratories and PCR processing times which delay result return and mean multiple post-partum visits are required for mother infant dyads potentially increasing the probability of LTFU (14,24,32,33). Point-of-care (POC) could circumvent many of the logistical challenges and allow for result return in a single visit whilst reducing the delay in initiating infected infants on ART. Two mathematical models relating to the cost effectiveness and impact of birth testing within the EID cascade by Lilian et al. and Franke et al. (pre-publication) have found that adding birth testing significantly increases the life years saved in infected infants by reducing mortality of infants in the early weeks of life (34,35).

Paediatric HIV testing in South Africa 2014

Over the past decade, there has been substantial research around the EID cascade. Focus has been placed on reducing the number of infant infections and understanding the scale of LTFU within the cascade. However, little headway has been made on increasing the number of infected infants linked to care and the proportion of infants currently receiving treatment lags far behind adults (36,37). In consideration of the high mortality rate in the neonatal period and high levels of loss to follow up at 6 week testing, the South African National Department of Health became the first high burden, resource limited country to implement HIV-PCR testing at birth in 2014 (Figure 1). Revised clinical guidelines for the PMTCT of HIV included HIV-PCR testing at birth for HIV-exposed infants thought to be at high risk of perinatal transmission (38). Risk criteria include low birth weight and prematurity as well as those born to mothers on ART for less than 3 months during pregnancy or with a viral load of above 1000 copies per milliliter of blood (14,39). Infants would be required to return for a follow up test at 6 weeks of age to detect intra-partum infections, the same requirement as for all HIV-exposed infants. However, there is a paucity of data on the diagnosis or treatment of infants during the neonatal period, it is therefore unknown what impact the addition of a PCR test at birth will have on the current yield and coverage of early infant diagnosis (EID) services.

Figure 1 Early infant diagnosis (EID) cascade in South Africa 2015: HIV-exposed non-low risk infants receive a birth test and require further follow up if the specimen is negative. They then enter the general EID cascade with all low-risk infants. This process includes; specimen collection, transport, and laboratory processing before the relay of results to both healthcare providers and infants' families/caregivers followed by linkage to care.



Background to proposed research – barriers to HIV-PCR testing at birth

As HIV-PCR testing at birth is only a conditional recommendation from the WHO, South Africa is the first high burden, resource limited country to implement HIV-PCR testing at birth (15,40). Currently little to no evidence is available to guide policy makers on amendments to resource provisions or counseling emphasis. The WHO have called for additional information on birth testing as previous research has highlighted the post-partum period as a weak link in the continuum of care. Qualitative research studies from South Africa suggest that despite a high level of awareness of MTCT, HIV knowledge gaps exist on infant diagnosis and many caregivers remain uncertain about six-week EID services and early initiation of ART in HIV-infected infants (28,41). Hassan et al report that post-partum care was often inadequately covered during the antenatal period during PMTCT services (26–29). The introduction of an additional test for paediatric HIV without changes to counseling procedures could cause confusion around the significance of the birth test result and a requirement to attend a second test at 6 weeks. In turn, this could negatively impact the loss to follow up for routine EID services at 6 weeks. The only previous research conducted on this topic, an observational study from the

Western Cape, found that only 49% of infants returned for follow up testing having previously received a birth test, far below the national estimates of EID coverage (42).

Introducing birth testing nationally in South Africa has been a somewhat controversial issue considering the shortage of evidence available for initiating ART in the first weeks of life and the unknown impact of adding an additional test to the cascade. Birth testing could nonetheless have significant implications for an HIV-exposed infant's health. Infants infected *in utero* could access treatment earlier, but for those infected *intra-partum* there could be severe consequences if levels of coverage at 6 week testing fall such that infants may miss diagnosis and are therefore unable to access ART in an adequate timeframe.

STATEMENT OF THE PROBLEM

It is apparent from the literature that there is little evidence around HIV-PCR testing at birth. The changes to the South African 2014 National Policies on PMTCT make it the first high burden, low resource country to implement HIV-PCR testing at birth. The impact of receiving a result within 48hours of life is complex and may have many repercussions, both positive and negative. As South Africa moves to a universal policy of testing all HIV-exposed infants at birth in 2016, the impact of birth testing needs to be evaluated to provide further evidence around the yield of positive results, linkage to care and to consider the possible consequences on coverage levels at routine follow-up EID visits.

The proposed study has the potential to make a significant contribution to improvements in EID services in South Africa. Birth testing could help to maximize the benefits of antiretroviral therapy for child health and could have a considerable effect on the number of children who are successfully linked to care. However, there is currently little research around the impact of birth testing on the EID cascade. If levels of loss to follow up are significantly different to those who did not receive a birth test, there could be further requirements for research on how best to counsel and manage services appropriately, especially for mothers of infants whose birth test is negative on the need to return for an additional test.

STUDY AIMS & OBJECTIVES

Study aim

The overall aim of the proposed research is to investigate attendance at EID testing among HIV-exposed infants born at Mowbray Maternity Hospital, comparing mother infant dyads who received an HIV-PCR test at birth, to dyads who did not receive an HIV-PCR test at birth.

Objectives

1. Examine the proportion of infants attending follow-up EID testing who received an additional HIV-PCR test at birth compared to those who did not receive a birth test.
    a) To examine the time at which EID testing takes place for each cohort.
    b) To investigate maternal and infant socio-demographic, obstetric, and clinical characteristics as possible predictors of EID presentation.
2. Determine the number and proportion of confirmed positive and negative HIV-PCR tests in relation to the number of HIV-exposed neonates:
    a) Within 48 hours of birth (*in utero* transmission rate)
    b) Follow up testing (*intrapartum* / early *postpartum* transmission rate)
    c) Compare the proportion of infants confirmed positive at routine EID testing between high risk infants who underwent birth testing and infants who did not receive a HIV-PCR at birth
3. Examine linkage to care of infected infants

Hypothesis

It is hypothesized that presentation at EID testing will be lower in those who received an HIV-PCR test at birth, but that due to the targeted HIV-PCR testing at birth, there will be a higher yield of infected infants at this time point.

METHODOLOGY

Study Design

In order to address these objectives a retrospective descriptive folder review and database search is proposed. Infants born to HIV-infected women at a secondary level obstetric hospital who were identified as high risk and therefore received an HIV-PCR test at birth are included in a hospital database. Infants born to HIV-infected women at the obstetric hospital not identified as high risk, who did not receive a birth test are recorded in PMTCT, labour and theatre registers. Infants born between July 2013 and August 2015 will be included, folder review for all infants will collect maternal obstetric and infant clinical characteristics. Infant HIV-PCR results will be followed until February 2016.

Study Population

All HIV-exposed infants who were delivered at Mowbray Maternity Hospital (MMH), who received an HIV-PCR test at birth between July 2013 and August 2015 will be included. A cohort of HIV-exposed infants who did not receive an HIV-PCR test at birth will be assembled retrospectively by review of routine data records from the hospital (PMTCT, labour and theatre registers). There will be no direct contact with any participants for this research.

Inclusion Criteria

Maternal HIV status for Mowbray Maternity Hospital (study site) is documented from confirmed finger-prick rapid test or documentation of HIV status for those women self-reporting HIV diagnosis in the Road to Health card. Participants who had no record of delivery at MMH and may have been transferred to the study site after delivery will be excluded from the analysis as date of birth cannot be confirmed.

Sample size

Over 500 infants were delivered at MMH and received an HIV-PCR test at birth over the 2 year study period, a control sample of HIV-exposed infants who did not receive a birth test will be taken as a comparison group.

A cohort of HIV-exposed infants who received a birth test and a cohort of infants who did not receive an HIV-PCR test at birth should be considered using 80% power ($\beta=0.2$) and a two-sided $\alpha=0.05$. Current estimates of coverage of EID before 3 months of age are between 50-80% (43–45). Sample size calculations generates a

required sample size of 539 minimum in each group. As 575 infants received a HIV-PCR test at birth, all will be included in the study to maximize the accuracy of estimates produced during the analysis.

Estimated sample sizes required under different assumptions

Proportion of mother infant dyads presenting to care in the cohort who received birth testing	Absolute increase in outcome, control minus intervention	Proportion of mother-infant dyads presenting to EID testing who did not receive a birth test	Sample size required (per group)
0.55	0.1	0.65	61
0.60	0.05	0.65	234
0.60	0.08	0.68	90
0.65	0.05	0.70	219
0.68	0.02	0.70	1330
0.70	0.05	0.75	199
0.70	0.02	0.72	1280
0.72	**0.03**	**0.75**	**539**
0.75	0.05	0.80	174
0.60	0.2	0.80	14

Study Setting

HIV-PCR tests on infants <48 hours old took at the Department of Neonatology at Mowbray Maternity Hospital (MMH). Specimen draw was conducted at MMH before samples were transported for processing at the National Health Laboratory Services (NHLS) laboratory at Groote Schuur Hospital (GSH) as standard of care (SOC). MMH is a secondary-level obstetric hospital with neonatal care facilities and a primary care component, meaning women with local addresses also deliver at this facility. Recent data indicates that around 11,000 births take place each year of which 13% are HIV-exposed and 3.5% of infants are estimated to contract HIV. National estimates state that between 60-70% of women using government facilities will require the services of a hospital at some point during their pregnancies. Referral to a secondary care facility occurs if the pregnancy is deemed to be high risk (irrelevant of HIV status), due to a women's obstetric history, general medical condition or if she presents with specific risk factors during her current pregnancy.

GSH is the current reference laboratory for much of the cape metropole area. HIV-PCR tests are conducted using the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 qualitative assay. Approximately 10,000 tests are conducted at this laboratory annually, with a test positive rate of ~2% and an indeterminate/equivocal rate of ~0.5%.

The neonatal department performed HIV-PCR tests at birth on infants deemed to be high risk of perinatal transmission of HIV as per provincial policy. In 2013 Option B+ (implemented nationally in South Africa) permitted clinicians to perform HIV-PCR testing at birth for infants deemed to be high risk of HIV transmission, at this time categorization of infants as high risk was subjective to clinician's judgement. In 2014 South African National Guidelines recommended the use of HIV-PCR testing at birth for HIV-exposed infants at high risk of vertical HIV transmission. High risk status was based on maternal characteristics, primarily late or no initiation of ART, clinically documented default status, or if the infant was born preterm or low birthweight. This was in line with the WHO technical expert panel that considered universal or risk based birth testing in 2013 (Appendix 4). After the successful roll out of high risk birth testing nationally, in late 2015 the South African guidelines were updated to include universal testing of all HIV-exposed infants.

Infant and maternal characteristics of those who received HIV-PCR testing within 48 hours of birth were recorded in a hospital database.

Data Collection

The study will be conducted in two phases: Data from 575 infants who received an HIV-PCR test at birth has been collated into a hospital database at MMH, infants who received a birth test will be matched to HIV-exposed infants who did not receive an HIV-PCR test at birth. The study schema is shown in Figure 2. Infants will be matched on date of birth as the criteria for receiving a birth tests was altered during the study period. Labour ward or theatre birth registers will be used to verify date of birth during the study. Date of birth is required to determine test results for infants enrolled in the study. Matching was therefore based on delivery method and date of birth.

Phase I – A random sample of 551 HIV-exposed infants who were born at Mowbray Maternity Hospital between July 2013 and August 2015 and did not receive a birth test will be matched to infants who received a negative result from the HIV-PCR test at birth, using date of birth (within +/- 2 weeks) and delivery method (natural vaginal delivery (NVD) or cesarean section (C/S)). PMTCT register, labour ward and theatre registers will be used to identify possible candidates for inclusion. Identified infants will be randomly selected (using random number draw if more than one possible match was possible) before retrospective review of routine medical records is used to identify key demographic variables. Abstracted chart data to be collected includes

(Appendix 3):

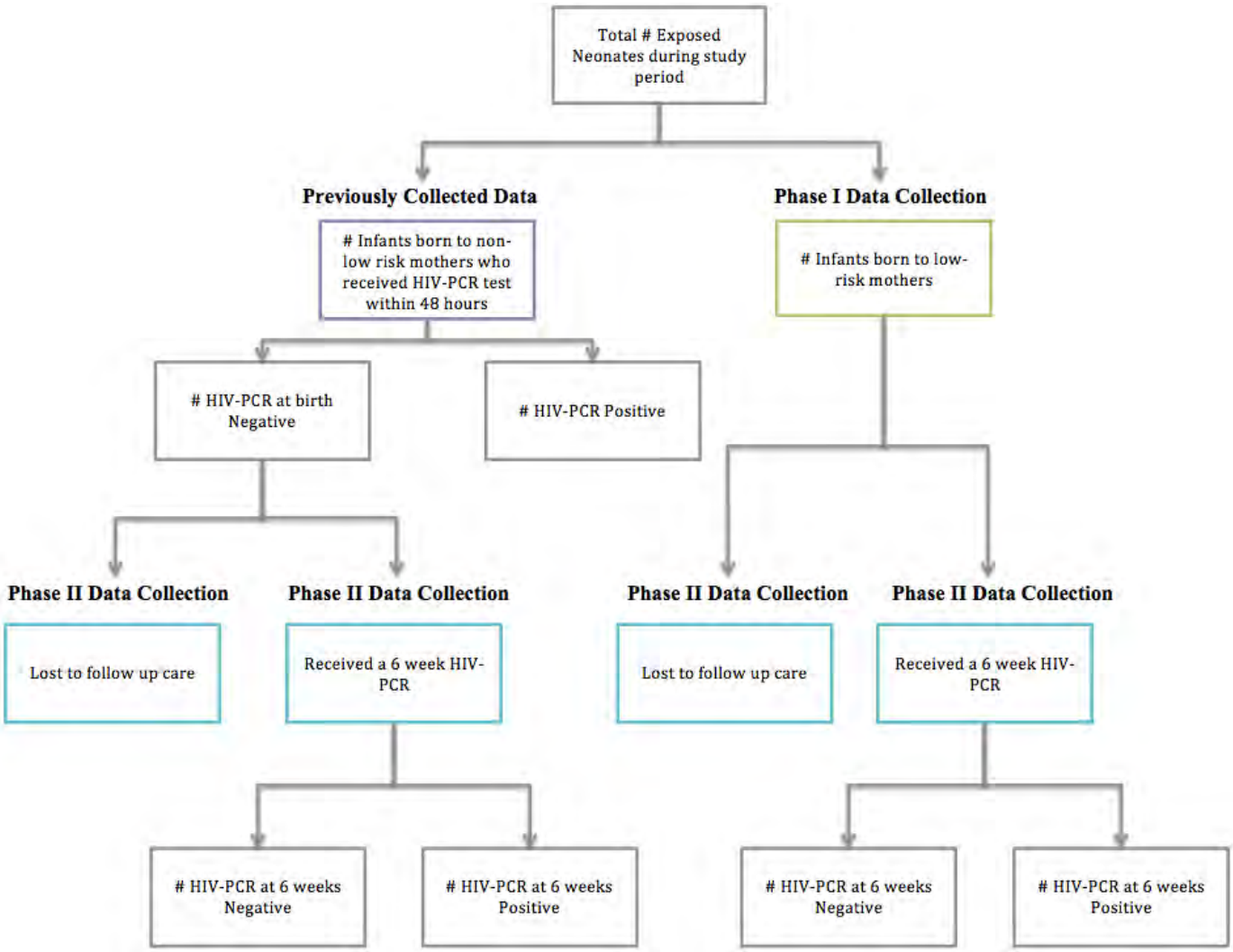
- Maternal demographic information (Name, Address, date of birth, population group, parity)
- Infant demographic information (Name, date of birth, birthweight, gestation)
- Maternal Clinical, immunological and virological data prior to labour
- Choice of feeding method

Phase II – Infant HIV status from HIV-PCR results and date of the test will be determined by review of National Health Laboratory Service (NHLS) online record database. All infants will be followed from the time of birth until the initial EID visit. A window period of 4-12 weeks will be specified as returning for routine EID testing, but all infants will be given the full study period (to February 2016) to return for follow-up testing. Linkage to care will also be determined by review of NHLS databases. Medical records of all infants who returned a positive result either at birth or at the 6week test will be reviewed to determine whether a confirmatory test was received, and if a further specimen related to HIV care was sent for processing at NHLS laboratories signifying linkage to care.

Outcomes of the proposed study will be defined as:

- EID presentation: mother/infant dyad accessing an HIV-PCR test after being discharged from hospital, recorded by HIV-PCR result documented on the NHLS system.
- Linkage to care: Specimen from an HIV-infected infant sent to the NHLS laboratory and documented on the online system after an initial HIV-PCR positive test result.

Figure 2: Study Process: Previously Collected Data: HIV-exposed high risk infants who received a negative result from their birth test required further follow up. This data has been collated into a hospital database**,** infant date of birth will be cross referenced to labour and theatre registers before capturing into study database.
Phase I: HIV-exposed low risk infants are matched on mode of delivery and date of birth to each high risk infant born at MMH with a negative birth test result from retrospective folder review. HIV-exposed infants will be identified from the PMTCT register and date of birth will be confirmed from labour and theatre registers before folder review for obstetric, and maternal socio-demographic characteristics for included infants.
Phase II will use the NHLS database to identify HIV-PCR results for all enrolled infants and follow up care for all HIV-infected infants to determine linkage to care.



Data Management

Data management will take place at Mowbray Maternity Hospital, following procedures established for multiple previous studies conducted within the hospital site. Data collected on paper forms will be entered into a custom designed Microsoft Access database, maintained in a firewall-protected UCT server with nightly backups. The study database will be password-protected following standard password protection procedures. All electronic files will be encrypted and password protected. Quality control will be through data checking original data collection tools or patient folders to identify out-of-range values, logic violations, and missing observations. Data editing will be based on reference to the form and/or source document in question; all data queries and responses will be logged, and edits will be implemented through separate program files.

Data Analysis

The data from this study will be analysed using Stata version 13.0 (Stata Corporation, USA). Data will be explored using univariate and bivariate descriptive statistics, focusing primarily on maternal characteristics stratified by receipt of an HIV-PCR test at birth. Standard approaches will be used (e.g. means with standard deviations or medians with interquartile ranges). Frequency tables will be used to examine proportions of HIV-infected infants, and those who did and did not attend EID testing, comparisons by receipt of birth testing will be made.

Objective 1 –To determine associations between maternal characteristics and presentation at routine EID testing, bivariate analysis using chi-square tests will be used. The EID statistic (dichotomized for presentation) will be the primary outcome of interest analysed using logistic regression to explore independent predictors for EID presentation; this will be adjusted for potential confounders including demographics characteristics such as maternal age, ART coverage during pregnancy, maternal age, maternal population group, infant birthweight or gestation. Outputs will be expressed as odds ratios, with 95% confidence intervals.

Objective 2 – Proportions of infants with positive HIV-PCR results will be compared to those with negative results, this will include comparisons within the group of infants that received a birth test and to those who did not.

Objective 3 – Proportion of infants linked to care after a positive birth test result will be compared to infants with positive results at 6 weeks.

ETHICAL CONSIDERATIONS

Ethical Approval

The study protocol and all data collection tools will be reviewed by the University of Cape Town Faculty of Health Sciences Research Ethics Committee (UCT-REC). All study procedures will be conducted in accordance with the protocol and to the standards of Good Clinical Practice guidelines. The study protocol will be presented to Mowbray Maternity Hospital Ethical board for site specific permission for the proposed study.

Potential Benefit

Participants will not receive any direct benefits; however, exploring the possible impact of introducing a test at birth could have wide ranging implication for policy makers. The proposed study could provide evidence around the benefits of birth testing to improve care for future HIV-exposed infants. Evidence in favour of birth testing could encourage other high burden settings to implement HIV-PCR testing at birth, leading to reduced mortality for infants infected in utero. In the event that the evidence shows a negative impact of birth testing, it is hoped that the information generated will be used by policy makers to adapt the guidelines and resources to ensure health care providers have adequate knowledge and time during PMTCT services to provide care that will benefit all HIV-exposed infants and their caregivers.

Potential Harm

The proposed study will present minimal risk to participants due to the use of routine clinical data during a retrospective folder review. Considerable precautions to avert the possibility of loss of confidentiality will be taken throughout the process.

Informed consent

As this is a retrospective folder review, it is not possible to gain consent from participants. Anonymous participant identification numbers will be used on all study documents. Collection of participant names and other identifiers will only be used for identification of infants to be included in the study. When data collection is completed all participant identifiers will be destroyed. As informed consent is not possible in this study, the need for confidentiality increases as the only potential risk is loss of confidentiality.

Confidentiality

The following steps will be taken to minimize the risk of any loss of confidentiality throughout study design and conduct.

- All personnel involved in data collection and management will undergo specific training for the study in confidentiality and related patient protection issues.
- Following standard practice, all patient- and study-related information will be kept in locked cabinets at the study site.
- Anonymous participant identification numbers will be used on all study documents. Collection of participant names and other identifiers will be destroyed after data collection is completed.
- All electronic records will be kept in password-protected files. All electronic communications of study data will be through password-protected, encrypted files. All data storage at the University of Cape Town will be within a firewall-protected SQL server.

Compensation

There is no individual compensation for participation in this study.

LIMITATIONS

The retrospective nature of this study and the collection of data from routine medical records means there may be variation in the information collected over the two-year study period.

Social and demographic factors such as maternal HIV-knowledge, household income, maternal education level or distance to clinic that could be associated with non-attendance at EID testing cannot be obtained from folder review and will therefore be unavailable for analysis.

Between July 2013 and October 2015 NHLS database is stratified by province. Before October 2015 study staff only had access to data from the Western Cape on the online database. Mother-infant dyads could present for care at an alternative facility in a different province and would not be captured, they would therefore be recorded as not-attending EID testing in this study, we may therefore overestimate loss to follow up. However, we are also unable to ascertain if results were successfully returned to caregivers and therefore may overestimate the completion of the EID cascade. We will not be able to determine the date of ART initiation for

infected infants and therefore must use a proxy for linkage to care which could overestimate the retention in care and linkage to ART.

This study will not evaluate barriers to follow-up from the patient perspective as no direct contact with participants will be made, nor will the study look at completion levels of the whole EID cascade (return for testing after cessation of breastfeeding) as timeline for completion of the study would not permit all infants to reach the 18 months of age boundary for possible MTCT.

The study is also not designed to evaluate health system constraints and perspective on knowledge of changes in guidelines and reporting of results and HIV-exposure in the Road to Health Card.

DISSEMINATION OF FINDINGS

The findings from the proposed study will be submitted in partial fulfilment of the requirements for the Master of Public Health (General) degree at the University of Cape Town, and to a peer reviewed journal for publication. Based on the result of a preliminary analysis an abstract could be submitted to relevant conferences for presentation. When analysis has concluded, a report or presentation of the findings can be offered to all interested stakeholders.

BUDGET

Table 3: Budget calculations for proposed study

Budget for Proposed Study			
Item	**Cost per unit**	**Number of units**	**Total cost**
Field Worker	R80/hour	50 hours	R4000
Photocopying	R0.30/page	300 pages	R90
Data Capture	R60/hour	40 hours	R2400
Total			R6490

TIMEFRAME

	October '15	November '15	December '15	January '16	February '16	March '16	April '16	May '16	June '16	July '16	August '16
Departmental and ethical approval											
Site (MMH) ethical approval											
Data Capture (Previously collected data)											
Data Collection (Phase 1)											
Data Collection (Phase 2)											
Data analysis											
Writing of manuscript											
Submission of preliminary findings to conference											
Submission of dissertation											
Dissemination of findings											

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PART B: LITERATURE REVIEW

INTRODUCTION

Enormous progress has been made towards tackling the HIV/AIDS epidemic, especially in low-income countries (1), but the global target to end AIDS by 2030 is an ambitious goal. Since the passing of the millennium development goal target date in 2015, UNAIDS has led the efforts to establish new targets for the scaling up of HIV-treatment (2). The new three part 90-90-90 target aims to identify 90% of all people living with HIV, treat 90% of those diagnosed with HIV infection and achieve virologic suppression in 90% of all individuals receiving antiretroviral treatment (ART) by 2020 (2,3). Previous changes in treatment regimes have increased the CD4 count threshold for initiation of HIV treatment in low income countries with almost all countries now adopting Option B+ (lifelong ART for all pregnant and breastfeeding women irrespective of CD4+ count) and slowly moving towards "test and treat", which modelling studies predict could be effective at reducing HIV transmission at a population level (4,5). The aim of the new global targets is to identify new infections, and provide high quality care to those living with HIV. Focus has been placed on retaining patients in care and achieving sustained virological suppression in order to reduce the morbidity and mortality associated with HIV infection (6,7). The same targets have been proposed for children, with the ideal of complete elimination of mother to child transmission leading to the emergence of an AIDS free generation, but in high burden settings, especially sub-Saharan Africa, programmes centred on diagnosis and treatment of infected infants continue to have the largest shortfalls (7–9).

South Africa has been a leader in the prevention of mother to child transmission (PMTCT) programmes, remarkably reducing the vertical transmission of HIV. Yet early infant diagnosis (EID) testing coverage reached only 72.6% of HIV-exposed infants in 2012 (10), and the linkage to care for infected infants was only 71% (11). Globally this was far worse, with only 49% of all HIV-exposed infants receiving a virologic test before 6 weeks of age in 2014 and only 30% of infected infants being effectively linked to care to initiate ART (12,13). Infected infants are known to be at high risk of mortality within the first year of life (14). Prompt initiation on ART has been shown to increase infant survival, but, without treatment, 50% will die before their second birthday (12). Despite the large-scale implementation of PMTCT and the pivotal role of EID within these programmes, the progress of infected infants is lagging far behind that of adults and the global targets for 2020 (7).

Understanding the gaps in HIV-related infant care, particularly around the retention of those HIV-exposed within the continuum of PMTCT services, allowing for diagnosis and treatment of infected infants remains one of the most important areas of investigation (8). The following review will explore the various factors relating to diagnosis, treatment and retention in care for HIV-exposed infants, along with barriers to presentation at EID testing.

OBJECTIVES OF THE LITERATURE REVIEW

The objective of this review is to examine major themes in the existing literature on HIV-testing in infants, looking specifically at the timing and frequency of tests, recent changes to local and global recommendations whilst also exploring the barriers to attaining sufficient coverage of EID testing. The review is also intended to assess the empirical evidence currently available and assess whether this is sufficient to help implement programmatic changes in National early infant diagnosis programmes.

LITERATURE SEARCH

Literature was collected by searching Medline (via Pubmed) Google Scholar and EMBASE databases for peer reviewed or non-peer reviewed publications using key words such as 'human immunodeficiency virus or HIV' 'infant HIV' 'antiretroviral therapy' 'early infant diagnosis' 'perinatal transmission' and 'birth testing' (full search terms included in appendix 10). There were no restrictions for geographical location or date applied. Only articles in English were included. All titles were screened and abstracts of relevant articles were read to identify full-texts for review. Articles were excluded if they examined effectiveness of specific ART drug regimes or assessed diagnosis of infants older than 18 months. In addition, the reference lists of relevant articles found were examined for any further texts. Conference abstracts for the Conferences of the International AIDS Society (2010 to 2016) and the Conference on Retroviruses and Opportunistic Infections for (2014/2015/2016) were searched to identify recent studies that may not yet have been published as full text. (Summary table of included studies attached as appendix 9).

SUMMARY OF THE LITERATURE

Global HIV/AIDS epidemic

It is estimated that over 36 million people were living with HIV globally in 2015, 71% of whom live in sub-

Saharan Africa (13). While the vast majority of new HIV infections in sub-Saharan Africa occur in adults, young women are disproportionately affected by HIV. Consequently, 1.2 million children were born exposed to HIV in 2013, all of whom required an HIV test during infancy (13, 14). In sub-Saharan Africa, an estimated 200,000 infants were infected with HIV in 2014, almost 90% of the global total, but with only 49% of HIV-exposed infants receiving a virologcal test, mortality rates for infected infants remain unacceptably high (4).

Prevention of mother to child transmission (PMTCT)

Women's access to antiretroviral treatment during pregnancy has increased extensively since 1994 with greater understanding around the pathogenesis of the virus and possible treatment mechanisms. Initially zidovudine was shown to reduce the risk of perinatal transmission by the PACTG 076 trial (15), followed by combined antiretroviral treatment in the late 1990's (16–18). Prevention of mother-to-child transmission (PMTCT) programmes no longer restrict access to medication based on the woman's health status, but rather, all HIV-infected pregnant women, irrespective of CD4+ count or clinical disease stage are initiated on lifelong ART treatment (19,20). Globally there have been major increases in the percentage of women receiving ART during pregnancy between 2000-2014, with substantial increases seen since the introduction of Option B+ in 2013 (21,22). Prior to ART use in pregnancy, over 25% of exposed infants would acquire HIV during pregnancy, labour or the postpartum period, but with access to ART, the transmission rate has dropped to below 2% in many settings, including Thailand and Cuba which have been certified by the WHO as having eliminated mother to child transmission of HIV (1,23,24). Yet despite the significant advances in PMTCT, vertical HIV-infection in infants continues to occur with an estimated 16,000 infants being diagnosed with HIV in South Africa in 2013 (4).

Diagnosis of paediatric HIV: Early Infant Diagnosis (EID)

The mortality rates of infected infants depends both upon the timing of HIV infection and the initiation of treatment. Perinatally infected untreated infants are at highest risk of rapid disease progression and mortality, with one in two untreated HIV+ infants dying before 2 years of age (25–27). In 2008, the Children with HIV Early ART (CHER) trial provided evidence that initiating treatment before 3 months of age reduced mortality by 76%, compared to waiting until clinical symptoms of AIDS or advanced immunosuppression developed (28). In order to benefit from very early ART initiation, infants must first be diagnosed with HIV, as a result, the World Health Organization (WHO) now recommends that HIV-exposed infants be tested by six weeks of life and those who test positive should be immediately be referred for initiation of ART (29,30).

PCR as a tool for EID

Inexpensive serological antibody assays that are routinely used for diagnosis in adults cannot be easily interpreted for HIV-exposed infants. Trans-placental transfer of maternal antibodies leads infants to be seropositive until 18 months of age (31,32). Infants under the age of 18 months require nucleic acid amplification tests (NAAT) to diagnose HIV infection. These assays recognize viral nucleic acids (HIV RNA or DNA), rather than anti-HIV antibodies produced as an immune response to infection by HIV. NAAT (otherwise known as polymerase chain reaction (PCR)) assays are more complicated, time consuming and costly than serological assays, but they have been shown to be both sensitive and specific in the detection of paediatric HIV infection (33,34).

Barriers to EID testing

NAAT tests require specimens to be stored in coldchain, and are typically performed using specialist laboratory equipment by highly trained personnel. Access to EID is therefore severely limited in many countries to locations with centralized facilities with UNAIDS estimating that of the 1.2 million HIV-exposed infants born in high burden settings, only 49% received a virological test in 2014 (35). Approaches to decentralize EID programmes and subsequently increase access to virological tests have been prioritized by the WHO and governments of countries with a high burden of paediatric HIV. Investments have been made in infrastructure and human resources to strengthen laboratory systems and transportation networks (36), whilst protocols of using dried blood spot (DBS) as means of specimen collection have decentralized testing and helped expand the coverage of EID in several high burden countries (37,38). DBS sample collection has no phlebotomy requirements, and specimens can be transported from peripheral sites to a central laboratory without refrigeration for up to 15 days, providing a cost-effective solution in resource-limited settings (13,37,38). Studies carried out in Cameroon, Tanzania, India, Côte d'Ivoire and South Africa have shown assays using DBS samples are highly sensitive and specific for infant HIV diagnosis and have been an important facet in the successful scale up of EID activities (39–42).

Cost and availability are not the only barriers to implementing successful EID programmes. Diagnosing paediatric HIV requires multiple clinic visits from mother infant dyads with each step providing an opportunity for infants to be lost from care. Edmonds et al describe difficulties in maintaining optimal service delivery at decentralized settings whilst Ciaranello et al. describe LTFU within the EID cascade, including the acceptance

of testing at a healthcare facility for HIV-exposed infants, specimen collection, transport, laboratory processing and results return to both healthcare providers and then infants' caregivers, with linkage to care to initiate antiretroviral therapy required for infected infants (Figure 1) (32).

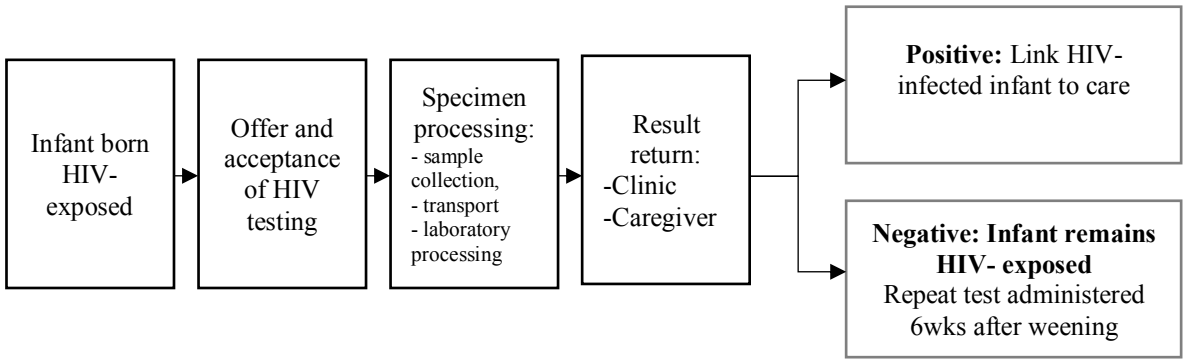

Figure 1. EID Cascade: Multiple steps are required to complete the EID cascade before caregivers are informed of an infant's final status. Infant's with a negative result remain HIV-exposed until the cessation of breastfeeding. (Adapted from Ciaranello 2011)

Experiences from the PMTCT cascade in low resource settings have shown both structural and operational aspects of the health system can lead to LTFU. A study from South Africa found women were more likely to both access and be retained in care if they were receiving treatment at a local clinic, whilst a study from Ethiopia suggested that patient load also has an effect on retention (43,44). Operationally it is known that the EID process can take months. In Kenya, Tanzania and Cote D'Ivoire samples took between 1-3 months to be returned to caregivers after the initial specimen collection had taken place (32,45,46). In South Africa, this has been found to be much quicker taking a maximum for 2 weeks for results to be returned to caregivers at primary care, and a matter of days in higher level facilities (47). Without point-of-care technology, which is not yet widely available for EID, the cascade requires multiple visits (48,49). Spanning these visits months apart could cause high levels of loss to follow up within the EID cascade. Even when results are available at clinics, there is often inadequate result return to caregivers. Patients often move between facilities or geographical areas and do not return to their original clinic, others fail to return to care at all and therefore are unable to receive the test result.

As EID programs are incorporated in both PMTCT and pediatric programs, many settings have failed to find procedures which take responsibility for result return and prevent bottlenecks within the EID cascade.

Ultimately the loss to follow up and delay in result return can prove fatal to infected infants; a study in Tanzania found that only 55% of caregivers returned for the result, and 14% of caregivers were returned a positive result after their infant had demised (50), with a more recent South African study finding 20% of HIV infected infants had either died or been lost to follow up by 6 weeks of age (51).

There is widespread recognition that every new paediatric HIV infection is the result of one or more shortcomings within the health system, but all too often there is limited response to a new infection. EID programs are incorporated in both PMTCT and pediatric programs, but the overlap leads to no single entity taking responsibility for result return and initiating action as and when required (52).

Barriers to retention are not only facility based. Individual, socioeconomic and psychosocial factors have been linked to LTFU in the PMTCT/EID cascades (53). As caregivers bear ultimate responsibility for EID presentation it is important to consider all possible influences on LTFU. Individual factors such as attending PMTCT services during pregnancy and illicit drug use have both been shown to reduce presentation at EID testing (54,55). These could be acting as proxies for health seeking behavior in women living with HIV. The most frequently cited barriers to EID presentation are sociodemographic factors; HIV knowledge, education level, maternal age and marital status have all been associated with lower presentation to HIV care (54,56,57). Ndondoki et al found that in Côte d'Ivoire acceptance from mothers was relatively high but paternal acceptance of EID testing was low, and social etiquette still dictates a key role for fathers in the decision making around infant health in west Africa. Stigma surrounding HIV also remains a huge barrier to women accessing treatment and preventing further transmissions, Braitstein, et al. described how women living with HIV struggle with fear of discrimination, a lack of autonomy and support from family and or the community which prevents many from disclosing their status. In Tanzania this was shown to be associated with not attending the HIV clinic (58).

Timing and frequency of HIV PCR testing in infants

Thanks to the expansion of PMTCT programs, a greater proportion of HIV-exposed infants will return a negative result. This can in turn provide reassurance to families (59,60), whilst providing integral surveillance data for the assessment of PMTCT programme effectiveness (42), but the main function of EID is to initiate infected infants on treatment. South African EID protocols schedule EID visits of specimen collection and result return to coincide with the early immunization visits at 6, 10 and 14 weeks of age, simplifying the logistics for

caregivers and reducing the number of postnatal follow up visits required (19). However, in the context of high infant and maternal prophylaxis, a single PCR test performed at 6 weeks of age may have reduced sensitivity and therefore fail to detect some *in utero* and *intrapartum* infections (51,61). Testing at 6 weeks of age is therefore too early to detect all infected infants when PMTCT coverage is high, but infants infected *in utero* are susceptible to rapid disease progression, with recently published data has showing the peak age of HIV-related mortality occurs around 8-12 weeks of age (62–64) This indicates that testing at 6 weeks is also too late to intervene before many infected infants demise (61). Further evidence from observational studies is needed to inform the optimal timing of EID testing in the first few weeks of life. This would need to consider, assay sensitivity, morbidity and mortality of infants and retention in care.

HIV PCR testing at birth

In the developed world, where there is a low prevalence of HIV, and ample access and availability of resources, guidelines recommend testing HIV-exposed infants at numerous time points during the neonatal period (8,65), but in South Africa and other high burden settings this is neither logistically or economically possible. Infants receive only one virological test during infancy followed by serological assay after the cessation of breastfeeding (29,66).

In 2012 Lilian et al demonstrated that 76% of all early HIV infections were detectable at birth (51), an increase to the yield found in previous studies (32). Improved PMTCT coverage at delivery has increased the proportion of *in utero* infections relative to *intra-partum* and very early *post-partum* infections that are identified by EID testing (67). More infants are detectable at birth and HIV-PCR testing within hours of delivery has become a more attractive option. However, birth testing necessitates a second test to detect the 25% of missed infections at birth and all intrapartum infections.

A recent modelling study evaluating the timing of early infant diagnosis concluded that performing a PCR test at birth and then again at 10 weeks would likely be the ideal diagnostic algorithm in South Africa (68). Further modeling studies have found this algorithm to be cost effective if result return occurs at scale on a reasonable timeline (61, Franke et al. Pre-publication). These studies take into account that in South Africa, 95% of women receive an HIV test and result during pregnancy, over 90% have a skilled birth attendant present at delivery and almost 70% of HIV-exposed infants are born to mothers who received ART during pregnancy (70). Although the CHER trial provided clear evidence around earlier initiation of ART in infants, there are no studies that have examined the mortality benefit for infants initiating ART within the first days of life. Cases that mirror that of

the Mississippi baby have forged a consensus from clinicians that initiating treatment earlier has the potential to lower viral reservoirs and prevent disease progression in early infancy (71,72), moreover no data exists around very early ART in premature or low-birthweight infants.

In 2013 South Africa adopted Option B+ for PMTCT programmes on a National scale. The PMTCT guidelines permitted clinicians to perform HIV-PCR testing at birth for infants deemed to be high risk of HIV transmission. The deviation from the EID testing algorithm at the time allowed for earlier treatment of infected infants or initiation of cARP for those with negative results at birth. It was an attempt to address the high level of childhood mortality associated with HIV. However, at this time categorization of infants as high risk was subjective to clinician's judgement.

In considering the limitations regarding 6 week testing, the perceived benefit from very early initiation of ART, and the current caveat option of birth testing within the PMTCT guidelines for Option B+**,** the 2014 South African National Guidelines recommended the use of HIV-PCR testing at birth for HIV-exposed infants at high risk of vertical HIV transmission (19). High risk status was based on maternal characteristics, primarily late or no initiation of ART, clinically documented default status, or if the infant was born preterm or low birthweight. This was in line with the WHO technical expert panel that considered universal or risk based birth testing in 2013. After the successful roll out of high risk birth testing nationally, in 2015 the South African guidelines were updated to include universal testing of all HIV-exposed infants. Over the previous year birth testing had been more commonly carried out in higher level facilities where health care professionals were more likely to be aware of high risk criteria (73), however the high risk criteria took into account risk factors for HIV transmission, and not simply in utero transmission, which lead to the vast majority of test results returned to be negative. Programmatically it may be easier for health care professionals to implement a universal policy effectively rather than restrict testing to high risk infants (8,61).

Barriers to birth testing

Loss to follow up (LTFU) has been well documented across the EID cascade in low resource settings, with evidence of LTFU occurring at each stage in the cascade (32). Adding additional steps to an already complicated cascade could result in attrition of routine EID services compared to the uptake when no birth test is available. An observational study from the Western Cape found that only 49% of infants returned for follow

up testing having previously received a birth test, far below the national estimates of EID coverage (74). There are recognsied weaknesses in the provision and uptake of PMTCT services during the *post-partum* period for women living with HIV and their newborn infants. Yet, there remains a paucity of data available on why mother infant dyads who do present to care for testing are lost from follow up visits further down the cascade (35,75,76). Woldesenbet et al. describe how inadequate maternal knowledge, fear of discrimination and lack of provider led counseling contributed to missed opportunities for EID in South Africa. Kenyan and Tanzanian studies report mortality to be a key reason for LTFU as well as disclosure, fear and not wanting to upset family members (56,77–79). Considering the scale of LTFU within the cascade it is surprising that more is not being done to examine and address these concerns to facilitate mother/infant dyads being retained in care.

PMTCT services are designed to offer support to women living with HIV, and provide them with information for both antenatal and postnatal care. Although much literature exists about the effectiveness of PMTCT programmes, there has been little research conducted into the quality of counseling provided around post-partum care. A report by the WHO details how counseling is provided by often busy, overworked staff members, who focus on the initial HIV test result and ART treatment. Despite numerous opportunities for follow up counseling, provision of accurate information about postpartum care including infant feeding and infant testing remains limited (60,79–81).

Linkage to care for infected infant

The substantial losses in retention of mother infant dyads from the cascade results in an extremely low number of infected infants linking to care. In 2014, UNAIDS estimated that of the 2.6 million children living with HIV globally, only 32% were receiving ART. This does not account for the hundreds of thousands more who died as a result of the disease. For HIV-infected infants the time between their HIV-test result and linkage to care is critical because of the rapid disease progression during the early weeks of life (82). Accessing antiretroviral treatment could allow children to survive into adolescence. However, a study in Cape Town, South Africa found that only 71% of HIV-infected infants who presented to care for EID testing were linked to care (11). In Malawi this figure was much lower at 36% of infected infants receiving ART (83, 84). Although many studies associate mortality with becoming lost to follow up after a positive test result, or, low result return to caregivers, a study from Kenya found that 30% of HIV-infected infants had not returned to the clinic for care because of disclosure issues including fear of family or community discrimination (47,56). In South Africa, although access to EID testing is high, not all clinics who offer testing are able to provide paediatric ART services (11). The

requirement for further clinic visits could not only increase the opportunity for loss to follow up to occur, but presents further worries around stigma and fear of discrimination for families (85). It is clear that there is a greater need to understand the reasons for infants becoming lost to follow up from a caregivers perspective in order to implement successful programmes integrating PMTCT, EID and childhood ART services that can achieve greater retention in care.

IDENTIFICATION OF GAPS IN THE LITERATURE

It is evident in the literature from high and low burden countries, that initiating earlier treatment to HIV-infected infants will increase survival, but there are a range of barriers which prevent complete coverage of EID testing, around costs and health system constraints, as well as loss to follow up across the cascade. In South Africa it is known that caregivers and infants fail to attend EID testing as a result of lack of HIV knowledge or as a result of the social context in which they live. Stigma is still abundant around HIV and many women suffer from a fear of discrimination that prevents them from seeking follow up treatment. There is a need to improve support and counseling services provided to women living with HIV during pregnancy to improve their understanding of HIV transmission, especially during the post-partum period.

Further evidence is also needed around testing during the neonatal period, possible attrition of services at 6 weeks, and whether this is the optimum time for follow up testing. This study aims to contribute to the growing body of literature around birth testing. Due to the limited evidence around diagnosis and treatments of HIV-infected infants immediately after birth, the WHO guidelines provide only a conditional recommendation to consider the use of birth testing as an additional test to the EID cascade. South Africa is therefore the first high burden country to implement universal birth testing for all HIV-exposed infants, but other countries such as Swaziland are soon to follow suit. Since there are very few published studies, public health programmes have little evidence on which to base their birth testing policies, and as a result there has been no change to counseling or result return procedures since the addition of an HIV-PCR test a birth.

Exploring the possible impact of introducing a test at birth could have wide ranging implication for policy makers. Birth testing could be shown to successfully link infected infants to care earlier, or with a greater yield

than currently seen at 6 weeks by bypassing possible losses associated with mortality or loss to follow up. However, it is also possible that introducing birth testing could have a profound effect on those infants that experience *intra-partum* infections, who may be less likely to return for follow up testing or subsequently link to care having previously received a negative result. Understanding the impact of introducing a birth test could allow policy makers to adapt the guidelines and resources to ensure PMTCT and EID programmes are effective for all HIV-exposed infants.

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PART C: JOURNAL MANUSCRIPT

Title: Impact of birth HIV PCR testing on the uptake at follow up early infant diagnosis (EID) services in Cape Town, South Africa

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*As per the MPH dissertation guidelines, co-authors are not listed on the journal ready manuscript. The contribution of collaborators and supervisors is listed in the acknowledgement section of this dissertation. This article is written according to the requirements in the Instructions for Authors for the Journal of Acquired Immune Deficiency Syndromes. These instructions are included as Appendix 6 following dissertation guidelines.

ABSTRACT

Introduction: PCR testing at birth ('birth-testing') is suggested by new World Health Organization guidelines for rapid diagnosis of infants infected with HIV *in utero*. However, there are few data on the implementation of this approach in sub-Saharan Africa and whether birth-testing affects uptake of subsequent routine early infant diagnosis (EID) testing at 6-10 weeks of age is unknown.

Methods: We reviewed 575 consecutive infants undergoing targeted high-risk birth-testing in Cape Town, South Africa, and matched those testing HIV-negative at birth (n=551) to HIV-exposed infants who did not receive birth-testing (n=551). Maternal and infant clinical and demographic data, including EID testing uptake, were abstracted from routine records. Data were analyzed using logistic regression models, examining independent predictors of presentation at follow-up EID testing.

Results: Overall 3.8% of all birth-tests conducted were positive, while later EID testing positivity rates were 0.5% for those infants testing HIV-negative at birth and 0.4% for those without birth-testing. Infants who underwent birth-testing were less likely to present for later EID compared to those without a birth-test (73% vs 85%; $p<0.001$). This difference persisted after adjusting for maternal and infant characteristics (adjusted odds ratio,0.60 95% confidence interval, 0.40-0.88) and across demographic and clinical subgroups. Infants undergoing birth-testing also presented for later EID at a significantly older age (mean age 60 vs 50 days, $p<0.001$).

Conclusions: While the yield of targeted high-risk birth testing in this setting appears high, neonates testing HIV-negative at birth may be less likely to present for subsequent EID testing. For birth-testing implementation to contribute to overall EID programme goals, structured interventions are required to support follow-up EID services after negative birth-test results.

INTRODUCTION

Prevention of Mother-to-Child Transmission (PMTCT) programmes have been one of the hallmarks of success in the fight against HIV/AIDS (1). In South Africa, access to antiretroviral therapy (ART) during pregnancy and infancy has steadily increased, leading to a 76% reduction in new infections among children (1). Currently, lifelong ART is available to all pregnant or breastfeeding women regardless of CD4 count or clinical stage of infection (2,3). Yet challenges remain in eliminating mother-to-child transmission (MTCT), with over 16,000 new paediatric infections detected in 2015 (4). It is known that those who acquire HIV *in utero (IU)* are at highest risk of morbidity and mortality (5), but strong evidence from the CHER trial indicated that prompt diagnosis and early introduction of ART can dramatically reduce mortality, disease progression and neurodevelopmental impairment in children infected with HIV (6,7). Early infant diagnosis (EID) is the key strategy for identifying infected infants and linking them to care. In high burden settings, however, coverage remains limited and the proportion of infected infants receiving treatment is below both adult rates and global targets (8–10).

Currently the World Health Organization (WHO) recommends HIV-exposed infants be tested by six weeks of life, with those who test positive being immediately referred for initiation of ART (11,12). Virological tests (HIV-PCR) required by infants for diagnosis of HIV are expensive for the health system and also require mother infant dyads to complete multiple clinic visits often spanning several months (13,14). Delays between sampling and return of test results may cause loss to follow-up (LTFU) and, consequently, fail to link many infants to life saving ART before the peak age of mortality at 11 weeks (15,16).

Although there are limited data on diagnosis and treatments of infants immediately after birth, there is consensus amongst clinicians that early initiation of ART has the potential to limit viral reservoirs and prevent disease progression early in infancy (17–19). It is argued that as ART coverage during labour increases the proportion of vertical infections that occur *in utero* increase relative to *intra-partum* infections (20). With high levels of LTFU in routine EID, a successful birth testing regime detecting 76% of all early vertical transmissions could increase the proportion of infected infants linked to care. A follow up test scheduled between 6-10 weeks of age would be required to diagnose *intra-partum* and very early *post-partum* transmissions undetectable at birth (21,22). In 2014, South African National Guidelines recommended the use of HIV-PCR testing at birth for

HIV-exposed infants at high-risk of vertical HIV transmission. High risk status was based on maternal characteristics, primarily late or no initiation of ART, clinically documented default status, or if the infant was born preterm or low birthweight (23). In mid-2015, the guidelines were further updated to include routine HIV-PCR testing at birth for all HIV-exposed infants (2).

Delivery of postnatal services and retention in care are recognized as weak links in the continuum of care for women and their infants accessing PMTCT services. Lack of knowledge and understanding of the need for infant testing are often cited as reasons for non-attendance at EID testing (24). The addition of an extra test to an already complicated cascade could result in attrition of routine EID services compared to the uptake when no birth test is available. Recent estimates indicate that only 49% of infants presented for follow-up EID testing after a negative PCR result at birth, far below the national estimates of EID coverage (25). We examined whether there is an impact from including a birth test within the EID testing protocol and looked to identify possible infant and maternal factors associated with presentation at EID testing.

METHODS

This study was a retrospective cohort study including 1,126 mother-infant dyads conducted at a single-site in the Western Cape Province, South Africa. All infants were delivered at Mowbray Maternity Hospital (MMH) between July 2013 and August 2015. MMH is a secondary-level obstetric hospital with neonatal care facilities. Of the 11,000 infants born at MMH each year, 13% are HIV-exposed and 2% are HIV-infected (14) (Ref Max Kroon Personal Correspondence). National estimates state that between 60-70% of women using government facilities will require the services of a hospital at some point during their pregnancies. Referral to a secondary care facility occurs if the pregnancy is deemed to be high risk, due to a women's obstetric history, general medical condition or if she presents with specific risk factors during her current pregnancy (26,27). MMH also has a primary care component allowing women with local addresses to deliver on site.

During the study period, all mothers were eligible for Option B+ (ART for all pregnant and breastfeeding women irrespective of CD4+ count). HIV-PCR testing at birth was targeted at infants identified as being at high risk of vertical transmission as per local guidelines. High-risk status was based on maternal characteristics, primarily unsuppressed viral load, late or no initiation of ART or known non-adherence during pregnancy (clinically documented default status), or infant characteristics (symptomatic, preterm delivery or if the infant

was abandoned). Birth tests occurred within 48 hours of delivery, and result return occurred prior to discharge. Provincial guidelines for the study period required all HIV-exposed infants to undergo HIV-PCR testing at routine postnatal immunization visits at 6 weeks of age. Interim HIV screening also occurred in children who presented to hospitals with opportunistic infections or symptoms related to HIV. The results of all HIV-PCR tests are hosted in the central data warehouse of the National Health Laboratory Service (NHLS), the sole provider of pathology services for the public health sector in South Africa.

All infants delivered at MMH who underwent a birth test were enrolled in the study. Infants with negative birth PCRs were matched on date of birth and mode of delivery to HIV-exposed infants who did not receive an HIV-PCR test at birth. Maternal antenatal features and obstetric characteristics of all neonates were abstracted via folder review from data collected routinely in the hospital for clinical monitoring and evaluation purposes. Using folder number, name, date of birth and maternal address, we identified the first HIV-PCR result on the NHLS system for each HIV-exposed infant before February 2016. All infants were given the complete time period of the study to return for testing.

Written informed consent was not feasible because of the retrospective nature of the research. All data was handled confidentially throughout the research process. The study, including waiver of informed consent was approved by the Human Research Ethics Committee of University of Cape Town Faculty of Health Sciences and the Mowbray Maternity Hospital Research Committee.

Data analysis

Data were analysed using Stata Version 13.0 (Stata Corporation, College Station, Texas, USA). We described patient characteristics using summary statistics, presented using mean/median or frequencies as appropriate. Associations between categorical variables were calculated using chi-squared tests whilst the Wilcoxon rank-sum test was used for continuous variables. The age of infants at follow up testing was calculated using their specimen date and their date of birth from labour or theatre registers at MMH. We defined EID presentation as mother/infant dyad accessing an HIV-PCR test after being discharged from hospital, with results hosted on the NHLS system. Analyses examined the characteristics of the infants included in the study by receipt of birth testing. Logistic regression models were used to examine the independent predictors of returning for an HIV-PCR test. The results are presented as odds ratios (OR) with 95% confidence intervals (CI). Variables in the model were selected based on prior evidence and findings from descriptive statistics. We defined a secondary

outcome, linkage to HIV care, as an infected infant having a specimen related to HIV care (eg. HIV RNA, CD4, viral load, etc.) sent to the NHLS for processing by either 3 or 6 months after the date of the positive EID assay.

RESULTS

Overall, 1,126 HIV-exposed infants were followed from birth until February 2016. Of these, 575 neonates underwent birth testing, of whom 4% (22) received a positive result, 0.3% (2) returned equivocal results and 96% (551) received negative results. Reasons for being considered at high risk of perinatal transmission of HIV and therefore receiving a birth test ranged from maternal characteristics, to abandonment, low birthweight or preterm birth. Table 1 lists the most common reason for receiving a birth test to be lack of maternal ART coverage during pregnancy, which was associated with 50% (286) of HIV-PCR tests performed at birth. This is followed by recorded maternal default on treatment and high viral load attributing to 28% (163) of infants receiving a birth test. One fifth of infants (119) were included due to low birthweight or preterm birth. No reason was stated for 6% (37) of infants who underwent a birth test.

Infants who had negative birth test were matched to 551 infants who did not receive birth testing. The demographic characteristics of the infants followed up for subsequent testing are summarized in Table 2. Of the 1,102 study subjects analysed, there were no differences found between the two groups with regards to infant sex, infant feeding mode or gravidity. Just over half, 50% (555) were female, and 53% (582) of all infants were delivered by normal vortex delivery. Over three quarters, 77% (843) of all mothers had previously given birth, and 82% (898) exclusively breastfed their infant. Clinical differences in the two groups could be found for maternal age, gestation and infant birthweight, which were all part of the criteria used to identify infants at high risk of HIV transmission. Maternal age ranged from 15 years to 48 years, with mean maternal age of 28.2 years for infants who underwent a birth test compared to 29.2 years for those infants who did not. Fewer infants who underwent a birth test were term gestation (73% (401) vs 88% (486)) and birthweight >2500g (66% (363) vs 92% (506)) than those who did not undergo a birth test. Maternal population group also differed, more infants were born to mothers identifying as black African in the cohort who did not receive a birth test compared to those who did (82% (433) vs 79% (454)). Available CD4 count also varied between the two groups, but 87% (481) of CD4 data for infants who did not undergo birth testing were unavailable due to changes in hospital reporting practices.

Of the 551 birth-tested infants who received a negative result at birth, 73% (401) presented for follow-up EID. Of the 551 not birth-tested, 85% (470) presented for follow-up EID, p<0.001. EID testing occurred at a significantly older age in birth-tested compared to not birth-tested infants: mean age 60 vs. 50 days, p<0.001. The proportion of positive results was lower at follow-up testing than at birth: 0.5% (2/401) of those who received a birth test and 0.4% (2/470) of those who only received routine EID vs. 4% (22/575) at birth returned a positive result. Of the 575 infants who received an HIV-PCR at birth a total of 24 infants were diagnosed as HIV-infected after completion of both EID tests, 92% (22) had detectable viraemia at birth signifying *in utero* infection and 2 were diagnosed at 6 weeks. At 3 months after a positive test, linkage to HIV care was 71% for truly infected infants diagnosed at birth and 75% for those diagnosed later; at 6 months after a positive test, these linkage rates were 86% (diagnosed at birth) as 3 infants linked to care in the additional 3month period and 100% (diagnosed later) as 1 infant linked to care in the additional 3month period (Table 3).

Unadjusted logistic regression analysis (Table 4) showed that only receipt of birth testing and ART coverage during pregnancy were associated with lower EID presentation. Maternal age, birthweight, gestation and population group were not associated with the outcome. From the crude analysis, it was estimated that those who received a birth test were 54% less likely to return for follow-up EID testing than those that did not receive a birth test (OR,0.46; 95%CI,0.34-0.62). The significant decrease in routine EID testing among children tested at birth persisted in multivariable analyses adjusting for maternal age, nadir CD4 cell count, ART use during pregnancy, gestational age, infant sex, maternal age, maternal population group, birthweight and infant feeding modality. Where possible confounding factors were included in the model which reduced the association between receipt of birth testing and EID presentation; the model estimated infants who received a birth test were 40% less likely to return for EID presentation than those that did not receive a birth test (AdOR,0.60; 95%CI,0.40-0.88), whilst those that had low ART use during pregnancy (≤12 weeks) were 30% less likely to return for EID presentation compared to those who had ≥12 weeks coverage (AdOR,0.70;95%CI, 0.46-1.06). Women who did not receive any ARTs during pregnancy were 57% less likely to report for EID presentation compared to those that had ≥12 weeks ART coverage, (AdOR,0.43; 95%CI, 0.23-0.78).

We also assessed the effect size in different subgroup populations (Figure 1). The trend of reduced EID presentation after receipt of a negative test result at birth persisted across subgroup populations. Those born with low birthweight (n=229), preterm (n=198) or born to mothers with younger maternal age (n=259) or who did

not identify as black African (n=124) had the greatest influence on the association between receipt of a birth test and EID presentation, however, all influential subgroups had small populations (n<260) with wide confidence intervals often spanning 1. In order to further assess these effects, we conducted a sensitivity analyses on 826 mother-infant dyads removing infants born with low birthweight or prematurity from the analysis. Infants born prematurely and with low birthweight have the highest risk of morbidity and mortality, but were also more likely to undergo birth testing. As these infants may have demised before routine EID testing could take place, they were excluded from both groups for this subgroup analysis. The negative association between birth-testing and EID presentation increased (AdOR, 0.54 95% CI 0.34-0.86).

DISCUSSION

These novel data are among the first to estimate routine EID test coverage of HIV-exposed infants who underwent birth testing in sub-Saharan Africa. There were two key findings. First, targeted birth testing successfully identified mothers at high risk for transmission to their infants, with diagnosis of HIV within 2 days of life possible for 92% of all infected infants who returned for complete EID testing. Second, neonates undergoing HIV testing at birth appear less likely to receive subsequent EID testing compared to infants who did not receive a birth test.

Our data have demonstrated that while birth testing is possible and could have a higher yield of positive infants if implemented correctly, it is important to focus resources on all recipients of HIV results rather than only the infected infants. Whilst implementing targeted birth testing in a setting with high maternal HIV prevalence could allow for earlier identification of infected infants, it is unlikely to be suitable for all settings. Birth testing could also lead to false reassurance for families whose infants return a negative result at birth and prevent subsequent engagement in care. It is essential that this reassurance comes after both *in utero* and *intra-partum* infection has been ruled out, rather than after limited and incomplete results from a single birth test (28–30).

We found routine EID coverage to be higher than national estimates of 73%, with 85% of HIV-exposed infants who did not receive a birth test (estimated to be 75% of all deliveries), and 73% of those who received a birth test, returning for a follow-up EID testing (31). For HIV-infected infants, levels of linkage to care were similar within 3 months for infants who were diagnosed at birth or 6 weeks, but were significantly lower at 6 months

for infants diagnosed at birth. This could be explained by survival bias as infants infected in utero are at higher risk of rapid disease progression, and infants who were born preterm or with a low birthweight were more likely to receive an HIV-PCR test at birth but have 6 fold increased risk of neonatal mortality (32,33). Infected infants may therefore have demised before possible linkage to care could occur.

Although the attendance at routine EID testing was higher than national estimates, the negative association between birth testing and EID presentation persisted through all sensitivity analysis. Unlike other studies looking at retention within the EID cascade, only receipt of an HIV-PCR test at birth and low ART coverage during pregnancy were associated with EID presentation. Predictors such as maternal age, WHO clinical stage, parity and preterm delivery were not associated (30,34–36). During the study period, HIV-PCR testing at birth was targeted to infants thought to be at high-risk of HIV transmission. Half of birth tests were attributed to limited ART coverage during pregnancy. Regression analysis found low ART coverage during pregnancy to be predictive of lower EID presentation. While the reasons behind this finding require further investigation, it is possible that low ART coverage could be a proxy for reduced antenatal visits and suboptimal patient care-seeking behavior. It may suggest that risk factors associated with being at high risk of perinatal HIV transmission require special consideration around retention within the PMTCT/EID cascades.

Substantial research has been conducted around the lack of retention within the EID cascade, with each step presenting an opportunity for loss to follow up to occur (13,37). Including a birth test in the EID algorithm adds additional steps into an already complicated cascade and could cause attrition in follow-up coverage. We have seen in other areas of the cascade that despite continuing minimal transmission risk from breastfeeding in the postpartum period (late postpartum infections can account for 8-25% of HIV-infections in infants) there is extensive loss to follow up after an initial HIV test, with very few women presenting for testing after the cessation of breastfeeding (38, 39,40). Receiving a negative result immediately after delivery may cause confusion around the need for further testing at 6 weeks, especially considering the likelihood that caregivers who receive a negative HIV result are significantly less likely to receive follow up counseling compared to those with a positive result (39).

PMTCT services are designed to offer support to women living with HIV, and provide them with information for both antenatal and postnatal care, however, a report by the WHO details how counseling is often provided by

busy, overworked staff members, who focus only on the initial HIV test result and ART treatment (2,40,41). It is possible that the association between receipt of a negative result and reduced EID presentation could be linked to a lack of HIV knowledge; evidence has suggested that women living with HIV in South Africa have inadequate knowledge of HIV transmission during the post-partum period and are unaware of EID testing schedules (24). The lack of focus away from the initial test result during PMTCT counselling sessions could limit caregivers' knowledge around post-partum care, and continued transmission risks after labour. There is a clear need for further emphasis on early infant follow-up, along with ensuring accurate health records and strong data management to enable timely identification of infants who remain HIV-exposed and require EID testing.

While the negative association between birth testing and EID presentation is of clear concern given recent policy changes within South Africa, the results have several limitations. First, generalizations regarding other settings and facility levels should be made with caution. The results reflect a single urban setting within South Africa. All participants were enrolled in a referral obstetric unit within a maternity service in Cape Town and required post discharge down referral back to the referring unit with consequent opportunity for communication failure and LTFU. Second, due to the retrospective nature of the research, we were unable to collect social and demographic variables such as HIV knowledge, employment and education of mothers that could have provided interesting insight into further predictors of EID presentation. We were unable to ascertain why mother/infant dyads were lost from care, qualitative studies focused on finding mothers lost from care and examining why they did not report for testing would provide greater understanding to the issues surrounding LTFU within the EID cascade. We were also unable to assess the process of result return to caregivers or if and when an infected infant was initiated on ART, meaning that levels of LTFU could be underestimated. Finally, during the study period HIV-PCR testing at birth was targeted at infants thought to be at high risk of perinatal transmission. It would be important to repeat the study in the light of the introduction of universal birth testing to examine further predictors of LTFU after birth testing and reflect on the impact of targeted birth testing in this setting.

In conclusion, these results show that implementation of targeted birth-testing in South Africa is extremely effective at identifying infected infants and has the potential to improve infant survival by increasing earlier access to ART for infants infected *in utero* who are most at risk of early death. However, there is a danger of reducing population coverage of routine EID services at later ages for infants testing negative at birth. PMTCT programmes' counseling curricula must emphasize adherence to the new infant and child testing schedule after

each negative result is returned to the caregiver. Where appropriate testing schedules, counseling curriculum and EID services need to be revised to integrate birth testing into the EID cascade, while PMTCT programmes must be routinely monitored, evaluated and revised whenever there is a significant shift in policy.

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TABLES AND FIGURES

Table 1: Possible reasons cited for infants receiving an HIV-PCR at birth. Infants were given more than one possible reason for fulfilling criteria for a birth test. All reasons given for each infant were included.

Reason For Birth Test	Frequency (N=655)	Percentage of Birth Tests (N=575)
No Reason	37	6.4%
ART <12weeks	221	38.4%
Low Birthweight / Preterm	119	20.7%
High Viral Load	85	14.8%
Defaulted from Treatment	78	13.5%
Unbooked	34	5.9%
No ARVs during pregnancy	31	5.4%
Seroconverted during Pregnancy	23	4.0%
Maternal Infection	23	4.0%
2nd Line Treatment	20	3.5%
Needle stick injury	3	0.5%
Substance Abuse	5	0.9%
Other	10	1.7%

Other: Respiratory distress syndrome, Meconium aspiration syndrome, voval cord paralysis, adoption, milksharing, AZT only, Low CD4

Table 2. Summary of population demographics by receipt of an HIV-PCR test at birth. (§Criteria used to identify high risk infants eligible for a birth HIV PCR test. #Criteria for matching HIV-exposed infants who received a birth test to HIV-exposed infants who did not.)

Variable	Birth Test N=551 (%)	No Birth Test N=551 (%)	Total N=1102 (%)	P-value
HIV PCR result for routine EID:				
Negative	399 (72.4)	468 (84.9)	867 (78.7)	
Positive	2 (0.4)	2 (0.4)	4 (0.4)	
Not tested	150 (27.2)	81 (14.7)	231 (21.0)	<0.001
Age at routine EID testing (among infants tested)				
Mean (SD)	59.6 (41.8)	49.5 (22.8)	54.1 (33.3)	<0.001
4-10 weeks	337 (84.0)	427 (90.9)	764 (87.7)	
Sex:				
Female	279 (50.6)	276 (50.1)	555 (50.4)	0.953
§ **Birthweight (g):**				
Mean (SD)	2781.4 (700.1)	3173.5 (566.1)	2977 (665.7)	
Low (<2500)	184 (33.4)	45 (8.2)	229 (20.8)	<0.001
# **Mode of Delivery**				
C/S	260 (47.2)	260 (47.2)	520 (47.2)	-
§ **Gestation (wks)**				
Preterm (<37)	148 (26.9)	50 (9.7)	198 (18.0)	<0.001
Infant Feeding				
Breastfeeding	438 (79.5)	460 (83.48)	898 (81.5)	0.241
§ **Maternal Age (years)**				
Mean (SD)	28.2 (5.9)	29.2 (5.3)	28.7 (5.6)	
Adolescent mothers (<24)	157 (28.5)	102 (18.5)	259 (23.5)	<0.001
Maternal population group				
Black African	433 (78.6)	454 (82.4)	887 (80.5)	<0.001
Gravidity				
Mean (SD)	2.5 (1.3)	2.5 (1.2)	2.5 (1.2)	0.829
§ **PMTCT Coverage**				
Received ART 12+weeks	276 (50.1)	551 (100)	827 (75.0)	<0.001
Received ART <12weeks	218 (39.6)	-	218 (19.8)	
No ART	57 (10.3)	-	57 (5.2)	
§ **Default on Treatment**				
Recorded default	75 (13.0)	-	75 (6.8)	-
§ **Viral Load**				
VL >1000	82 (14.2)	-	82 (7.4)	-

Table 3. HIV-PCR results for all HIV-exposed infants. Infants deemed to be high risk of HIV-transmission underwent HIV-PCR testing at birth, NHLS database was examined to determine if a confirmatory HIV-PCR or VL was run after the initial result. Linkage to care for truly infected infants looked for a subsequent specimen sent to NHLS for processing. Routine HIV PCR results reflect mother/infant dyad accessing an HIV DNA-PCR test after being discharged from hospital. (CT: Confirmatory Test)

	Birth Test N=575 (%)		
Birth HIV PCR result:			
Negative Result	551 (95.7)		
Equivocal Result	2 (0.3)		
Positive Result	22 (3.8)		
Confirmatory Test	21 (95.5)		
Possible False Positive from CT	1 (4.8)		
Linkage to HIV care w/in 3 months	15 (71.4)		
Linkage to HIV care w/in 6 months	3 (14.3)		
	Birth Test N=401 (%)	**No Birth Test N=470 (%)**	**Total N=871 (%)**
HIV PCR result for routine EID:			
Negative Result	399 (99.5.)	468 (99.6)	867 (99.5)
Positive Result	2 (0.5)	2 (0.4)	4 (0.5)
Confirmatory Test	2 (100)	2 (100)	4 (100)
Possible False Positive from CT	0	0	0
Linkage to HIV care w/in 3 months	1 (50)	2 (100)	3 (75.0)
Linkage to HIV care w/in 6 months	1 (50)	-	1 (100)

Table 4. Logistic regression analyses for predictors of EID presentation for HIV-exposed infants born at MMH in Cape Town. Variables found to be independently associated with the outcome of EID presentation were included in the multivariate model along with variables found to be significant from previous research.

		Univariate Model		Multivariate Model	
Risk Factors	**Categories**	**Crude OR**	**95% CI**	**Adjusted OR**	**95% CI**
Exposure to Birth Test	**No birth test**	**-**	**-**	**-**	
	Received birth test	**0.46**	**(0.34-0.62)**	**0.60**	**(0.40-0.88)**
Sex	**Male**	-	-		
	Female	0.92	(0.69-1.23)		
Birthweight	**Weight (g)**	1.00	(0.99-1.00)		
	Normal birthweight	-	-	-	-
	Low birthweight	0.79	(0.56-1.12)	0.97	(0.67-1.42)
Maternal Age	**Age (years)**	1.01	(0.98-1.03)		
	Mature mother (≥24)	-	-	-	-
	Adolescent mother (<24)	0.90	(0.62-1.29)	1.02	(0.70-1.49)
Gestation	**Gestation (weeks)**	1.00	(0.95-1.07)		
	Full gestation	-	-		
	Preterm (<37)	0.93	(0.64-1.35)		
Prima Gravida	**No**	-	-		
	Yes	0.88	(0.62-1.25)		
Race	**Black**	-	-		
	Coloured	0.69	(0.43-1.09)		
	Foreign	0.37	(0.06-2.25)		
Infant Feeding	**Breastfeeding**	-	-		
	Formula	1.11	(0.75-1.66)		
PMTCT Coverage	**Received ART 12+weeks**	-	-	-	-
	Received ART <12weeks	**0.49**	**(0.35-0.69)**	0.70	(0.46-1.06)
	No ART	**0.31**	**(0.18-0.54)**	**0.43**	**(0.23-0.78)**
Default on Treatment	**No default recorded**	-	-		
	Recorded default	0.94	(0.52-1.69)		
Viral Load	**VL <1000**	-	-		
	VL >1000	0.70	(0.42-1.17)		

Figure 1. Forest plot of the association between receipt of an HIV-PCR test at birth and routine EID presentation

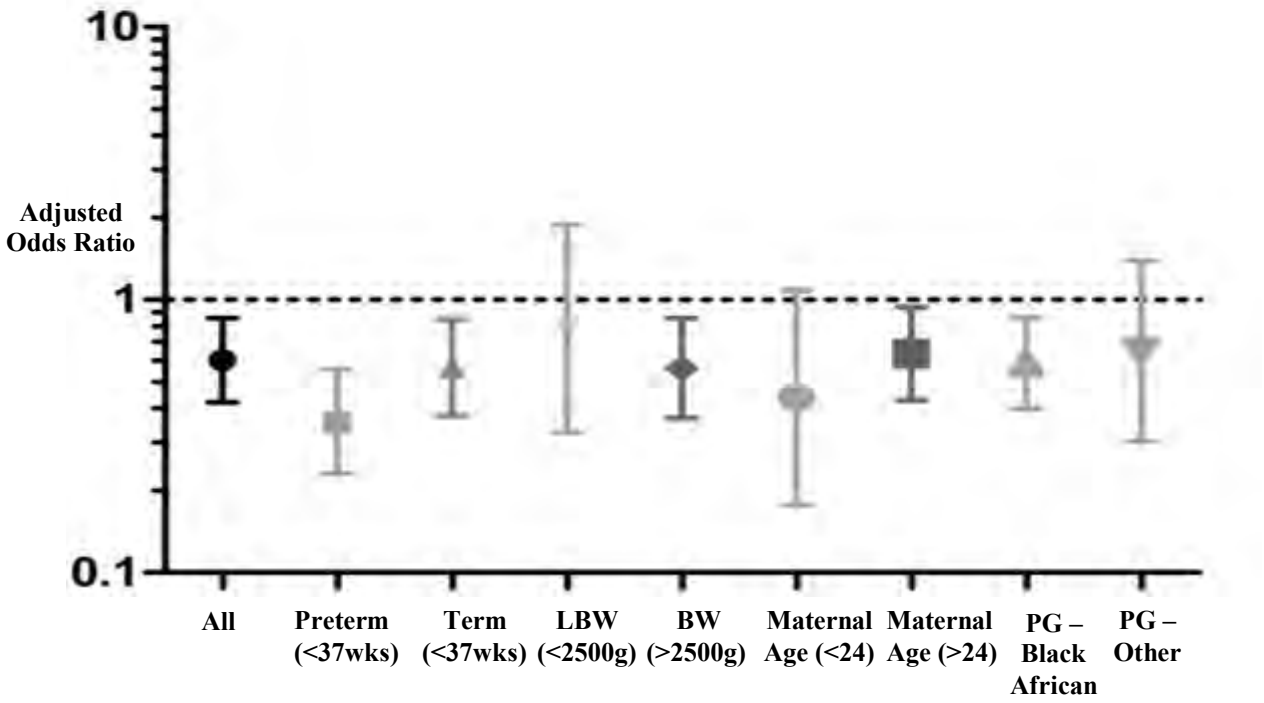

FIGURE CAPTION

Figure 1. The adjusted odds ratio for EID presentation having received an HIV-PCR test at birth is shown in multiple subgroup populations. In each subgroup analysis the population was restricted to include infants only with the desired population. Regression analysis was then performed to estimate the association within each subgroup controlling for confounding variables.

(Abbreviations: LBW: low birthweight, BW: birthweight, Maternal Age: Years, PG: Population Group)

PART D: APPENDICIES

1. Ethical Approval Letter

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

03 November 2015

HREC REF: 730/2015

Prof L Myer
Epidemiology and Biostatistics
School of Public Health & Family Medicine
FHS

Dear Prof Myer

PROJECT TITLE: ANALYSIS OF BIRTH TESTING AS PART OF THE EARLY-INFANT DIAGNOSIS SYSTEM FOR HIV EXPOSED INFANTS IN CAPE TOWN-(MPH-candidate-L Dunnings) sub-study linked to 628/2014 & R040/2014

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has formally approved the above-mentioned study.

Approval is granted for one year until the 30th November 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the following student:-Lorna Dunnings is also involved in this project.

Please quote the HREC reference no in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

Signed by candidate

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

2. Site Specific Ethical Approval

Dear Lorna

Thank you for your request to perform the research at MMH. I note that it has UCT HREC approval and is a retrospective record review.

The MMH research committee would support this research being performed at MMH.

Our medical records department is in an extreme state of chaos, so that 2013 maternal folders may not be easily procured. The records department is currently in the process of being sorted but this project is not nearing completion. Retrieval of folders for clinical or research audits has not been given priority. It may be that you have funds that might allow an intern to be paid specifically to locate the required folders.

Mother and baby are linked on clinicom. All babies who stayed with their mothers will have their records in the mother's folder. All who were admitted to the nursery will have their own folder

I am copying the request to our CEO, Janine Joemat, for her information also.

Best wishes

Sue Fawcus

MA, MBBS, FRCOG, Consultant obstetrician

3. Table 1: Data Collection Tool

MMH INFANT TESTING REGISTER

SURNAME AND NUMBER AND ADDRESS	DOB AND GENDER	BIRTH WEIGHT AND GESTATION (BALLARD)	DELIVERY MODE (NVD; VACUUM; EmC/S; ElecC/S)	MATERNAL					INFANT OR MATERNAL COMORBIDITY/MORBIDITY (INSERT ABBREVIATION FROM LIST BELOW)	FEEDING BF FF PEBM DEBM	INFANT TEST DATE		RESULT (P=POS N=NEG E=EQUIVOCAL)	
				DATE OF HIV DX	CD4 AND DATE	VL AND DATE	ART START DATE	RACE			Birth	Later EID	Birth	Later EID

ABBREVIATIONS: NEONATAL: NEONATAL TUBERCULOSIS – NTB; CONGENITAL CYTOMEGALOVIRUS – CCMV; THROMBOCYTOPAENIA – TCP; CARDIOMYOPATHY – CMO; SEVERE BIRTH ANAEMIA – Hb<8; HYPOGLYCAEMIA – GLUC<2; INS; BIRTH DEFECT – BD (PLS SPECIFY); OTHER (PLS SPECIFY); MATERNAL: MATERNAL TUBERCULOSIS – MTB; CHORIOAMNIONITIS – MCA.

4. High Risk Criterai For Birth Testing

Western Cape Government
Health

DIRECTORATE: HAST

REFERENCE: 19/4/4

ENQUIRIES: Ms M.H. DYESHANA AND C.GOOSEN

Director HAST: JO Arendse

TO: **HEAD OF DEPARTMENT**
DDG: CHIEF OPERATIONS OFFICER
HOSPITAL CHIEF EXECUTIVE OFFICERS
CHIEF DIRECTORS: HEALTH PROGRAMMES; METRO DISTRICT SERVICES; RURAL DISTRICT HEALTH SERVICES; STRATEGY AND HEALTH SUPPORT
EXECUTIVE DIRECTOR CITY OF CAPE TOWN
DIRECTORS: FACILITY BASED SERVICES
COMMUNITY BASED SERVICES
DISTRICTS AND SUB-STRUCTURES
NURSING SERVICES
PHARMACY SERVICES
DEPARTMENT OF CORRECTIONAL SERVICES
NGO PARTNERS

Circular No: H116/2013

CANCELLATION AND REPLACEMENT OF CIRCULAR H106/2013: IMPLEMENTATION OF THE PMTCT CLINICAL GUIDELINES 2013

This circular cancels and replaces Circular H106/2013: Implementation of the PMTCT Clinical Guidelines 2013. Appendix 1, the PMTCT Clinical Guidelines Update, was amended.

This circular should be read in conjunction with:

The Western Cape Antiretroviral Treatment Guidelines 2013.
Circular H166/2012: Infant Feeding Counselling Guideline.
Circular H186/2012: Criteria for Safe Infant Feeding by HIV-infected Mothers.

This circular serves to inform you of the changes to the Western Cape PMTCT Guidelines and to communicate the PMTCT Clinical Guidelines Update document (Appendix 1).

Change in PMTCT policy

With immediate effect, all pregnant and breastfeeding HIV-infected women qualify for lifelong antiretroviral treatment (ART). For easy reference, the revised guidelines are summarized in the algorithm on page 5 of the PMTCT Clinical Guidelines Update (Appendix 1).

Implementation

a) Service integration

Ideally, lifelong ART for HIV-positive pregnant and breastfeeding women should be initiated on the day of HIV diagnosis (new client) / at the first visit (known HIV not on ART).

1st Floor, 8 Riebeeck Street, Cape Town, 8001 P O Box 2060, Cape Town, 8000
tel: +27 21 483 9881 fax: +27 21 483 6033 www.westerncape.gov.za

However, since all services are not fully integrated at all facilities, the integration of ART services with antenatal and postnatal care will depend on existing capacity. Midwives and staff working in PMTCT services will be prioritized for Nurse Initiated Management of ART (NIMART) training and mentoring to support integrated services.

b) Training

PMTCT Clinical Guidelines Update
The Provincial Office will provide Train-the-Trainer sessions to district and substructure trainers (identified by districts and substructures). These trainers will be responsible for cascading the training to subdistrict and facility level. The Provincial Office will provide further training on request.

NIMART
The Provincial office will amend the training package with current service provider for NIMART mentoring and training to provide the platform to scale up NIMART training for midwifes. Capacity should be created by service providers for current and new NIMART mentors to enable the scale-up of the NIMART mentor process.

c) Product supply

Kindly note that ALL antiretrovirals (ARV's) must be ordered from the ARV depot. The CMD will no longer stock ARV's.

Facilities who have not previously ordered from the ARV depot should please complete and submit the following two documents to Ms Natalie M Jacobs: Fax: 021 483 5558; Email: Natalie.Jacobs2@westerncape.gov.za
- New Demander Source Document (Appendix 2)
- ARV depot ordering authority (Appendix 3)

Please find the ARV depot ordering form attached (Appendix 4).

Adverse events

Please submit an Adverse Drug Reaction Reporting Form (Appendix 5) should any adverse drug events be suspected.

The content of this circular should be brought to the attention of all relevant staff.

Signed by candidate

PROF KC HOUSEHAM DR EH ENGELBRECHT
Western Cape Government: Health
Acting Head of Department
DATE: 2013-07-04

5. CARE OF HIV-EXPOSED INFANTS

If symptomatic or classified as high risk (attending clinician discretion)

If NVP resistance likely or no prelabour ARVs, consider combining AZT and NVP for infant prophylaxis. Discuss further management with an expert.

Perform an HIV-1 DNA PCR test soon after birth.

If PCR positive
- Fast track for ART.
- Do a baseline viral load but do not delay ART initiation while waiting for the result.

If PCR negative
- Repeat the HIV-1 DNA PCR test at 6 weeks.

Abandoned infants / Orphans
- Immediately perform a rapid HIV test to determine if the infant was exposed to HIV.
- If the rapid HIV test is positive, perform an HIV-1 DNA PCR test and manage as above.
- These infants qualify for donated expressed breast milk (if available) or formula feeding.

AT BIRTH

Give all infants oral Nevirapine (NVP) urgently after birth (within 72 hours) (Table 4).

Table 4. Nevirapine (NVP) doses for prophylaxis at birth

	Birth Weight	Dosage	Volume
Nevirapine (NVP) syrup (10mg/ml)	<2.0kg	2mg/kg	0,2 ml/kg
	2.0 – 2.5kg	10mg	1ml
	>2.5kg	15mg	1.5ml

IN HEALTH FACILITY

Provide daily NVP as per dosing schedule (or other ARV prophylaxis regimen under expert guidance) (Tables 5 and 6).

Draft 20131129 MMH Protocol: Recognition And Management Of Mother/Newborn Pairs At Increased Risk Of HIV Transmission

Adequate maternal antiretroviral therapy (ART) and adherence eliminates vertical transmission and must be optimised urgently in pregnancy.

Further gains in PMTCT and better linkage to care and treatment are possible by focusing on high risk scenarios.

Vertical transmission risk is high if no maternal ART: 5-10% before labour; 15% during 12 hours of labour and 1% per month of breastfeeding (BF).

Infant multiple antiretroviral post exposure prophylaxis (multi-ARV PEP) targets and reduces intra-partum risk by 50% if no pre-delivery ARVs.

Infants at increased risk of HIV infection should be identified at birth by clinical and laboratory parameters, be tested early and receive multi-ARV PEP.

Prompt diagnosis and treatment of HIV infected infants reduces morbidity and mortality.

In the high risk scenario, maternal ART/adherence must be optimised urgently to reduce BF transmission risk and improve maternal health and survival. BF risk per month is low with ARV cover. Objective review of feeding choice must consider this low risk and aim to promote HIV free survival.

Identification Of Infants At Increased Risk:

There is a potential increased risk of HIV infection in the following situations:

Maternal

- Maternal antiretroviral therapy < 8 weeks (especially if no prelabour ARVs)
- Maternal viral load > 400 copies/ml (not always available)
- Maternal viral rebound (treatment interruption, poor adherence, true resistance)
- Maternal comorbidity (Tuberculosis, Opportunistic Infections, chorioamnionitis)
- Maternal substance abuse (alcohol or drugs)
- Incident infection (Initial HIV test negative and subsequently tests positive)
- Adolescent pregnancy (recent/incident/vertically transmitted infection, more likely to have problems with follow up)
- Likely Non-nucleoside reverse transcriptase inhibitor (NNRTI) resistance (2^{nd} line ART, failing 1^{st} line ART; multiple sdNVP previously)

Infant

- Symptomatic (severe growth restriction, lymphadenopathy, hepatosplenomegaly, thrombocytopaenia, pancytopaenia, congenital CMV)
- Preterm delivery regardless of cause and/or LBW infants (<2500g; <37 weeks gestation)
- Abandoned infants (if Alere Determine or ELISA positive)

5. **Journal Submission Guidelines**

JAIDS: Journal of Acquired Immune Deficiency Syndromes

Online Submission and Review System

The Editor-in-Chief of the Epidemiology and Prevention section is Dr. William A. Blattner.

SCOPE

JAIDS: Journal of Acquired Immune Deficiency Syndromes is a peer-reviewed, multidisciplinary journal directed to an audience of physicians and researchers. The journal publishes original work in the form of Original Articles, Implementation and Operational Research*, Rapid Communications, Critical Reviews, Brief Reports, and Letters to the Editor*. *JAIDS* does not publish case reports. (*published online only)

MANUSCRIPT SUBMISSION

A submitted manuscript must be an original contribution not previously published (except as an abstract or preliminary report), must not be under consideration for publication elsewhere, and, if accepted, must not be published elsewhere in similar form, in any language, without the consent of Lippincott Williams & Wilkins. Each person listed as an author is expected to have participated in the study to a significant extent. Although the editors and referees make every effort to ensure the validity of published manuscripts, the final responsibility rests with the authors, not with the journal, its editors, or the publisher.
All submissions will be rigorously peer-reviewed by members of the Editorial Board and by other specially qualified individuals as well. In the interests of rapid reviewing of contributions, only one of the Editors-in-Chief will, in general, make the final determination as to the acceptability of a submission, after collecting the referee's comments. Contributors may recommend specific names of reviewers from the Editorial Board, as well as other individuals they deem especially well qualified. However, the Editors-in-Chief will not be bound to follow such suggestions.

In general, the instructions for preparation of manuscripts should follow the International Committee of Medical Journal Editors (ICMJE) Uniform Requirements for Manuscripts Submitted to Biomedical Journals. In case of questions, please feel free to contact the Editorial Office of any one of the Editors-in-Chief.

Authors must submit their manuscripts to the relevant section through the Web-based tracking system:

Basic and Translational Science (**http://www.editorialmanager.com/jaids**)
Clinical Science (**http://www.editorialmanager.com/jaids**)
Epidemiology and Prevention (**http://www.editorialmanager.com/jaids**)

The site contains instructions and advice on how to use the system, guidance on the creation/scanning and saving of electronic art, and supporting documentation. In addition to allowing authors to submit manuscripts on the Web, the site allows authors to follow the progression of their manuscript through the peer review process. Authors should not send hard copies of the manuscript or artwork to the editorial office. Address all inquiries regarding manuscripts not yet accepted or published to the Journal's editorial office. The editorial office will acknowledge receipt of your manuscript via e-mail.

PREPARATION OF MANUSCRIPT

Manuscripts that do not adhere to the following instructions will be returned to the corresponding author for technical revision before undergoing peer review.

ARTICLE LIMITATIONS – BEGINNING WITH JULY 15, 2010 SUBMISSIONS:

Article type	Limitations	Abstracts

Original Articles	3500 words + 5 figures/tables – if more then use Supplemental Digital Content	Structured; 250 words

*If a figure/table is more than one page it will count for multiple figures (ie: if 1 figure totals 2 pages it will count as 2 figures; if 1 figure takes 3 page, it will total 3 figures, etc.)

ARTICLE TYPES

Original Articles

The above guidelines apply to the original article format. Articles should be limited to 3500 words + 5 figures/tables. If additional space is needed, then use Supplemental Digital Content options. There should be a structured abstract of 250 words or less.

Implementation and Operational Research (NEW ARTICLE TYPE)

JAIDS is now accepting manuscripts for a new focus area of interest: Implementation and Operational Research. In the context of HIV/AIDS with advances in HIV therapy and care, expansion of global access to treatment, care and prevention Implementation and Operational Research, while having particular relevance to global health is an important domestic focus as well. However the lessons learned through this research discipline are particularly relevant to guiding best practices in low-resource settings as antiretroviral drug access is expanded. Articles that encompass the translation of knowledge, practices, and technologies into clinical care of adult and pediatric patients with HIV/AIDS and their evidence-based effectiveness in "real world settings" are of particular interest.
All manuscripts should be submitted through one of the existing three sections: Basic and Translational Science, Clinical Science, or Epidemiology and Prevention using the article type Implementation and Operational Research. Structure of article is the same as Original Article.

If accepted for publication, articles are published ONLINE ONLY with titles appearing in the print and online edition table of contents.

Online Submission

Manuscript files must be uploaded into the Editorial Manager online interface. Most word-processing file formats are acceptable. Editorial Manager will then create PDF files of the authors' submission, and the author must view and approve the files before they will be submitted to the editorial office. Please be sure that the manuscript file contains complete text for your submission (title page and abstract), as this is the file that will be downloaded by the reviewers and publisher. Please see the sections below for instructions regarding Figure and Table files.
Once the paper has been accepted for publication, and final versions of the manuscript, figures, and table files have been uploaded to the Editorial Manager interface, PDF files will not be used for typesetting. This is important to note for Table and Figure files, which may lose formatting when converted to PDF, but will remain intact in their original file format.

Title Page

A title page must be included in the manuscript file. Include on the title page: *a*) complete manuscript title; *b*) authors' full names, academic degrees, and affiliations (the affiliation should reflect the institution where the actual work was done and, if different, the present or permanent address should be indicated as a footnote to that author's name); *c*) name and address for correspondence, including fax number, telephone number, and e-mail address; *d*) address for reprints if different from that of corresponding author; *e*) meetings at which parts of the data were presented (including title of conference, city, and date); *f*) sources of support; and *g*) a running head of no more than 40 characters.
The title page must also include disclosure of funding received for this work from any of the following organizations: National Institutes of Health (NIH); Wellcome Trust; Howard Hughes Medical Institute (HHMI); and other(s).

Abstract and Key Words

The abstract should be structured and limited to 250 words depending on article type. It must be factual and comprehensive. Limit the use of abbreviations and acronyms, and avoid general statements (eg, "the significance of the results is discussed"). List 3 to 6 key words or phrases.

Text
Organize the manuscript file into sections with appropriate section headings. The sequence should be as follows: title page, abstract/key word page, introduction, methods, results, discussions, acknowledgments, references, tables, figures and figure captions.
Authors should type, whenever possible, all mathematical and chemical symbols, equations, and formulas, and identify all unusual symbols the first time they are used. Define abbreviations at first mention in text and in each table and figure. If a brand name is cited, supply the manufacturer's name and address (city and state/country).

Abbreviations
For a list of standard abbreviations, consult the Council of Biology Editors Style Guide (available from the Council of Science Editors, 9650 Rockville Pike, Bethesda, MD 20814) or other standard sources. Write out the full term for each abbreviation at its first use unless it is a standard unit of measure.

References
The authors are responsible for the accuracy of the references. Key the references (double-spaced) at the end of the manuscript. (If using End Note, set the style output to *JAMA*.) Cite references in text in order of appearance. Cite unpublished data, such as papers submitted but not yet accepted for publication, or personal communications, in parentheses in the text. If there are more than 3 authors, list only the first 3 authors and then use et al. Refer to the List of Journals Indexed in Index Medicus for abbreviations of journal names. Sample references are given below:

Journal Article
76 Schambelan M, Benson CA, Carr A, et al. Management of metabolic complications associated with antiretroviral therapy for HIV-1 infection: recommendations of an International AIDS Society-USA panel. *J Acquir Immune Defic Syndr.* 2002;31:257–275.

Book Chapter
76 Wortmann RL, Bentzel CJ. Renal handling of uric acid. In: Massry SG, Glassock RJ, eds. *Massry and Glassock's Textbook of Nephrology*. Phiadelphia: Lippincott Williams & Wilkins, 2001;90–92.

Entire Book
76 Mandell GL, Mildvan D, eds. *Atlas of AIDS*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2001.

Software
76 Epi Info [computer program]. Version 6. Atlanta: Centers for Disease Control and Prevention, 1994.

Online Journals
76 Friedman SA. Preeclampsia: a review of the role of prostaglandins. *Obstet Gynecol* [serial online]. January 1988;71:22–37. Available from: BRS Information Technologies, McLean, VA. Accessed December 15, 1990.

Database
76 CANCERNET-PDQ [database online]. Bethesda, MD: National Cancer Institute, 1996. Updated March 29, 1996.

World Wide Web
76 Panel on Clinical Practices for the Treatment of HIV Infection. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents. Department of Health and Human Services and Henry J. Kaiser Foundation, January 28, 2000. Available at: http://www.hivatis.org/guidelines/AA599.pdf.

Paper Presented at a Conference
8. Koenig L, Ellerbrock T, Pratt-Palmire M, et al. Prospective predictors of medication adherence: a study of the first six months of highly active antiretroviral therapy (HAART) using electronic monitoring [WePeB5818]. Presented at: XIV International AIDS Conference; 2002; Barcelona.

Figures
Cite figures consecutively in the text, and number them in the order in which they are discussed. Submit all artwork

in camera-ready form through Editorial Manager. Authors must submit figures as separate electronic files. High-quality hard copies may be requested once the manuscript has been accepted for publication. Lettering should be large enough that it will remain legible after figure reduction; typewritten or unprofessional lettering is unacceptable. Figure parts (A, B, C) may be left unlabeled (but clearly marked on back) for professional placement by the journal's printer.

Figure Legends

Legends must be submitted for all figures. They should be included in the manuscript file, should be brief and specific, and should appear on a separate manuscript page after the references. Use scale markers in the image for electron micrographs, and indicate the type of stain used.

Color Figures

The journal accepts for publication color figures that will enhance an article. Authors who submit color figures will receive an estimate of the cost for color reproduction. If they decide not to pay for color reproduction, they can request that the figures be converted to black and white at no charge.

Digital Figures

76 **Creating Digital Artwork**

1 Learn about the publication requirements for Digital Artwork: http://links.lww.com/ES/A42
2 Create, Scan and Save your artwork and compare your final figure to the Digital Artwork Guideline Checklist (below).
3 Upload each figure to Editorial Manager in conjunction with your manuscript text and tables.

B) Digital Artwork Guideline Checklist

Here are the basics to have in place before submitting your digital artwork:

- Artwork should be saved as TIFF, EPS, or MS Office (DOC, PPT, XLS) files. High resolution PDF files are also acceptable.
- Crop out any white or black space surrounding the image.
- Diagrams, drawings, graphs, and other line art must be vector or saved at a resolution of at least 1200 dpi. If created in an MS Office program, send the native (DOC, PPT, XLS) file.
- Photographs, radiographs and other halftone images must be saved at a resolution of at least 300 dpi.
- Photographs and radiographs with text must be saved as postscript or at a resolution of at least 600 dpi.
- Each figure must be saved and submitted as a separate file. Figures should not be embedded in the manuscript text file.

Remember:

- Cite figures consecutively in your manuscript.
- Number figures in the figure legend in the order in which they are discussed.

6. Cover Letter for submission to JAIDS

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Dear JAIDS Editors,

We are pleased to submit an original article entitled "Impact of birth HIV PCR testing on uptake of follow-up EID services for HIV-exposed infants in Cape Town" for consideration for publication in JAIDS (Implementation and Operational Research).

Despite substantial investment in the elimination of mother to child transmission of HIV (MTCT) paediatric HIV remain a global burden, with more than 200,000 infants identified as infected in 2014. Prompt diagnosis and early introduction of ART have been shown to dramatically reduce mortality in children infected with HIV, but identification of infected infants through virologic diagnostic testing remains a complex process.

- The World Health Organization (WHO) currently recommends that all infants born to HIV-infected mothers be tested for HIV by 6 weeks of age.
- There are growing concerns, that in the context of high maternal and infant prophylaxis the sensitivity of the assay at this age is reduced.
- The current early infant diagnosis (EID) cascade already requires multiple postpartum visits, and sees high levels of loss to follow up.
- Published studies across sub-Saharan Africa argue that delays in result return mean infected infants often demise before the age of six weeks or become loss to follow up before successful linkage to care can occur.

The **South African National Guidelines recently recommend routine HIV PCR testing of all HIV-exposed newborns ('birth-testing') to help detect *in utero* HIV transm**ission. However, birth testing necessitates a follow up test to detect intrapartum infections, and **there are concerns around the paucity of data for diagnosis and treatment of infants in the neonatal period and lack of awareness on the impact of introducing another test into the EID cascade**.

We conducted a retrospective cohort study using routine clinical and laboratory data from a large obstetric hospital in Cape Town to determine the yield of birth testing in this setting and examine if receipt of a negative birth test result would affect the level of follow-up testing at 6-10 weeks

- We found that 92% of infants infected with HIV were detectable at birth and levels of linkage to care within 3 months were similar amongst those who were diagnosed at birth or those diagnosed at 6 weeks of age.
- The data also indicated that infants who underwent birth-testing may be less likely to return for a subsequent EID test. 73% of infants returned for follow up testing after receipt of a birth test, compared to 85% of infants who did not receive a test at birth (OR,2.17; 95%CI,1.61-2.93). Those that did return, arrived at a significantly older age on average if they had received a birth test previously (mean age 60vs50 days, $p<0.01$).

The **implementation of birth testing is being more widely considered in high burden settings**, it is important that **policy makers consider the impact of introducing a birth-testing into the EID cascade**. Successful testing to detect infants infected with HIV is an essential step towards improving life expectancy in this vulnerable population. It is our hope that this paper will help to broaden the discussion round birth testing in neonates undergoing HIV testing at birth and will contribute to the progress being made in the elimination of mother to child transmission.

Preliminary results were presented as an oral abstract at the Paediatric HIV workshop in July 2016. Our work reflects the first analysis of HIV-exposed infants receiving a birth test and the possible impact on follow up programmes from a South African birth cohort. The manuscript has not been accepted for publication elsewhere, and there is no overlap of the content of this paper with any prior publication. All authors have seen and approved the content and have contributed significantly to the work.

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Thank you for your consideration.

Signed by candidate

7. Completed Demograohic Table for HIV-exposed infants stratified by recipt of an HIV-PCR test at birth

Table 2: Summary population demographics by receipt of birth testing (§Criteria used to identify high risk infants eligible for a birth HIV PCR test. #Criteria for matching HIV-exposed infants who received a birth test to HIV-exposed infants who did not.)

Variable	Birth Test N=551 (%)	No Birth Test N=551 (%)	Total N=1102 (%)
6 week HIV PCR result:			
Negative	399 (72.4)	468 (86.8)	867 (78.7)
Positive	2 (0.4)	2 (0.4)	76 (0.4)
Not Tested	150 (27.2)	81 (14.7)	231 (21.0)
Age if returned for 6wk test (days):			
Mean (SD)	59.6 (41.8)	49.5 (22.8)	54.1 (33.3)
less than 28 days	4 (1)	10 (2)	14 (1.3)
4wks-10wks	337 (84)	427 (90.9)	764 (87.7)
10wks-6months	50 (12.5)	30 (6.4)	80 (9.2)
6-9months	76 (1.3)	2 (0.4)	7 (0.8)
9-12months	5 (1.3)	1 (0.2)	6 (0.7)
Sex:			
Missing	0 (0)	76 (0.7)	76 (0.3)
Male	272 (49.4)	271 (49.2)	543 (49.3)
Female	279 (50.6)	276 (50.1)	555 (50.4)
§ Birthweight (g):			
Mean (SD)	2781.4 (700.1)	3173.5 (566.1)	2977 (665.7)
Missing	4 (0.7)	1 (0.2)	76 (0.4)
Normal (>2500)	363 (65.9)	505 (91.7)	868 (78.8)
Low (1500-2500)	161 (29.2)	40 (7.3)	201 (18.2)
Very Low (1000-1500)	17 (3.1)	76 (0.5)	20 (1.8)
Extremely Low (<1000)	6 (1.1)	2 (0.4)	8 (0.7)
# Mode of Delivery			
Nvd	291 (52.8)	291 (52.8)	582 (52.8)
C/S	260 (47.2)	260 (47.2)	520 (47.2)
Method of Delivery:			
Elective C/S	20 (3.6)	14 (2.5)	34 (3.1)
Emergency C/S	240 (43.5)	246 (44.6)	486 (44.1)
NVD	287 (52.1)	291 (52.8)	578 (52.5)
Vaginal Breech	4 (0.7)	0 (0)	4 (0.4)
§ Gestation (wks)			
Mean (SD)	38.0 (2.9)	38.8 (1.9)	38.4 (2.5)
Missing	2 (0.4)	15 (2.72)	17 (1.5)
Term (>37)	401 (72.8)	486 (88.2)	887 (80.5)
Late Preterm (34-37)	99 (18.0)	39 (7.1)	138 (12.5)
Moderate Preterm (32-34)	24 (4.4)	6 (1.1)	30 (2.7)
Very Preterm (<32)	25 (4.5)	5 (0.9)	30 (2.7)
Infant Feeding			
Missing	13 (2.4)	76 (0.7)	17 (1.5)
Breastfeeding	438 (79.5)	460 (83.48)	898 (81.5)
Formula	100 (18.2)	87 (15.8)	187 (17.0)

§ Maternal Age (years)			
Mean (SD)	28.2 (5.9)	29.2 (5.3)	28.7 (5.6)
Missing	2 (0.4)	3 (0.5)	5 (0.5)
15-19	39 (7.1)	18 (3.3)	57 (5.1)
20-24	118 (21.4)	84 (15.3)	202 (18.3)
25-39	184 (33.4)	185 (33.6)	369 (33.5)
30-34	120 (21.8)	166 (30.1)	286 (26.0)
35-39	70 (12.70)	83 (15.1)	153 (13.9)
40+	18 (3.27)	12 (2.2)	30 (2.7)
Maternal Race			
Missing	43 (7.8)	62 (11.25)	105 (9.5)
Black	433 (78.6)	454 (82.4)	887 (80.5)
Coloured	73 (13.25)	32 (5.8)	105 (9.5)
Foreign	2 (0.4)	3 (0.5)	5 (0.5)
Gravidity			
Mean (SD)	2.5 (1.3)	2.5 (1.2)	2.5 (1.2)
Missing	1 (0.2)	1 (0.2)	2 (0.2)
1	123 (22.3)	126 (22.2)	249 (22.6)
2	172 (31.2)	183 (31.38)	355 (32.2)
3	153 (27.8)	132 (27.66)	285 (25.9)
4+	102 (18.5)	109 (18.6)	211 (19.2)
§ CD4			
Mean (SD)	382.3 (291.5)	443.5 (218.3)	390.0 (283.9)
Missing	64 (11.6)	481 (87.3)	547 (49.6)
< 200	110 (22.6)	8 (11.4)	118 (10.7)
200 – 350	157 (32.2)	18 (25.7)	175 (15.9)
351 – 500	104 (23.8)	24 (28.3)	128 (11.6)
>500	116 (23.8)	24 (34.3)	140 (12.7)
§ PMTCT Coverage			
Received ART 12+weeks	276 (50.1)	551 (100)	827 (75.0)
Received ART <12weeks	218 (39.6)	-	218 (19.8)
No ART	57 (10.3)	-	57 (5.2)
§ Default on Treatment			
No default recorded	75 (13.0)	-	75 (6.8)
Recorded default			
§ Viral Load			
VL <1000	82 (14.2)	-	82 (7.4)
VL >1000			

Criteria for matching HIV-exposed infants who received a birth test to HIV-exposed infants who did not
§Criteria used to identify high risk infants eligible for a birth HIV PCR test.

8. Demographic Characteristics of HIV-exposed infants stratified by EID presentation.

Table 3: Description of infant characteristics eligible for an EID test at 6 week based on test attendance (§Criteria used to identify high risk infants eligible for a birth HIV PCR test. #Criteria for matching HIV-exposed infants who received a birth test to HIV-exposed infants who did not.)

Variable	Not Tested N=231 (%)	Tested N=871 (%)	Total N=1102	P value
Cohort:				
Birth test	150 (27.2)	401 (72.8)	551	<0.001
No birth test	81 (14.7)	470 (87.3)	551	
Sex:				
Missing	1 (0.4)	76 (0.3)	76 (0.4)	0.84
Male	110 (47.6)	433 (49.7)	543 (49.3)	
Female	120 (52.0)	435 (50.0)	555 (50.7)	
Birthweight (g):				
Mean (SD)	2901.6 (653.6)	2996.3 (706.8)	2977.7 (665)	**0.08**
Missing	2 (0.9)	3 (0.3)	5 (0.4)	
Mode of Delivery				
Nvd	118 (51.1)	402 (46.2)	520 (47.2)	0.24
C/S	113 (48.9)	469 (53.9)	582 (52.8)	
Gestation (wks)				
Mean (SD)	38.4 (2.9)	38.4 (2.4)	38.4 (2.5)	0.75
Missing	1 (0.4)	16 (1.84)	17 (1.5)	
Infant Feeding				
Missing	6 (2.6)	11 (1.3)	17 (1.5)	0.30
Breastfeeding	189 (81.2)	709 (81.4)	898 (81.5)	
Formula	36 (15.6)	151 (17.3)	187 (17.0)	
Maternal age (years)				
Mean (SD)	28.6 (5.7)	28.7 (5.6)	28.7 (5.6)	0.67
Missing	3 (1.3)	2 (0.2)	5 (0.5)	
Maternal race				
Missing	24 (10.39)	81 (9.30)	105 (9.5)	0.27
Black	177 (76.6)	710 (81.5)	887 (80.5)	
Coloured	28 (12.1)	76 (8.8)	105 (9.5)	
Foreign	2 (0.9)	3 (0.3)	5 (0.5)	
Gravidity				
Mean (SD)	2.56 (1.3)	2.47 (1.2)	2.5 (1.2)	0.31
Prima gravida				
Missing	1 (0.4)	1 (0.1)	2 (0.2)	0.46
Yes	48 (20.8)	201 (23.1)	249 (22.6)	
No	182 (78.8)	669 (76.8)	851 (77.2)	
CD4				
Mean (SD)	395.4 (236.9)	388.2 (298.3)	390 (283.9)	0.79
Missing	90 (44.3)	414 (58.9)	504 (45.7)	
Date of birth				
q4_2013	7 (3.0)	33 (3.8)	40 (3.6)	
q1_2014*	27 (11.7)	112 (12.9)	139 (12.6)	0.33
q2_2014	28 (12.1)	94 (10.8)	122 (11.1)	
q3_2014	39 (16.9)	126 (14.8)	165 (15.0)	
q4_2014	56 (24.2)	165 (18.9)	221 (20.5)	
q1_2015	11 (4.8)	61 (7.0)	72 (6.5)	
q2_2015**	63 (27.8)	280 (32.2)	343 (31.1)	

* Data from February 2014 not included in cohort

** Data included up to August 2015

9 . Table 4: Summary of Included Literature

Author, year	Setting	Study Design	Population	Outcome Measured	Cascade Point	Key Findings
Adebimpe, 2013	Nigeria	Review	HIV-exposed infants	Barriers to successful EID programmes	Identification of infants for testing	EID programmes are hindered from effective EID due to health system constraints along with fear of discrimination
Adeniyi, 2015	South Africa, rural	Semi-structured interviews	HIV-infected mothers	Perspectives of mothers of accessing EID	Complete cascade	Mothers have fairly good knowledge of HIV and risks of transmission but face barriers of fear, stigma and access
Ahmed, 2013	Resource limited	Review	HIV-infected infants	Barriers to case finding HIV-infected infants and children	Missed HIV-infected infants	Improved efforts are needed to find infants missed by EID testing
Aliyu, 2014	Nigeria, rural	Prospective cohort study	HIV-infected pregnant women	Probability of initiation of EID	Presentation for testing	Presentation for EID testing depended upon location, access to care during pregnancy
Ambia, 2016	Global	Systematic Review	HIV-infected pregnant women and their infants	Interventions to improve rention in care and uptake of EID testing	PMTCT/EID overlap	Male partner involvement and mobile phone reminders may have important roles in retention in care, more evidence needed
Ananworanich, 2014	USA	Viewpoint	HIV-infected infants	Viral replication in infants infected with HIV	Achieving remission in HIV-infected infants	Amount of latent virus, and markers for on-going viral replication in infants may be important in achieving HIV remission
Anoje, 2012	Nigeria,	Retrospective cohort study	HIV-infected pregnant women and their infants	EID presentation and result at 6 weeks	PMTCT/EID overlap	PMTCT interventions reduce transmission of HIV, infant feeding practices need to be addressed according to access to treatment
Ayele, 2015	Ethiopia	Retrospective cohort study	HIV-infected pregnant women	Treatment outcome	PMTCT cascade	All people living with HIV/AIDS should initiate ART as early as possible

Barron, 2013	South Africa,	Review	HIV-infected pregnant women and their infants	Lessons learnt in the elimination of MTCT	PMTCT/EID overlap	Despite excellent progress, challenges remain in the EMTC
Becquet, 2012	Global	Systematic review and meta analysis	HIV-infected infants	Survival of infants initiating ART	Linkage to care and survival	There remains an urgent need to urgency of provide ART to women during pregnancy to prevent early vertical transmission
Bedelu, 2007	South Africa, rural	Prospective evaluation of a pilot intervention	HIV-infected pregnant women	Treatment outcomes for women on ART	PMTCT	Task shifting of PMTCT services can improve treatment outcomes. Barriers to treatment include location of facility, staff capacity and counselors
Ben-Farhat, 2013	Sub-Saharan Africa	Longitudinal analysis	HIV-infected infants	Two-year mortality and programme attrition rates per 1000 person-years stratified by age group	Retention in care for HIV-infected infants	Earlier diagnosis and treatment of infected infants is needed to reduce mortality.
Bhardwaj, 2014	South Africa	Prospective evaluation of novel intervention	HIV-infected pregnant women	Facility-level bottlenecks to PMTCT	PMTCT	District-level, data-driven quality improvement processes at a national scale to improve the performance of the PMTCT programme
Bingawaho, 2013	Rwanda	Programme evaluation for scale up of EID	HIV-infected pregnant women and their infants	Linkage to care, result retrun to caregivers	EID Presentation to result return	Scale up of EID programmes nationally have allowed improvements in result return but barriers exist to linkage to care
Bonner, 2013	Global	Systematic review	HIV-infected adults	Viral load to monitor adherence to ART	-	Viral load can be used to monitor adherence to ART
Bourne, 2009	South Africa, urban	Retrospective cohort study	Perinatal deaths under 1 year of age	Postnatal mortality	-	Intrauterine and intrapartum infection may contribute to peak age of mortality of 10/11 weeks
Braitstein, 2011	Kenya	Prospective evaluation study	HIV-infected pregnant women and their infants LTFU	Reasons for LTFU	Retention within EID cascade	Mortality, fear of discrimination and disclosure issues were related to becoming LTFU
Braun, 2011	Malawi, urban	Retrospective observational	HIV-infected infants	HIV-prevalence, linkage to care and	Retention in care for infected infants	High attrition rates from care. Initiation of antiretroviral therapy

		cohort.		retention in care		increased the likelihood of survival
Cassol, 1991	USA	Validation of new sampling technique	HIV-exposed specimens	Sensitivity and Specificity	Diagnosis of HIV using DBS	DBS PCR will prove valuable for large-scale implementation of EID
Chamla, 2015	Global	Systematic Review	HIV-exposed women and their infants	Uptake of EID services, possible barriers to presentation	EID presentation	HIV testing during immunization clinic visits is acceptable and feasible as a possible model for service delivery
Chatterjee, 2011	Africa and Asia	National Programme review	Participants of the EID cascade	Uptake of EID, linkage to care, Age of infants tested	Complete EID cascade	Retention in care for HIV-exposed infants important whilst ensuring infected infants initiate treatment in a timely manner
Cherutich, 2008	Kenya	Policy Review	-	Identification of HIV-infected infants, treatment and PMTCT	PMTCT/EID overlap	Focus is not the integration of PMTCT with symptomatic infants, testing not widely available
Chetty, 2012	South Africa, urban	Retrospective cohort study	HIV-exposed infants	Outcomes of infants within EID cascade Age at LTFU	Retention in care for HIV-exposed infants	LTFU major barrier in knowledge of HIV transmission
Chiduo, 2013	Tanzamia	Retrospective cross-sectional study	HIV-infected infants	Overall proportion of tested infants, prevalence	Retention in care for HIV-exposed infants	Despite oncrease in testing of HIV exposed infants challenges remain for turnaround times and LTFU
Ciaranello, 2011	Resource limited	Review of the literature	HIV-exposed infants	LTFU within the EID cascade	Complete EID cascade	Even with highest reported levels of uptake nearly half of HIV-infected infants may not complete the cascade successfully
Connor, 1994	USA, France	Randomized control trial	HIV-infected pregnant women	Reduction of maternal-infant transmission	PMTCT	Zidovudine treatment reduced transmission to infants
Cook, 2011	Mozambique, rural	Retrospective cohort study	HIV-exposed infants	Follow up testing rates for EID and age at first test	EID presentation	Three of four HIV-infected women in rural Mozambique did not bring their children for early infant HIV diagnosis

Coovadia, 2009	South Africa	Viewpoint	PMTCT programmes	Call to action for information	PMTCT/EID	Further commitment needed to PMTCT programmes
Cotton, 2013	South Africa	Randomized control trial	HIV-infected infants	Time to failure of first-line ART	Linkage to care	Early time-limited ART had better clinical and immunological outcomes than deferred ART,
Coulibaly, 2014	Burkina Faso	Cross-sectional	HIV-exposed infants	Bottlenecks in EID, ART initiation	EID cascade	Limited coverage and many bottlenecks in PMTCT cascade
Creek, 2007	Resource limited	Viewpoint	HIV-exposed infants	reviews challenges and progress in EID	EID cascade	Decisions about when and how to test infants, should be made on a country basis for the best outcomes
Creek, 2008	Bostwana	Validation of new sampling technique	HIV-exposed specimens	Sensitivity and Specificity	Diagnosis of HIV using DBS	DBS PCR will prove valuable for large-scale implementation of EID
Davies, 2015	South Africa	Viewpoint	HIV-infected infants and adolescents	Achieving 90-90-90	Linkage to care	challenges require multiple players to attain better treatment for infants
Davies, 2015	South Africa	Viewpoint	HIV-infected neonates	Challenges to neonatal care	EID cascade	Evidence is lacking to inform guidelines and programme development for
Davies, 2015	Sub-Saharan Africa	Systematic review	HIV-infected infants	ART treatment for infected infants	Linkage to care	There is an urgent need to address barriers to ART for infants
Donahue, 2012	Malawi, urban	Qualitative study – semi structured interviews	HIV-infected pregnant women and their infants	Identify and explore barriers women face in accessing HIV testing and care for their infants	LTFU from care	lack of knowledge regarding EID and infant ART, fear of disclosure and lack of psychosocial support all limit retention in care
Dube, 2012	Malawi	Programme evaluation for scale up of EID	HIV-infected pregnant women and their infants	Linkage to care, result retrun to caregivers	EID Presentation to result return	Scale up of EID programmes nationally have allowed improvements in result return but barriers exist to linkage to care
Dunning, 2015	South Africa	Viewpoint	HIV-infected infants	Linkage to care and sensitivity of diagnositic device	Linkage to care	Sensitivty isn't everything in diagnosis if logistical challenges removed
Edmonds, 2015	DRC	Retrospective cohort study	HIV-infected pregnant women and their infants	HIV/CD4 testing and EID testing coverage	PMTCT/EID coverage	Optimal service delivery hard to maintain at decentralized facilities

Eley, 2006	South Africa	Retrospective cohort study	HIV-infected infants	Outcomes of infants on ART	Retention in care on ART	HAART can improve the health of many HIV- infected children with advanced disease
Essajee, 2015	Global	Commentary	HIV-infected infants	Outcomes of infants on ART	Retention in care on ART	New strategies for diagnosis could improve identification and retention in care of HIV-infected infants
Evans, 2016	Global	Review	HIV-exposed infants	HIV infection and co-morbidities	-	PMTCT/EID services should be integrated into wider newborn child healthcare
Feucht, 2015	South Africa	Retrospective cohort study	HIV-infected infants	HIV infection and co-morbidities	Retention in care on ART	Due to high levels of LTFU new interventions for paediatric HIV case finding are required
Francke, 2016	South Africa	Modelling study	HIV-exposed infants	Timing of EID test	Presentation to testing	Cost-effective to implement a birth test as increase HIV-infected infants survival
Ghadrshenas, 2013	Resource limited	Review	HIV-exposed infants	Barriers to EID testing	EID cascade	Coverage of EID remains low in many settings, for where it isn't too few services are focused on retention of HIV-exposed infants in care
Goga, 2012	South Africa	Program evaluation using facility based survey	HIV-exposed infants	Effectiveness of PMTCT programme, transmission, retention, treatment	PMTCT	Virtual elimination of paediatric HIV infection is possible with intensified effort
Gourlay, 2013	Sub-Saharan Africa	Systematic review	HIV-infected pregnant women	Barriers to treatment	PMTCT	health-systems issues and community level factors prevent women accessing treatment
Gouveia, 2014	Brazil	Retrospective cohort study	HIV-exposed infants	Predictors of LTFU	PMTCT/EID overlap	Health system constraints can affect presentation to care for EID
Granuch, 2009	South Africa	Modelling	Adults living with HIV	Transmission of HIV	-	Universal test and treat could affect transmission at the population level
Hassan, 2012	Kenya, rural	Prospective cohort study	HIV-infected pregnant women and their infants	Uptake and LTFU within EID cascade	PMTCT/EID overlap	Lack of knowledge, inadequate service provision and fear of discrimination lead to LTFU

Hlarlaithe, 2014	Resource limited	Review	HIV-infected pregnant women and their infants	Uptake and LTFU within EID cascade	PMTCT/EID overlap	Social and economic factors, physiological status and psychological conditions lead to LTFU
Horwood, 2010	South Africa	Qualitative, semi-structured interviews	HIV-infected pregnant women and their infants	Comparison of recorded and reported information	EID presentation	Poor integration of PMTCT services into routine care, lack of clarity about health worker roles cause LTFU to occur
Hsiao, 2016	South Africa	Validation of new diagnostic technique	HIV-exposed specimens	Sensitivity and Specificity	Diagnosis of HIV using DBS	POC PCR will prove valuable for large-scale implementation of EID
Hsiao, 2013	South Africa	Retrospective cohort study	HIV-infected infants	Linkage to care after positive test result	Linkage to care	A large proportion of infected infants remain unlinked to antiretroviral therapy services and
Ines, 2014	South Africa	Retrospective cohort study	HIV-infected infants	Linkage to care and treatment outcomes for infected infants	Linkage to care	ART initiation by three months of age may not adequately prevent disease progression
Jani, 2014	Mozambique, rural	Validation of new diagnostic technique	HIV-exposed specimens	Sensitivity and Specificity	Diagnosis of HIV using DBS	POC PCR will prove valuable for large-scale implementation of EID
Johnson, 2012	South Africa	Modelling	HIV-infected infants	Linkage to care and treatment outcomes for infected infants	Linkage to care	ART initiation needs to occur earlier to have an impact on survival
Jones, 2005	South Africa	Retrospective cohort study	HIV-exposed infants	Retention in care, possible barriers	EID presentation	Socio-economic factors contributor to poor follow-up
Kageha, 2012	Kenya	Validation of new sampling technique	HIV-exposed specimens	Sensitivity and Specificity	Diagnosis of HIV using DBS	DBS PCR will prove valuable for large-scale implementation of EID
Kalk, 2016	South Africa, urban	Retrospective cohort study	HIV-exposed infants	EID presentation, coverage of HIV-PCR testing at birth	EID Presentation	LTFU could be increased by addition of an HIV-PCR test at birth
Kebede, 2014	Ethiopia	Retrospective cohort study	HIV-exposed infants	potential risk factors for not achieving early diagnosis.	EID Presentation	Barriers exist causing LTFU, reduction in test turnaround time, from sample collection, to

						laboratory, to the return of test results, is urgently required.
Khamadi, 2008	Kenya	Program evaluation – pilot study	HIV-exposed infants	Barriers to implementing EID testing	EID presentation	Coordinated training of health workers, rapid testing from a central laboratory and quick dispatch of results can minimize the complexities of PCR testing
Kiyaga, 2015	Uganda	Retrospective cohort study	HIV-exposed infants	potential risk factors for not achieving early diagnosis.	EID Presentation	Barriers exist causing LTFU, reduction in test turnaround time, from sample collection, to laboratory, to the return of test results, is urgently required.
Kroon, 2015	South Africa	Viewpoint	HIV-infected infants	Recognizing and managing HIV-infected infants	Linkage to care	Improved knowledge and management of neonates and women during pregnancy is required to improve outcomes
Lerebo, 2014	Ethiopia	Cross-sectional, modelling	HIV-exposed infants	potential risk factors for not achieving PMTCT.	PMTCT	Barriers exist causing LTFU, including health system and individual, geographic
Lilian, 2014	South Africa	Modelling	HIV-infected infants	Timing of infant testing	Linkage to care	Six weeks may no longer be the optimal age to diagnose perinatal HIV infection. Birth and 10 weeks optimal
Lilian, 2012	South Africa	Prospective cohort study	HIV-exposed infants	Timing of detection of HIV infection	Specimen Processing	76.3% of all infants with early HIV infections at birth and by 4 weeks were 96% sensitive
Lilian, 2013	South Africa	Prospective cohort study	HIV-exposed infants	Timing of detection of HIV infection	Specimen Processing	Six-week testing delayed antiretroviral therapy initiation beyond the time of early HIV-related infant mortality and missed one-fifth of perinatally HIV-infected infants. Earlier
Mayaux, 2011	India	Prospective cohort study	HIV-exposed infants	Follow up of infants, outcomes, comorbidities	EID cascade	Careful virological monitoring of HIV infected infants could help improve outcomes
Makau, 2015	Kenya	Cross-sectional	HIV-exposed infants	Barriers to EID testing	EID Presentation	Barriers exist causing LTFU, including health system and individual, geographic

Maritz, 2016	South Africa	Retrospective cohort study	HIV-exposed infants	EID presentation after a birth test	EID Presentation	Low uptake of routine EID after a birth test
Marston, 2011	Sub-Saharan Africa	Systematic Review	HIV-infected infants	Survival of perinatally and postnatally infected infants	Retention in care	Those children infected perinatally had a much higher risk of dying than those infected through breastfeeding
Mccollum, 2012	Malawi	Retrospective cohort study	HIV-exposed infants	Age and acceptability of infant testing	EID Presentation	Testing at immunization clinics simpler and more acceptable.
Mofnson, 2010	Resource Limited	Systematic Review	HIV-exposed infants	Barriers to EID testing	PMTCT/EID cascade	Greater expansion and commitment to PMTCT/EID programmes are required to overcome gaps in knowledge, HIV resistance and further transmissions
Mugglin, 2013	Resource Limited	Systematic Review	HIV-infected infants	Retention in care, possible barriers	EID presentation	Urgent need to improve access to care for infected infants, majority eligible for ART initiation
Myer, 2016	South Africa	Retrospective cohort study	HIV-infected pregnant women	Virological episodes during pregnancy	PMTCT	Interventions required to improve adherence, virologic episodes common during pregnancy
Myer, 2015	South Africa	Retrospective cohort study	HIV-infected pregnant women	ART initiation	PMTCT	Different models of service delivery can impact ART initiation
Naiwatanakul, 2016	Thailand	Retrospective cohort study	HIV-exposed infants	EID coverage, MTCT rates	EID Presentation	Increase in national EID programme uptake and coverage, however linkage to ART remains somewhat limited, active case finding required
Newell, 2004	Africa	Combined randomized intervention trials	HIV-exposed infants	Mortality of infants	EID cascade	Mortality varied by geographical region, and was associated with maternal death, and HIV infection
Nondoki, 2015	Côte d'Ivoire	Cross-sectional	HIV exposed infants	Maternal and Paternal acceptance of EID testing	EID acceptance and testing	Paternal consent was poor and result return was low. Links between PMTCT and PPOC require strengthening to improve

						children accessing ART
Nutall, 2015	South Africa	Viewpoint	HIV-infected infants	ART initiation	Linkage to acre	Safety and efficacy data on neonatal Cart is currently very limited
Nuwagaba-Biribonwoha, 2007	Uganda	Qualitative, Semi-structured interviews	Health care workers, PMTCT programme	Experiences of implementing PMTCT programme	PMTCT/EID cascade	Staff had positive attitudes, many challenges with HIV-disclosure and management of infected infants
Nuwagaba-Biribonwoha, 2010	Tanzania	Validation of new sampling technique	HIV-exposed specimens	Sensitivity and Specificity and timing of testing	Diagnosis of HIV using DBS	DBS PCR will prove valuable for large-scale implementation of EID
Penazzato, 2014	Resource limited	Viewpoint	HIV-exposed infants	Overview of EID cascade	PMTCT/EID cascade	Little cost-effectiveness evidence exists about different strategies to deliver early infant diagnosis services
Phelps, 2013	Resource limited	Review	HIV-exposed infants	Overview of EID cascade	PMTCT/EID cascade	Key individual, institutional, and systems barriers to diagnosing children with HIV exist
Pillay, 2001	South Africa, urban	Prospective cohort study	HIV-infected infants	Outcome of infected infants	Linkage to care	HIV progresses rapidly in newborns with coinfections to HIV
Prendergast, 2015	Global	Viewpoint	HIV-exposed infants	Outcomes of HIV-exposed infants and meeting global targets	EID cascade	Despite significant progress challenges remain for EMTCT
Rollins, 2014	Resource limited	Review	HIV-exposed infants	Defining retention in care and reports on how to measure	EID cascade	Retention-in-care, and the ability to correctly measure it, is highly dependent on the challenges faced by health systems
Rujumba, 2013	Uganda	Qualitative – Semi structured interviews	Pregnant women	Experiences of routine counseling and testing	PMTCT	Strengthen post-test and follow up counselling for both HIV positive and negative women is required
Sawe, 2016	Tanzania, urban	Retrospective cross-sectional	HIV-exposed infants	Documentation of pretest and post-test counselling	EID presentation and counseling	Counselling was much more likely to be documented when the test result was positive.

		study				
Shapiro, 2010	Sub-Saharan Africa	Commentary	HIV-exposed infants	Childhood mortality	EID cascade	More information is needed for HIV-exposed uninfected infants, HIV-infected high levels of mortality
Sherman, 2005	South Africa	Validation of new sampling technique	HIV-exposed specimens	Sensitivity and Specificity and timing of testing	Diagnosis of HIV using DBS	DBS PCR will prove valuable for large-scale implementation of EID
Sherman, 2015	South Africa	Viewpoint	HIV-exposed infants	Timing of testing and linkage of HIV infected infants to care	EID presentation	6 week testing is no longer the optimum timing for diagnosis, too late to impact peak age of mortality and too early to detect all infections.
Sherman, 2005	South Africa	Retrospective cohort study	HIV-exposed specimens	Sensitivity and Specificity and timing of testing	EID testing	a single 6-week HIVDNAPCR test can increase identification of HIV-infected children
Sherman, 2005	South Africa	Prospective longitudinal study	HIV-exposed infants	Costing and validation and	EID testing	A marginal additional investment by government to access an earlier HIV diagnosis for infants could triple the efficacy of PMTCT programmes
Sidze, 2015	Cameroon	Prospective longitudinal study	HIV-exposed infants	LTFU among women/infant pairs required to attend follow up visits	EID presentation	Factors associated with LTFU differ according to maternal HIV serostatus.
Sidibe, 2016	Thailand	Commentary	HIV-exposed infants	Elimination of Mother to child transmission	EID cascade	WHO certified that Thailand had eliminated mother-to-child transmission of HIV
Smit, 2014	Global	Systematic Review	HIV-exposed specimens	Sensitivity and Specificity	Diagnosis of HIV using DBS	DBS PCR will prove valuable for large-scale implementation of EID
Smith, 2014	South Africa, rural	Cross-sectional	HIV-infected infants	Retention in care and linkage to care, infants receiving ART after HIV diagnosis	Linkage to care	Proportions of infected infants linked to care are extremely low.

Sohn, 2013	Global	Review Article	HIV-infected infants	Retention in care, passing into adolescence	Retention in care	Challenges related to perinatally infected infants moving into adolescence, success is possible but there are consequences to lifelong ART requirements
Tsague, 2014	Resource limited	Commentary	HIV-infected pregnant women	ART treatment experiences	PMTCT	Health system constraints remain barriers for complete access
Varga, 2015	South Africa, urban	Qualitative Study	HIV-infected mothers	Experiences of mothers regarding infant testing and diagnosis	EID cascade	Diagnosis had both beneficial and detrimental psychosocial effects
Violari, 2008	South Africa	Randomized control trail	HIV-infected infants	Treatment outcomes for infants accessing ART, delayed or otherwise	ART Treatment	Earlier initiation of treatment improves outcomes for infected infants
Wang, 2016	Global	Programmes evaluation using national estimates	HIV-infection globally	Incidence, prevalence and mortality of HIV	-	Despite scale ups in treatment, incident infections have not been prevented. Further effort required for 90-90-90
Wagner, 2015	Kenya	Prospective cohort study	HIV-exposed infants	HIV prevalence, number needed to test, infant age at testing, and turnaround time for tests	EID presentation	Diagnosis of HIV infections before symptom onset can increase survival of infected infants
Willcocks, 2016	UK	Qualitative	HIV-infected mothers	Perceived challenges and facilitating factors for mother-infant bonding	EID cascade	More targeted psychological support is required to ensure adequate mother infant bonding
Woelk, 2016	Rwanda	Retrospective cohort study	HIV-exposed infants and their mothers	Retention in care	EID presentation Linkage to care	Strategies need to be developed to identify, provide support and trace these women at risk of loss to follow-up
Woldesenbet, 2015	South Africa	Cross sectional	HIV-exposed infants and their mothers	Barriers to retention in care	EID presentation	Missed opportunities for EID were attributed to poor documentation of HIV status, inadequate maternal knowledge about mother-to-child HIV transmission, fear of

						discrimination
Yapo, 2013	Côte d'Ivoire	Cross sectional	HIV-exposed infants	Diagnostic performance of assays	EID testing	Good performance using DBS for infant diagnosis

10. Table 5: Literature Search Strategy

1	HIV Infections[MeSH] OR HIV[MeSH] OR hiv[tiab] OR hiv-1*[tiab] OR hiv 2*[tiab] OR hiv1[tiab] OR hiv2[tiab] OR hiv infect*[tiab] OR human immunodeficiency virus[tiab] OR human immunedeficiency virus[tiab] OR human immune-deficiency virus[tiab] OR human immune-deficiency virus[tiab] OR ((human immune*[tiab]) AND (deficiency virus[tiab])) OR acquired immunodeficiency syndrome[tiab] OR acquired immunedeficiency syndrome[tiab] OR acquired immune-deficiency syndrome[tiab] OR acquired immune-deficiency syndrome[tiab] OR ((acquired immune*[tiab]) AND (deficiency syndrome[tiab])) OR "sexually transmitted diseases, Viral"[MeSH:noexp] OR PMTCT OR mother to child transmission
2	Child* OR infant* OR newborn OR baby OR babies OR neonatal OR birth OR postpartum
3	Diagnosis OR Early Infant Diagnosis OR EID OR identification OR prevalence OR testing
4	Linkage to care OR retention OR continuum of care OR attrition OR los* to follow up OR LTFU OR patient dropout OR LFU OR initiation OR access
5	Barriers OR challenges OR facilitate*
6	Pregnancy[MeSH] OR pregnan* OR OR matern* OR prevention of mother to child transmission OR PMTCT OR MTCT OR EID OR Early Infant Diagnosis
7	#1 AND #2 AND #3
8	#1 AND #4 AND #6
9	#1 AND #2 AND #4
10	#1 AND #5 AND #6